海外のリスク評価機関における評価結果等に関する調査

報告書

令和3年3月

一般財団法人残留農薬研究所

#### 調査の概要

2018年12月に改正された農薬取締法に基づき、2021年度から農薬の再評価制度が 開始される。これに伴い、食品安全委員会は、リスク管理機関からの諮問を受け、既 登録農薬の再評価を行うことになる。

我が国で再評価が予定されている農薬に関し,再評価制度を先んじて導入していた 海外のリスク評価機関である欧州食品安全機関(以下「EFSA」という。)及び米国環 境保護庁(以下「EPA」という。)でのこれまでの再評価に関する情報は,我が国での 再評価に向けて、大変有益である。このため、両機関における、再評価の評価書及び 再評価の関連文書を収集するとともに、農薬(有効成分)及び機関ごとにそれらを整 理した。

令和3年3月

茨城県常総市内守谷町4321番地 一般財団法人残留農薬研究所

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#### 1. 調査の目的

2018年12月に改正された農薬取締法に基づき、2021年度から農薬の再評価制度が 開始される。これに伴い、食品安全委員会は、リスク管理機関からの諮問を受け、既 登録農薬の再評価を行うことになる。

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#### 2. 再評価の評価書及び関連文書の収集

本調査では、再評価が予定されている既登録農薬のうち、内閣食品安全委員会事務 局担当官が指定する4有効成分(表1)を調査対象とし、これらの有効成分の欧州委 員会(以下「EC」という。)又はEPAでのこれまでの農薬登録の有無を調査し、過去 に登録されたことのある農薬については、以下に示す再評価の評価書及び関連文書を 収集し整理した。

整理項目としては、下記のとおりである。

- 再評価開始前の事前のやり取り(企業と政府機関との相談・連絡)に関する文書
- ② 再評価時に追加要求された試験項目及びその内容に関する文書
- ③ ②の試験成績の提出の有無及びそれに係る再評価での結果に関する文書
- ④ 再評価前の評価書、再評価の評価書(例えば,欧州の場合ならDAR/RAR及び EFSA評価書は少なくとも入手、公表文献の評価に関する文書もあれば入手)、 再評価後の評価書の追補文書(存在する場合のみ)
- ⑤ 再評価結果を反映した規制の内容(欧州にあっては再評価結果を踏まえた EC に よる登録状況(再評価前、再評価後、現在)、その他の規制の内容及び EU 各国 での規制の内容)に関する文書

番号	農薬名	英名	種類
1	フィプロニル	fypronil	フェニルピラゾール系殺虫剤
2	フェンメディファム	phenmedipham	カーバメート系除草剤
3	プロスルフォカルブ	prosulfocarb	カーバメート系除草剤
4	ピメトロジン	pymetrozine	ピリジンアゾメチン系殺虫剤

表1 調査対象の農薬

なお、該当する情報を収集するために現地のレギュラトリー制度に精通している専門家 のスキルが必要となり、ヒト健康影響評価に資する農薬の再評価の評価書及び再評価の 関連文書の情報収集における正確性、信頼性を確保するため、以下の会社に再委託を行 った。

- 米国: Landis International 社 (3185 Madison Highway, Valdosta, Georgia, 31603-5126, USA)
- 欧州: Mendel-Kreusel Consult 社 (Auf der Heide 8, 53783 Eitorf, Germany)

### 3. 一覧表の作成

2項で収集した情報について、それぞれの概要についてエクセル及びワードを用いてまとめた一覧表を作成した。

## 4. ガイダンス等の翻訳

以下のガイダンス等について、和文和訳(図表等を含める)を作成した。

- US EPA: U.S. : Office of Pesticide Programs 'Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides. (2016)
- EFSA: Scientific Opinion of the PPR Panel on the follow-up of the finding of the External Scientific Report 'Literature review pf epidemiological studies linking exposure to pesticides and health effects'. EFSA Journal 2017; 15(10): 5007
- EFSA: Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013: EN-497
- LaKind JS, et.al.: A proposal for assessing study quality : Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environment International 73 (2014) 195–207
- IPCS Harmonization Project Document Document No.4 (2007)
   PART 1: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS AND CASE-STUDIES
   PART 2: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS

#### 5. 残留農薬のリスク評価に当たっての毒性試験結果の解釈に係る知見の収集

以下の4点について、海外のリスク評価機関や残留農薬のリスク評価に関する国際 機関のガイダンス等の文書、信頼性の高い公表文献等を対象に情報を収集し、それぞ れの概要(入手資料の該当ページに関する情報を含む)を日本語で作成した。

- ① 各種毒性試験における毒性を解釈する上で共通する考え方(適応性変化等)
- ② 毒性判断に用いる統計学的手法、背景データの利用
- ③ 血液学的検査、血液生化学的検査及び尿検査に対する基本的な考え方及びこれらの検査において統計的に得られる正常範囲
- ④ 体重、摂餌量及び臓器重量に対する基本的系な考え方及びこれらの重量について統計学的に得られる正常範囲

#### 6. 作業実施者の要件

3~5項に規定する概要及び翻訳の作成に当たっては、作業内容に応じて、以下の 要件を一つ以上満たした作業者が実施した。

- ・毒性学、体内動態学に関する科学的知見を有する者(学位等)
- ・化学物質のリスク評価(手法)に関する調査等の実務経験を有する者
- ・毒性学、生化学、農芸化学、生物学、有機化学、医学、薬学等の分野における論 文(英文、邦文)の検索・要約作成等の業務経験(研究等を含む)を有する者

#### 7. 専門家の意見の聴取

(ア) 2項で収集した情報の取捨及び3項の一覧表の概要の作成に当たっては、農薬の再評価制度に加えて以下の分野についての専門知識を有する者(各分野で1名程度)の意見を聴取の上、行った。(表 2)
 ・動物代謝 ・毒性 ・遺伝毒性 ・疫学

専門分野	氏名	肩書	所属
動物代謝	永田 清	教授	東北医科薬科大学
			薬学部 環境衛生学系 環境衛生学教室
毒性	山手 丈至	教授	大阪府立大学生命環境科学研究科
			獣医病理学教室
遺伝毒性	太田 敏博	名誉教授	東京薬科大学
疫学	福島 哲仁	教授	福島県立医科大学
			医学部 衛生学・予防医学講座

表2 専門家のリスト

(イ) 4項のガイダンスの翻訳に当たっては、以下の分野についての専門知識
 を有する者(各分野で1名程度)の意見を聴取の上、行った。(表 3)
 ・毒性
 ・ 褒学

専門分野	氏名	肩書	所属
疫学	福島 哲仁	教授	福島県立医科大学
			医学部 衛生学・予防医学講座
疫学	圓藤 陽子	所長	圓藤労働衛生コンサルタント事務所
疫学	横山 和仁	教授	国際医療福祉大学大学院公衆衛生学専
			攻
毒性	堀本 政夫	教授	千葉科学大学危機管理学部動物危機管
			理学科

表3 専門家のリスト

(ウ) 5項で収集した情報の取捨及び概要の作成に当たっては、①~④の各項目について以下の分野の専門知識を有する者の意見を聴取の上、行った。(表 4)

① 毒性を解釈する上での共通的な考え方

・動物代謝 ・毒性(2名以上) ・病理

- ② 毒性判断に用いる統計学的手法、背景データの利用
  - ・毒性(2名以上) ・病理
- ③ 血液学的検査、血液生化学的検査及び尿検査に対する基本的な考え方 及びこられの検査において設定される正常範囲
  - ・毒性(血液を専門とする者を含む2名以上) ・病理
- ④ 体重、摂餌量及び臓器重量に対する基本的な考え方及びこれらの重量
   について設定される正常範囲
  - ・毒性(2名以上) ・病理

表4 専門家のリスト

専門分野	氏名	肩書	所属
毒性	赤池 昭紀	名誉教授	京都大学
毒性	小野 敦	教授	岡山大学 医歯薬学総合研究科・薬学系
			毒性学研究室
毒性	桒形 麻樹子	室長	国立医薬品食品衛生研究所
			安全性生物試験研究センター 毒性第二
			室
毒性	堀本 政夫	教授	千葉科学大学
			危機管理部 動物危機管理学科

毒性	松本 清司	特任教授	信州大学
(血液)			基盤研究支援センター
病理	浅野 哲	教授	国際医療福祉大学
			薬学部 衛生化学部門
病理	美谷島 克宏	教授	東京農業大学 応用生物科学部
			食品安全健康学科 食品安全評価研究室
病理	義澤 克彦	教授	武庫川大学
			食物栄養科学部 食創造科学科
動物代謝	小澤 正吾	教授	岩手医科大学
			薬学部

## 8. 調査結果の報告会の開催

下記の日程において、調査結果の報告会を開催した。

- 日 時: 令和3年3月25日 14:00~16:00
- 形 式: Web 会議
- 参加者: 吉田 緑 委員及び食品安全委員会 事務局 (一般財団法人)残留農薬研究所

9. 調査結果

# 9.1.1. フィプロニル

【人の健康影響評価についての要約】

- ▶ 米国
  - ✓ EPA レビューページ(別紙1)

Regulations.gov - Docket Browser

▶ 初年度登録 1996年

年月	評価資料	食品経由での人健康影響エンドポイント
		・追加要求
2020年8月	Comment submitted by Fipronil Task	◇FTF(フィプロニル・タスクフォース)からの生
	Force, LLC	態影響評価、健康影響評価、食品経由での
	<b>F</b>	リスク評価に対するコメント
	[FPU2]	・バイエル社コメント:皮膚吸収試験結果から
	PDF A	吸収率は 0.65% であり、 EPA が 1% 未満と
	Comment.pdf	数値を丸める必要はない(P.12)。
2020年3月	Draft Risk Assessment for	◇オス成体、妊娠ラット、非妊娠メスラットの経
	Registration Review	口投与で投与後1~2週間後に甲状腺ホル
		モンの恒常性の乱れが認められ、暴露の継続
	[FPU4]	により、甲状腺に有害な顕微鏡的変化をもた
	PDF 8	らした。
	EPA-HQ-OPP-201	◇ラット、マウスの経口投与では肝毒性が、甲
	1-0448-0076.pdf	状腺ホルモン及び神経行動学的臨床徴候の
		変化を誘発する用量でのみ認められた。
		・変異原性を示さなかったが、慢性曝露により
		ラット(オス/メス)で甲状腺濾胞細胞腫瘍が
		発生した。その結果、ヒト発がん性物質(グル
		ープ C)に分類された。
		◇飲料水への残留が予想されたことにより食
		品経由のリスク要因となった。
		$\Diamond$ CTA(Cumulative Thyroid Assay)
		を毒性データベースに追加したことで、本有効
		成分及び代謝物/分解物の暴露による感受
		性の増加における胎児及び乳幼児のリスク評
		価が十分となったため、FQPA 安全係数を X1
		とした。
2020年3月	Exposure Assessment (DP 433141)	飲用水経由のリスク評価補遺

	for Deviation Deviation	
	for Registration Review	◇非農業用途を追加した DWA (Drinking
	(	Water Assessment)の更新
	[FPU5]	◇飲料水での曝露が、ヒト健康リスク評価の
		主要因(P.21)。
	EPA-HQ-OPP-201 1-0448-0072 cont	
2020年3月	Review of Additional Rice Field Trial	◇BASF 社は、玄米の輸入での残留基準設
	Data to Support an Import Tolerance	定のため、追加の水稲の圃場試験データを提
	and the Registration Review of	出。水稲への使用登録は取り消されており、
	Fipronil. Summary of Analytical	輸入品としての玄米に対する基準値を維持し
	Chemistry and Residue Data.	たいと考えている(P.2)。
		◇HED(Health Effects Division)代謝
	【FPU6】	委員会は、作物及び畜産物の基準値設定に
	PDF &	対象となる残留物は、親、代謝物 MB
	EPA-HQ-OPP-201	46136 及び MB 45950、光分解物 MB
	1-0448-0073_cont	46513と判断(P.2)。
2019年12月	Data evaluation record (DER) for a	◇現在、CTA 試験に関する OCSPP(the
	non-guideline comparative thyroid	Office of Chemical Safety and
		Pollution Prevention) のガイドラインはな
	【FPU7】	いが、本試験は EPA の 2005 年甲状腺測
	PDF 2	定ガイドラインに従って実施された。
	EPA-HQ-OPP-201	◇母動物の LOAEL は、仔動物における T4
	1-0448-0105_cont	の減少と甲状腺病理学のわずかな変化に基
		づいて 1.0mg/kg/日であり、NOAEL は
		0.3mg/kg/日である。
		◇生殖毒性試験の LOAEL は 3.0mg/kg/
		日、NOAELは 1.0mg/kg/日である。
		◇仔動物の LOAEL は、甲状腺ホルモンレベ
		ルの変動及び甲状腺病理学的変化に基づい
		て 3.0mg/kg/、NOAELは 1.0mg/kg/
		日。である。
		◇予備試験では、6-PTUと本有効成分への
		経口暴露による甲状腺毒性が報告されたが、
		母乳中に本有効成分とフィプロニルスルホンが
		含まれている証拠があることから、授乳中に仔
		動物へ移行することが示唆された。
		(P.2)
2016年11月	Data evaluation record (DER) for 28-	◇EPA は 90 日間経皮毒性試験を要求し、

	day dermal toxicity study	FTFは28日間経皮毒性試験を提出して該
		当試験の免除を要求。
	[FPU8]	◇EPAは、甲状腺影響及び重量が報告され
	2	ていないことでガイドラインを満たしていないと結
	EPA-HQ-OPP-201	論付けた(P.2)。
	1-0448-0068_cont	
2015年4月	Requirement for a Comparative	◇EPA は CTA 試験が提出されていないため、
	Thyroid Assay, and Limited Review	FQPA 係数を X10 にすることを議論。
	to Determine Incidental Oral	◇CTA試験要求は、発達神経毒性試験の
	Endpoints.	谷田県などのに、りたどいには、日本にのであった。     結果から成体の標的臓器が甲状腺であった。
		とと、仔動物の甲状腺機能の評価が行われて
	【FPU9】	いないことに基づく。
	PDF	(P.2)
	EPA-HQ-OPP-201	
	1-0448-0043_cont	
2013年7月	GENERIC DATA CALL-IN NOTICE	◇データコールイン
	[FPU10]	・CTA についての試験要求
	EPA-HQ-OPP-201 1-0448-0045_cont	
2013年5月	Fipronil Focus Meeting Notes	♦ HSAPOC (Hazard and Science
		Policy Committee)は以下の理由で 90
	[FPU11]	日間経皮毒性試験が必要であることを EPA
	PDF	と合意した。①使用パターンと更新された使用
	EPA-HQ-OPP-201	方法により、1~6カ月の連続暴露が生じるこ
	1-0448-0041_cont	と、②暴露期間に比例して毒性が増加してい
		るため、21 日間の試験では 6 カ月までの暴
		露を評価できない、③ラット亜急性経口試験
		からの外挿は適切ではない。
		従って、HASPOC はウサギでの 90 日間経皮
		毒性試験を推奨し、標的臓器(甲状腺と肝
		臓) の毒性に関連する適切なパラメーター(ホ
		ルモン、酵素、体重、病理組織学的解析)の
		測定を要求する(P.3)。
2011年12月	Final Work Plan	◇2011年6月20日の文書からの追加
		は、ペットへの使用に関する表示と関連する懸
	[FPU12]	念事項の追加のみ。

	POF	◇ヒト健康影響評価で要求される可能性のあ
	2	◇CFIE成影響評価で安水される可能性のの るデータは以下の通り(P.7)。
	EPA-HQ-OPP-201 1-0448-0039_cont	・使用方法
	_	・水稲作物残留試験(分析対象:フィプロニ
		ル、フィプロニルスルフィド、フィプロニルスルホン、
		MB46513)
		·90 日間経皮毒性試験
		・90日/128日吸入毒性試験
		・免疫毒性試験
2011年11月	HED Response to Registration	◇6月に公表された Summary
	Review Comments	Document for Registration Review $\land$
		の関係企業からのコメントに対する HED の回
	[FPU13]	答。主としてペット用駆虫薬用途に関するも
	PDF	日。1000007771mm/23来/11座に因9900 の。
		・潜在的なリスク評価をするためには、処理さ
	EPA-HQ-OPP-201 1-0448-0036_cont	れた害虫に対する子供の暴露が、28日間以
		上になる可能性があるため、より長期の皮膚
		試験が必要。さらに経皮毒性の可能性も否
		定できない。
2011年6月	Summary Document Registration	◇PWP(Preliminary Work Plan)、ファ
	Review	クトシート、データギャップが掲載。
		◇ヒト健康影響評価で要求される可能性のあ
	[FPU14]	るデータは以下の通り(P.7)。
	PDF 2	·使用方法
	EPA-HQ-OPP-201	・水稲作物残留試験(分析対象:フィプロニ
	1-0448-0003_cont	ル、フィプロニルスルフィド、フィプロニルスルホン、
		MB46513)
		MB46513) ・90 日間経皮毒性試験
		·90 日間経皮毒性試験
		・90 日間経皮毒性試験 ・90 日/28 日吸入毒性試験
		・90 日間経皮毒性試験 ・90 日/28 日吸入毒性試験 ・免疫毒性試験
		・90 日間経皮毒性試験 ・90 日/28 日吸入毒性試験 ・免疫毒性試験 ◇、EDSP(内分泌かく乱物質スクリーニング
2009年1月	Technical Fact Sheet	<ul> <li>・90 日間経皮毒性試験</li> <li>・90 日/28 日吸入毒性試験</li> <li>・免疫毒性試験</li> <li>◇、EDSP(内分泌かく乱物質スクリーニング プログラム)において優先リスト中の58 種類の</li> </ul>
2009年1月	Technical Fact Sheet	<ul> <li>・90 日間経皮毒性試験</li> <li>・90 日/28 日吸入毒性試験</li> <li>・免疫毒性試験</li> <li>◇、EDSP(内分泌かく乱物質スクリーニング プログラム)において優先リスト中の58 種類の 農薬有効成分の中には含まれていない。</li> </ul>
2009年1月	Technical Fact Sheet Fipronil Technical Fact Sheet	<ul> <li>・90 日間経皮毒性試験</li> <li>・90 日/28 日吸入毒性試験</li> <li>・免疫毒性試験</li> <li>◇、EDSP(内分泌かく乱物質スクリーニングプログラム)において優先リスト中の58 種類の 農薬有効成分の中には含まれていない。</li> <li>ファクトシートの記載事項要約(評価結果で)</li> </ul>
2009年1月		<ul> <li>・90 日間経皮毒性試験</li> <li>・90 日/28 日吸入毒性試験</li> <li>・免疫毒性試験</li> <li>◇、EDSP(内分泌かく乱物質スクリーニング プログラム)において優先リスト中の58 種類の 農薬有効成分の中には含まれていない。</li> <li>ファクトシートの記載事項要約(評価結果で はない)</li> </ul>

	顔面痙攣、頭部痙攣を示した。
	・ラットおよびマウスに単回経口または吸入暴
	露した場合の急性毒性の徴候は、活動性や
	歩行の変化、猫背の様相、震え、痙攣および
	痙攣等。
	・6 週間の摂餌投与によるマウスの毒性の臨
	床徴候は、過活動、過敏性、異常な歩行ま
	たは姿勢、体の震え、痙攣、および死亡等。
	・52 週間のラット慢性毒性試験における毒
	性の徴候は、給餌および食物変換効率の低
	下、体重増加の減少、発作および発作に関
	連した死亡、甲状腺ホルモンの変化、肝臓お
	よび甲状腺の質量増加、腎臓への影響等。
	【ヒト】
	・本有効成分を摂取した後に報告された臨床
	徴候および症状には、発汗、吐き気、嘔吐、
	頭痛、腹痛、めまい、激越、脱力感、および強
	直間代発作等。暴露の臨床徴候は一般に可
	逆性であり、自然に消失する。
	・ある症例報告では、50歳の男性が圃場にフ
	ィプロニル製剤を5時間散布した後、頭痛、
	吐き気、めまい、脱力感を訴えた。症状は2
	時間後に発症し、自然に消失したと報告。著
	者らは、結膜炎や皮膚刺激の兆候は見られな
	かったが、曝露経路として吸入または皮膚接
	触を示唆。
	◇RfD (Reference Dose)
	NOAEL(0.5ppm、0.019mg/kg/日)と
	不確実係数×100 に基づいて
	0.0002mg/kg/日。
	◇内分泌かく乱作用
	・ラット、ウサギ、マウス、イヌを対象とした短期
	及び長期毒性試験の結果からは、「いかなる
	内分泌かく乱作用も示唆していない」。
	・「甲状腺への直接的な影響ではなく、クリアラ
	ンスの増加に起因する」と結論づけた。
	・ラットを用いた2年間慢性毒性試験では、
	最高用量(300ppm)で甲状腺、下垂体

に関連した甲状腺腫瘍が観察された。結果は
ラットに特異的であると判断された。
・0、0.5、1.5、30.0、および 300.0ppm の
用量で2年間に摂餌投与したところ、最高用
量ではオス、メスともに甲状腺の良性および悪
性濾胞性細胞腫瘍の発生率が増加。
・ラットにフィプロニルデススルフィル(光分解
物)を 0、 0.5、 2.0、 10.0ppm(オス: 0、
0.025、0.098、0.050mg/kg/日、メス:
0、0.032、0.13、0.55mg/kg/日)で2
年間摂餌投与した。10 ppm の雄ラット及び
10 ppm の雌ラットでは、発がん性の証拠は
なく、臨床的な毒性の兆候が見られた。
・ヒトリンパ球、チャイニーズハムスターV79 細
胞、サルモネラ菌(エイムズテスト)、マウス小
核を用いた試験系で突然変異を起こさなかっ
た。
◇生殖毒性
30 ppm の摂餌投与試験(オスで:2.54
mg/kg/日、メス:2.74 mg/kg/日)では
生殖への影響は認められなかったが、甲状腺
及び肝臓重量の増加(オス及びメス)、下垂
体重量の減少(メス)、甲状腺肥大症の発
生率の増加(メス)を含む全身毒性が観察
された。生殖への影響が観察された最低用量
は 300 ppm(オス:26.0 mg/kg/日、メ
ス: 28.4 mg/kg/日)であり、仔動物にお
ける特定できない臨床症状、産仔数の減少、
体重の減少、交尾の減少、繁殖力の低下、
着床後及び子孫の生存率の低下、身体的発
達の遅れに基づく。
・短期間の発達神経毒性試験では、授乳中
の子犬の体重の有意な減少、およびオスの性
発達遅延の兆候に基づき、LOAELは
0.90mg/kg/day。
◇動物代謝
(吸収)

・経皮吸収はラットを用いた試験において1%
未満
・ヒト、ウサギ、ラットの皮膚表皮を用いた <sup>14</sup> C-
フィプロニルの in vitro 試験では、200g/L 溶
液塗布の8時間後の浸透率は、0.08%(ラ
ット)、0.07%(ウサギ)及び 0.01%(ヒ
ト)であった。0.2g/L の溶液塗布では、
0.9%(ラット)、13.9%(ウサギ)、0.9%
(ヒト)の吸収率であった。別の in vitro 試
験では、24 時間後にとトの皮膚表皮で 0.15
~3.00%、ラットの皮膚表で1~35%)の
浸透性であった。
・犬と猫を対象とした <sup>14</sup> C-フィプロニルのスポット
オン処理では、有効成分は主に皮膚表層に
分布し、真皮や表皮下層(脂肪組織)では
検出されなかった。
・ラット皮膚に <sup>14</sup> C フィプロニルデススルフィル
(光分解物)0.08~7.20mg を処理する
と24 時間後に塗布した量の約 0.2~7.0%
皮膚に浸透した。
・標識したフィプロニルを 0.05、2.00、
10.00ppm の用量でヤギの飼料経由で 7
日間投与したところ、吸収率は 15~33%で
あった。ラットを対象とした研究では、経口投与
後の吸収率は 30~50%であった。
【分布】
・本有効成分は哺乳類の体内に広く分布し、
主に脂肪組織に検出される。単回経口投与
されたラットでは、胃、消化管(GI)、脂肪、
副腎での濃度が最も高かった。中等度の濃度
は肝臓、膵臓、甲状腺、卵巣に認められた。
低濃度は筋肉、脳、心臓、心臓の血液中に
認められた。
・犬と猫を対象としたスポットオン処理では、治
療後2ヶ月後に皮脂腺、毛の周囲の上皮
層、毛軸の露出部に <sup>14</sup> C-フィプロニルが検出
され、毛と皮膚を覆う皮脂中に拡散しているこ
とが示唆された。スポットオン型のフィプロニル製
していか吸C1 いこ。 ヘルットオノ空のノ1ノロール器

品を犬に塗布し、毎日5分間、綿の手袋を         使って犬を激しく撫でた。手袋に移行した残留         物は、処理後24時間後に589(±206)         ppmでピークを迎え、時間の経過とともに減         少し(8日後には448±118ppm)、36         日後には検出されなくなった。         【代謝】         ・ラットにおける全血中の半減期は、4mg/kg         の経口投与で約6.2~8.3日であり、         150mg/kgの経口投与では2.1~2.3日         【排泄】         スパロコルの経口投与では2.1~2.3日
<ul> <li>物は、処理後 24 時間後に 589 (±206)</li> <li>ppm でピークを迎え、時間の経過とともに減少し (8 日後には 448±118ppm)、36</li> <li>日後には検出されなくなった。</li> <li>【代謝】</li> <li>・ラットにおける全血中の半減期は、4mg/kgの経口投与で約 6.2~8.3 日であり、</li> <li>150mg/kgの経口投与では 2.1~2.3 日</li> <li>【排泄】</li> </ul>
ppm でピークを迎え、時間の経過とともに減 少し(8 日後には 448±118ppm)、36 日後には検出されなくなった。 【代謝】 ・ラットにおける全血中の半減期は、4mg/kg の経口投与で約 6.2~8.3 日であり、 150mg/kg の経口投与では 2.1~2.3 日 【排泄】
<ul> <li>少し(8日後には448±118ppm)、36</li> <li>日後には検出されなくなった。</li> <li>【代謝】</li> <li>・ラットにおける全血中の半減期は、4mg/kgの経口投与で約 6.2~8.3 日であり、</li> <li>150mg/kgの経口投与では 2.1~2.3 日</li> <li>【排泄】</li> </ul>
日後には検出されなくなった。 【代謝】 ・ラットにおける全血中の半減期は、4mg/kg の経口投与で約 6.2~8.3 日であり、 150mg/kg の経口投与では 2.1~2.3 日 【排泄】
【代謝】 ・ラットにおける全血中の半減期は、4mg/kg の経口投与で約 6.2~8.3 日であり、 150mg/kg の経口投与では 2.1~2.3 日 【排泄】
・ラットにおける全血中の半減期は、4mg/kg の経口投与で約 6.2~8.3 日であり、 150mg/kg の経口投与では 2.1~2.3 日 【排泄】
の経口投与で約 6.2~8.3 日であり、 150mg/kg の経口投与では 2.1~2.3 日 【排泄】
150mg/kgの経口投与では 2.1~2.3 日 【排泄】
【排泄】
・フィプロニルの経口投与を受けたラットでは、
糞中に 45-75%、尿中に 5-25%のフィプロ
ニルが排泄された。親化合物と酸化生成物で
あるフィプロニルスルホンは、糞及び尿中に存在
した。
・授乳中のヤギに7日間摂取した場合には、
18~64%が糞中に、1~5%が乳中に排泄
され、8~25%が体組織中に残存した。
・フィプロニルーデスルフィニルをヤギに投与した地
合には、糞中に 20~50%、尿中に 3~7%
を排泄した。

- ▶ 欧州
  - ✓ EUデータベース(別紙1)
     <u>https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-</u> substances/?event=as.details&as\_id=1002
  - ✓ ECHA データベース(別紙1)
     <a href="https://echa.europa.eu/de/substance-information/-/substanceinfo/100.102.312">https://echa.europa.eu/de/substance-information/-/substanceinfo/100.102.312</a>
  - ✓ ミツバチ保護に関する EU ウェブサイト
     <a href="https://ec.europa.eu/food/animals/live\_animals/bees/health\_en">https://ec.europa.eu/food/animals/live\_animals/bees/health\_en</a>

年月	評価資料	食品経由での人健康影響エンドポイント /追加要求	
	New Certified Reference Material for fipronil in egg <u>https://ec.europa.eu/jrc/en/scienc</u> <u>e-update/new-certified-reference-</u> <u>material-fipronil-egg</u> (URL のみ)	<ul> <li>◇JRC (Joint Research Centre) のウェブ サイト</li> <li>・2017 年に卵粉末中にフィプロニルが検出され たことにより、残留分析の品質保証を担当。</li> </ul>	
2020年4月	CERTIFICATION REPORT (FPE1) KJNA30157ENN.e n.pdf	◇JRC が発行した「卵粉末に検出されるフィプロ ニル及びフィプロニルスルホンの標準分析方法」	
2019年10月	Official Journal (FPE2) CELEX_32019R17 92_EN_TXT (1).pc	<ul> <li>◇アミトロール、フィプロニル、フルピルスルホンメチル、イマゾスルフロン、イソプロツロン、オルトスルファムロン、トリアスルフロン等の MRL に関する公告。</li> <li>◇フィプロニルの有効成分としての認可は 2017年9月30日に失効。MRL は、検出限界値(LOD)に設定(P.67)。</li> </ul>	
2019年6月	SUMMARY REPORT Standing Committee on Plants Animals, Food and Feed	PAFF (Plants, Animals, Food and Feed) 委員会議事録 ◇アミトロール、フィプロニル、フルフェノックスロン、	

	(FPE3) sc_phyto_201906 13_ppr_sum.pdf	フルピルスルフロンメチル、イマゾスルフロン、イソプ ロツロン、オルトスルファムロン、トリアスルフロン等 の MRL に関する決定事項。 ◇すべての MRL を定量限界値(LOQ)に移 行。 ◇経過措置について提案。 (P.17)
2018年5月	2018年5月 SCIENTIFIC REPORT (FPE6) j.efsa.2018.5164. pdf	<ul> <li>◇養鶏場におけるフィプロニルの誤用が確認され、加盟国と欧州委員会は、PAFF 委員会において、特定のモニタリングプログラムを組織することで合意。</li> <li>◇2017年9月1日から2017年11月30日までに、5,439サンプルの結果が提出された。</li> <li>そのうち、742サンプルはMRLを超過。</li> <li>◇MRL 超過は主にフィプロニル。</li> <li>(P.24)</li> </ul>
2017年12月	Factsheet FIPRONIL IN EGGS (FPE8) jrc110632_final.p df	<ul> <li>◇フィプロニル事件は、EU 加盟国と欧州外において、殺虫剤に汚染された卵と卵製品が発見されたこもの。</li> <li>◇フィプロニルはイヌ、ネコのノミ、ダニに対する認可はあるが、食用のニワトリ等には使用禁止されている。</li> <li>◇適切な管理をするために、JRCは、EU法に規定されているフィプロニルの定量について公的検査機関の能力を評価するための支援を要請。</li> </ul>
2017年11月	SUMMARY REPORT (FPE9)	<ul> <li>PAFF 委員会議事録</li> <li>◇2019~2021 年の EU 協調複数年計画に</li> <li>関するモニタリング草案を提示。</li> <li>◇動物由来の食品に関する附属書 I にグリホ</li> <li>サートとフィプロニルを追加。</li> <li>(P.8)</li> </ul>
2017年9月	Conclusions from the Ministerial Conference on the follow up of the fipronil incident (FPE10)	◇EU 閣僚会議議事録 2017 年夏のフィプロニルによる汚染事件に関連 して、9月26日の閣僚会議での合意事項。

	PDF	
	rasff_fipronil-inci dent_conclusions_	
2017年9月	SUMMARY REPORT (FPE11) reg-com_ani-nutr it_20170911_sum	PAFF 委員会議事録 ◇ 産卵鶏農場でのフィプロニルの違法使用に関 連した汚染事件報告 ・フィプロニルによる違法な処理を行った農場を特 定。残留量が EU-MRL である 0.005mg/kg を下回らない限り、これらの農場からは出荷停 止。 ・2016 年から違法使用は行われ、高濃度のフ ィプロニルが残留する含む動物製品が出荷されて いる可能性が高い。 ・特に、産卵鶏の羽毛から高濃度のフィプロニル
2017年8月	SUMMARY REPORT (FPE12) reg-com_toxic_2 0170830_sum.pdf	が検出された。 PAFF 委員会議事録 ◇産卵鶏農場におけるフィプロニル含有製剤の 違法使用に関する意見交換・議論:状況の評 価と管理措置について
2017年7月	BfR Opinion (FPE13) Mealth-assessme nt-of-individual-m	BfR (ドイツ連邦リスク評価研究所)からの報告 ◇ベルギー産の卵と卵製品からフィプロニルの高 濃度残留検出された旨の RASFF の通知に基 づいてリスク評価を実施。 ◇分析結果は、鶏卵では 1kg あたり 0.0031 ~1.2mg、鶏肉では 1kg あたり 0.0015~ 0.0156mg。 ◇ARfD はラットを用いた発生毒性試験に基づき、0.009mg/kg 体重。 ◇最も高い残留量(卵 1kg あたり 1.2mg) のワーストケースとして試算し、欧州の消費データ を考慮すると、子供用の ARfD を超えると判断 した。 ◇必ずしも鶏卵の消費が具体的な健康リスクを もたらすことを意味するものではないが、これらの 汚染された鶏卵を消費することにより潜在的に子

		供の健康リスクが増加する可能性はある。 (P.4)
2017年7月	SCIENTIFIC REPORT (FPE14) j.efsa.2017.4929. pdf	<ul> <li>◇2016年にJMPRは、12の有効成分のリス ク評価を実施。EFSAは、CODEXのMRL、毒 性評価の提案に対するコメントを作成。</li> <li>・JMPR評価(2000年)</li> <li>ADI: 0.0002mg/kg/day</li> <li>ARfD: 0.003mg/kg/day</li> <li>・EFSA評価(2006年)</li> <li>ADI: 0.0002mg/kg/day</li> <li>ARfD: 0.009mg/kg/day</li> <li>(P.30~P.33)</li> </ul>
2017年4月	APPLICATIONS FOR RENEWAL (FPE15) pesticides_ppp_a pp-proc_air-3_sar	再登録認可 ◇最終適用 2014 年 9 月 29 日 ◇今回のドシエ提出なし(P.27)。
2016年12月	[SUMMARY REPORT] (FPE16) sc_phyto_201612 06_pppl_sum.pdf	<ul> <li>PAFF 委員会議事録</li> <li>◇フィプロニルのレビューの現状と次のステップ</li> <li>◇認可は 2017 年 9 月 30 日に失効。</li> <li>◇欧州委員会は、EFSA がこれ以上のレビューを継続しないことを決定。</li> <li>◇RMS : オーストリア (P.17)</li> </ul>
2016年11月	Official Journal (FPE17) CELEX_32016R20 35_EN_TXT.pdf	<ul> <li>◇フィプロニルの認可期間は、2017年9月30</li> <li>日から2018年7月31日まで延長されたが、</li> <li>再評価用の資料は提出されていない。</li> <li>◇従って、認可は、2017年9月30日に失効する。</li> </ul>
2016年10月	SUMMARY REPORT (FPE18) sc_phyto_201610 06_pppl_sum.pdf	PAFF 委員会議事録 ◇欧州委員会は、AIR(Annex I Renewal) III の作業計画の進捗状況につ いてドシエ審査が遅れているため、いくつかの有効 成分で認可延長が必要とする旨指摘。 ◇フィプロニルとマネブについては、申請書類が提 出されていないため、期限が後ろ倒しになり、有

		効期限が延長される。
		(P.3)
2016年9月	SUMMARY REPORT (FPE19) sc_phyto_201609 21_pppl_sum.pdf	<ul> <li>PAFF 委員会議事録</li> <li>◇内分泌かく乱化学物質を確認するための科学的基準関する委員会の意見交換。</li> <li>◇例えば、毒性学的懸念があり、関連する分析法が利用可能な場合には、MRL はデフォルト値よりも低いレベルで設定される可能性。(例:フィプロニルの MRL0.005)</li> <li>(P.7)</li> </ul>
2016年6月	COMMISSION STAFF WORKING DOCUMENT (FPE20) SWD-2016-211-F 1-EN-MAIN-PART	<ul> <li>◇内分泌かく乱性を検証するため、農薬用途</li> <li>324 有効成分、殺生物用途 98 有効成分を</li> <li>スクリーニング。</li> <li>◇フィプロニルについては、Option3 カテゴリーに</li> <li>分類された(P.16)。</li> </ul>
2015年3月	Office Journal (FPE21) CELEX_32015R04 08_EN_TXT.pdf	◇代替品候補リストにフィプロニル掲載。ADI が、それぞれの物質/用途分類のグループ内で認 可されている他の有効成分よりも有意に低い。 (P.1)
2014年10月	Office Journal (FPE23) CELEX_32014R11 27_EN_TXT.pdf	<ul> <li>◇フィプロニルの既存の MRL について、アブラナ 科野菜の花と頭頂部、豚、牛、羊、ヤギの脂肪 と肝臓、豚の腎臓、家禽の肝臓と卵は引き下げ ることを推奨。その他他は、既存の MRL を引き 上げるか、維持するよう勧告。</li> <li>◇ドイツは 2012 年 2 月 10 日にコメツキ種の 発生を理由に、フィプロニルを含む農薬製品の一</li> <li>時的な認可を欧州委員会に通告。その結果、</li> <li>家禽の脂肪の MRL の引き上げを求める要請を 通知。</li> <li>◇潜在的長期的健康リスクは排除できないと結 論付け、キャベツとケールへの使用認可は申請 者により取り消された。 (P.1~P.2)</li> </ul>
2014年1月	REASONED OPINION	MRL 改訂

	(FPE24) j.efsa.2014.3543. pdf	<ul> <li>◇前回の意見書で取り上げられた動物加工食</li> <li>品中の MRL を再計算し、提案されたすべての</li> <li>MRL の安全性に関する EFSA の結論を更新。</li> <li>◇ドイツで認可されたジャガイモに対する緊急認</li> <li>可と、欧州でのキャベツとケールに対する認可が</li> <li>最近取り消されたことも考慮。</li> <li>◇必要とされるデータ不足は認められず、消費</li> <li>者へのリスクは確認されなかった。</li> <li>(P.22)</li> </ul>
2012年5月	RESONED OPINION (FPE29) j.efsa.2012.2707. pdf	<ul> <li>◇ドイツから、ジャガイモへの緊急認可に伴う家 禽脂肪中の MRL の引き上げを求める要請。</li> <li>◇ジャガイモへの使用は残留データから十分に裏 付けられており、MRL は 0.01mg/kg と提案。</li> <li>家畜飼料に関する試験結果から、ジャガイモに 使用するには、家禽脂肪だけでなく、反芻動物の の肉、反芻動物の脂肪、反芻動物の肝臓、豚 の肉、豚の肝臓、豚の腎臓、家禽の肉、および 牛乳について既存の MRL を修正する必要があ る。</li> <li>◇リスク評価の結果、食品経由での暴露は、</li> <li>ADI を超える可能性がある</li> <li>◇ジャガイモへの使用については、動物性食品</li> <li>中の残留物に起因する長期的な消費者の健康</li> <li>リスクを排除できないと結論付けた。 (P.19)</li> </ul>
2011年5月	Office Journal (FPE32) CELEX_32011R05 40_EN_TXT.pdf	<ul> <li>新規則 Regulation (EC) No 1107/2009 での認可継続</li> <li>・純度: ≥ 950g/kg</li> <li>・発行日: 2007 年 10 月 1 日</li> <li>・有効期限: 2017 年 9 月 30 日</li> <li>◇必要に応じてリスク軽減措置を含めことが認 可条件。</li> <li>◇加盟国は、草食性鳥類及び哺乳類、並びに ミツバチ、特に八チ群体に対するリスク評価を確 認するための試験提出を認可から 1 年以内に 申請者が提出するよう要請する。</li> <li>(P.63~P.64)</li> </ul>

2010年7月	Office Journal	◇残留物の定義をフィプロニル及びスルホン代謝
		物の合算で MRL を設定(P.24)。
	(FPE35)	
	FDF	
	CELEX_32010R07	
	50_EN_TXT.pdf	
2010年3月	Office Journal	◇ミッバチのコロニーが大幅に失われたことによ
		り、加盟国は予防措置を講じ、これらの有効成
	(FPE36)	分を含む農薬製品の上市を一時的に停止して
	PDF 2	いる。
	CELEX_32010L00	◇フィプロニルは、種子処理用の殺虫剤としての
	21_EN_TXT.pdf	使用のみが認可されていが、加盟国からの事故
		報告は不適切使用が原因。
		◇将来の事故を避けるために、クロチアニジン、チ
		アメトキサム、フィプロニル、イミダクロプリドについ
		て、適切なリスク軽減手段を含む追加規定を定
		める。
		◇加盟国は 2010 年 10 月 31 日までに、クロ
		チアニジン、チアメトキサム、フィプロニル、イミダクロ
		プリドを含む農薬製品の認可を修正または撤回
		する。
2010年3月	Review report for the active	◇本レビュー報告書は、農薬製品の上市に関す
	substance	る既存有効成分レビューのための作業計画の中
		で、フィプロニルの再評価の結果として ANNEX I
	(FPE37)	に含まれる可能性を視野に入れて作成された。
	PDF	◇委員会は、レビュー結果に基づき、曝露された
	appendix 01_EC	種に対するリスクは許容可能であることに同意す
	Review Report Fir	る。EFSA による更なるレビューは必要ないと考え
		られる。
		(P.3)
2008年7月	Office Journal	◇MRL更新
		◇残留物の定義をフィプロニル及びスルホン代謝
	(FPE38)	物の合算で MRL を設定。
	PDF 2	(P.90)
	CELEX_32008R08	
	39_EN_TXT.pdf	
2008年1月	Office Journal	◇MRL 更新
		◇残留物の定義をフィプロニル及びスルホン代謝

	(FPE39)	物の合算で MRL を設定。
		(P.237)
	2	(F.237)
	CELEX_32008R01 49_EN_TXT.pdf	
2007年8月	Office Journal	Annex I 改訂
2007 1073		◇RMS はフランスで、2004 年 2 月 10 日に申
	(FPE40)	→ KH3 (6) ) ) / (C 200+ + 2) ] 10 日に+ 請資料提出済。
		ள員种迎山//。 ◇純度:≥950g/kg
	2	○/代度:29009/kg 施行日:2007年10月1日
	CELEX_32007L00 52_EN_TXT.pdf	有効期限:2017年9月30日
		◇種子処理としての殺虫剤としての使用のみが
		認可される。種子コーティングは、専門的な種子
		処理施設でのみ行う。これらの施設は、保管、
		輸送及び散布中の粉塵雲の放出を確実に排除
		するために、利用可能な最善の技術を適用す
		◇総合評価において、加盟国は特に以下の点
		に注意を払わなければならない。
		・懸念される光分解生成物の発生を回避するた
		めの市販製品の包装。
		・土壌や気候条件が脆弱な地域に適用した場
		合、特に親化合物よりも難分解性の高い代謝
		物による地下水汚染の可能性。
		・草食性鳥類や哺乳類、水生生物、非標的節
		足動物、ミツバチの保護。
		・土壌への処理を確実にする適切な機器の使用
		と、散布中の流出を最小限に抑えること。
		◇認可の条件には、必要に応じてリスク軽減措
		置を含めること。加盟国は、草食性鳥類及び哺
		乳類、並びにミツバチ、特にハチ群体に対するリ
		スク評価のための成績提出を要請すること。
		(P.6)
2006年5月	Scientific Report	ピアレビューレポート
	Conclusion on the peer review of	◇フィプロニルは、第二段階のレビュープログラム
	fipronil	の 52 物質の一つである。 RMS フランスは、
		2004 年 2 月 10 日に EFSA に DAR を提出
	(FPE41)	し、その後レビューを開始。
		2005年2月9日の評価会議で追加データの

PDF 2	必要性を合意。2005年6月と7月に開催さ
j.efsa.2006.65r.p	れた加盟国の専門家との一連の科学的な会合
df	では、残りの問題点や届出者が要求に応じて入
	手可能な追加データを評価。2006年2月7
	日に加盟国の代表者との間で専門家の協議の
	結果について最終的な議論が行われ、本報告
	書に記載されている結論に至った。
	◇申請者が提案した代表的用途は、ヒマワリとト
	ウモロコシの土壌害虫と線虫を防除するための種
	子処理。
	◇迅速かつ広範囲に吸収・分散され、生体内に
	蓄積する可能性はあるが、容易に代謝され、糞
	便を介してゆっくりと排泄される。
	◇わずかに皮膚や目の刺激性があり、感作性は
	弱い。
	◇毒性試験:短期試験では、中枢神経系、肝
	臓、甲状腺に悪影響が認められ遺伝毒性や発
	がん性は示されていない。
	甲状腺腫瘍の誘発メカニズムはラットに特有のも
	のであり、ヒトには外挿できないとの結論。生殖
	毒性、発生毒性なく、神経系での病理組織学
	的所見は観察されていない。ADI は
	0.0002mg/kg/日、許容作業者暴露レベル
	(AOEL)は 0.0035mg/kg/日、ARfD は
	0.009mg/kg 体重であり、安全係数は 100。
	◇植物代謝試験:土壌散布または種子処理
	を用いて、穀類、豆類、油糧種子、根茎及び塊
	茎を代表とする 5 作物について実施。3 作物グ
	ループに共通の代謝経路を定義し、関連代謝
	物(スルホン代謝物 MB 46136)を同定。🔷
	光分解物 MB 46513 の急性毒性が懸念され
	ていたが、種子処理用途では影響がないと判
	断。光分解プロセスを防ぐために、処理された種
	子を暗所で保管することのラベルの制限を提案。
	◇家畜の摂取量は 0.1mg/kg を大きく下回っ
	たが、フィプロニルは脂溶性であり、ADI が非常に
	低いため、畜産物への残留を考慮する必要があ
	る。種子処理によるフィプロニル及びフィプロニルス

			ルホン残留物の暴露か	消費者に高い慢性的な
			リスクをもたらす可能性は低いと考えられる。	
			(P.1~P.6)	
2004年4月	DAR(2005年1月	]改訂)	Draft Assessment Report	
			(毒性·代謝:Vol.3 B6 part1	
			$\sim$ part5)	
	(FPE42)			
	PDF 2	PDF J-	POF	PDF J
	Fipronil_DAR_01_ Vol 1_public.pdf	Fipronil_DAR_02_ Vol 2_public.pdf	Fipronil_DAR_03_ Vol 3_B1-B5_publ	Fipronil_DAR_04_ Vol 3_B6_part_1_
	Fipronil_DAR_05_ Vol 3_B6_part_2_	Fipronil_DAR_06_ Vol 3_B6_part_3_	Fipronil_DAR_07_ Vol 3_B6_part_4_	Fipronil_DAR_08_ Vol 3_B6_part_5_
	Fipronil_DAR_09_ Vol 3_B7_part_1_	Fipronil_DAR_10_ Vol 3_B7_part_2_	Fipronil_DAR_11_ Vol 3_B8_public.p	Fipronil_DAR_12_ Vol 3_B9_public.p

# 別紙1.データベース

# ▶ 米国

✓ EPA レビューページ

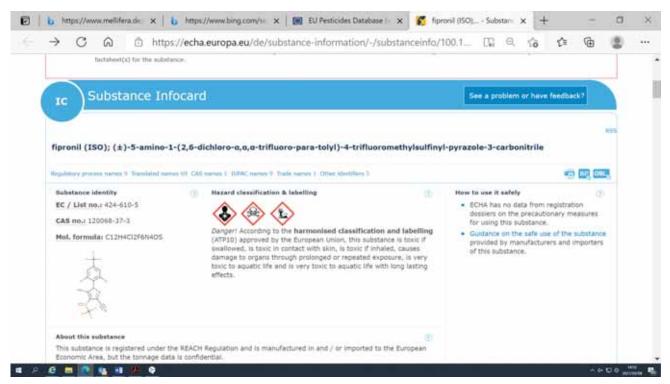
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▶ 欧州

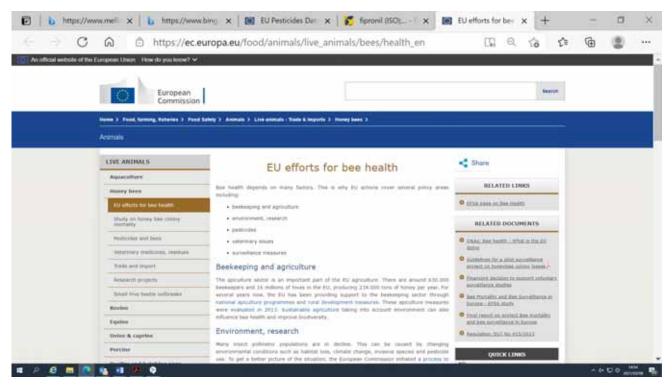


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	Active substance								
	Active substance Fipronil Real constants	Status under	Reg. (EC) No 1107/200	5 <u>-60</u>		Note	pproved		
	Fipronil	preprinting Direction (B)	NARC <sup>(1)</sup>		Apro. III.I. No. 22 Betalen au	unn u	pproved		
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	Fipronil Net contact Bran ander Reg Anterinetien Maximum Residue Levels	preprinting Direction (B)	NARC <sup>(1)</sup>	Old legislation	Bara, 11U1 312 340	umuu Barres	pproved		
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# ECHA データベース



## ミツバチ保護に関する EU ウェブサイト



#### 9.1.1. 一覧表:米国 EPA におけるフィプロニルの再評価概要と関係資料

<ol> <li>事前のや</li> </ol>	り取りに関する文章	<ol> <li>② 追加要求された試験項目及び その内容に関する文章</li> </ol>	<ol> <li>②の提出の</li> </ol>	有無、再評価結果	④ 再評価評価書	⑤ 規制内容/その他
						【Pesticide Docket for Registration Review】 Regulations.gov - Docket Browser (URLのみ)
2020 年 8 月 [COMMENT] Comment submitted by Bayer CropScience LP Regulations.gov - Comment (FPU1) 2020 年 8 月 [COMMENT] Comment submitted by Fipronil Task Force, LLC Regulations.gov - Comment	◇フィプロニル・タスクフォース (FTF)からの生態影響評 価、健康影響評価、食品経 由でのリスク評価に対するコメ ント		2020 年 3 月 【MEMORANDUM】 Draft Ecological Risk Assessment for Registration Review Regulations.gov - Supporting & Related Material Document (FPU3)	生態影響リスク評価のト'ラフト		
(FPU2)	値の取扱い		2020年3月 【MEMORANDUM】 Draft Risk Assessment for Registration Review Regulations.gov — Supporting & Related Material Document (FPU4)	<ul> <li>◇オス成獣、妊娠ラット、非妊娠ススラットの経口投与で投与後1~2週間後に甲状腺ホルモンの恒常性の乱れが認められ、暴露の継続により、甲状腺に有害な顕微鏡的変化をもたらした。</li> <li>◇ラット、マウスの経口投与では肝毒性が、甲状腺ホルモン及び神経行動学的臨床微候の変化を誘発する用量でのみ認められた。</li> <li>・変異原性を示さなかったが、慢性曝露によりラット(オス/メス)で甲状腺濾胞細胞腫瘍が発生した。その結果、とト発がん性物質(ゲループ C)に分類された。</li> <li>◇飲料水への残留が予想されたことにより食品経由のリスク要因となった。</li> <li>◇Cumulative Thyroid Assay (CTA)を素性デーラ ベースに追加したことで、本 有効成分及び代謝物/分解物の暴露による感受性 の増加について胎児及び 乳幼児のリスク評価に十分 となり、FOPA 安全係数を</li> </ul>		

評価	① 事前のやり取りに関する文章	<ol> <li>追加要求さ</li> </ol>	れた試験項目及び	<ol> <li>③ ②の提出の</li> </ol>	有無、再評価結果	④ 再評価評価書	<ol> <li>5 規制内容/その他</li> </ol>
機関		その内容	に関する文章				
EPA		2020年3月 【MEMORANDUM】 Review of Additional Rice Field Trial Data to Support an Import Tolerance and the Registration Review of Fipronil. Summary of Analytical Chemistry and Residue Data. <u>Regulations.gov</u> — <u>Supporting &amp; Related</u> <u>Material Document</u>	◇BASF 社は、玄米の輸入 での残留基準設定のため、 追加の水稲の圃場試験テ- タを提出。水稲への使用登 録は取り消されており、輸 入品としての玄米に対する 基準値を維持したいと考え ている。 ◇HED 代謝委員会は、作 物及び畜産物の基準値設 定に対象となる残留物は、 親、代謝物 MB 46136 及び MB 45950、光分解物 MB	2020年3月 [MEMORANDUM] Addendum to the Drinking Water Exposure Assessment (DP 433141) for Registration Review Regulations.gov - Supporting & Related Material Document (FPU5)	<ul> <li>         飲用水経由のリスク評価補遺         ◆ 非農業用途を追加した DWA の更新          </li> <li>         会飲料水での曝露が、ヒト健         康リスク評価の主要因         </li> </ul>		
		(FPU6)         2015年4月         MEMORANDUM]         Requirement for a         Comparative Thyroid Assay,         and Limited Review to         Determine Incidental Oral         Endpoints.         Regulations.gov —         Supporting & Related         Material Document	46513と判断。 ◇EPAはCTA試験が提出 されていないため、FQPA係 数をX10にすることを議論。 ◇CTA試験要求は、発達神 経毒性試験の結果から成 獣の裸的臓器が甲状腺で 機能の評工にないたか、すびにないな いことに基づく。	2019 年 12 月 【MEMORANDUM】 Data evaluation record (DER) for a non-guideline comparative thyroid <u>Regulations.gov -</u> <u>Supporting &amp; Related</u> <u>Material Document</u> (FPU7) 2016 年 11 月 【MEMORANDUM】 Data evaluation record (DER) for 28-day dermal toxicity study <u>Regulations.gov -</u> <u>Supporting &amp; Related</u> <u>Material Document</u> (FPU8)	◇現在、CTA 試験に関する OCSPP がイドラインはないが、 本試験は EPA の 2005 年 甲状腺測定がイドラインに従っ て実施された。 ◇母動物の LOAEL は、仔 動物における T4 の減少と 甲状腺病理学のわずかな 変化に基づいて 1.0 mg/kg/日である。 ◇生殖毒性試験の LOAEL は 0.3 mg/kg/日である。 ◇生殖毒性試験の LOAEL は 0.3 mg/kg/日である。 ◇子動物の LOAEL は、甲 状腺病担学的変化に基 づいて 3.0 mg/kg/NOAEL は 1.0 mg/kg/目である。 ◇仔動物の LOAEL は、甲 状腺病理学的変化に基 づいて 3.0 mg/kg/NOAEL は 1.0 mg/kg/目。である。 ◇予備試験では、6-PTU と 本有効成分への経口暴露 による甲状腺毒性が報告さ れたが、母乳中に本有効成 分と747 ロニルスルホンが含まれ ている証拠があることから、 授乳中に日動物へ移行す ることが示唆された。 ◇EPA は 90 日間経皮毒性 試験を要求し、Fipronil Task Foree (FTF)は 28 日間経皮 毒性試験を提出して該当試 験の免除を要求。 ◇EPA は、甲状腺影響及び 重量が報告されていないこ とでがイトラインを満たしていな いと結論付けた。		

評価	<ol> <li>事前のや</li> </ol>	① 事前のやり取りに関する文章		① 事前のやり取りに関する文章 ② 追加要求された試験項目及び		された試験項目及び	③ ②の提出	の有無、再評価結果	④ 再評価評価書	⑤ 規制	内容/その他
機関			その内容	に関する文章							
EPA	2013 年 5 月 【Fipronil Focus Meeting Notes】 Regulations.gov - Supporting & Related Material Document (FPU11)	◇Hazard and Science Policy Committee は次の理 由で 90 日間経皮毒性試験 が必要であることを EPA と 合意した。①使用パターンと更 新された使用方法により、1 ~6カ月の連続暴露が生じ ること、②暴露期間に比例 して毒性が増加しているた め、21 日間の試験では 6 カ 月までの暴露を評価できな い、③ ラパ亜慢性経口試験 からの外挿は適切ではな い。	2013年7月 【GENERIC DATA CALL-IN NOTICE】 Regulations.gov - Supporting & Related Material Document (FPU10)	AC関サる文早							
	2011年12月 【FWP】	従って、HASPOC はウサギ での 90 日間経皮毒性試験 を推奨し、標的臓器(甲状 腺と肝臓)の毒性に関連す る適切なバラ→ - クー(ネルモン、 酵素、体重、病理組織学的 解析)の測定を要求する。 ◇2011 年 6月 20 日の文書 からの追加は、ベゥへの使									
	Final Work Plan <u>Regulations.gov –</u> <u>Supporting &amp; Related</u> <u>Material Document</u> <b>(FPU12)</b>	用に関する表示と関連する 懸念事項の追加のみ。 ◇ トt健康影響評価で要求さ れる可能性のあるデータは以 下の通り。 ・使用方法 ・水稲作物残留試験(分析 対象:?ィプロニル、フィプロニルスル フィト、フィブオニルスルフォン、 MB46513) ・90 日間経皮毒性試験 ・90 日/28 日吸入毒性試験 ・免疫毒性試験									
	2011 年 11 月 【MEMORANDUM】 HED Response to Registration Review Comments Regulations.gov - Supporting & Related Material Document (FPU13)	◇6月に公表された Summary Document for Registration Review への関 係企業からのコシンドに対する HED の回答									

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	<ol> <li>追加要求された</li> </ol>	試験項目及び	③ ②の提出の有無、再評価結果	④ 再評価評価	書 ⑤ 規制内容/その他
機関			その内容に関す	する文章			
(機) EPA	2011年6月 [Summary Document Registration Review] Regulations.gov - Supporting & Related Material Document (FPU14) 2011年5月 [MEMORANDUM] Registration Review - Preliminary Problem Formulation for Ecological Risk and Environmental Fate. Endangered Species, and Drinking Water Assessments Regulations.gov - Supporting & Related Material Document	◇PWP、Fact Sheet、Data Gap が含まれている。 ◇とけ健康影響評価で要求される可能性のあるデータは以下の通り。 ・使用方法 ・水稲作物残留試験(分析 対象:フィブロール、フィブロールスル フイド、フィブロールスルフィン、 MB46513) ・90 日間経皮毒性試験 ・90 日間経皮毒性試験 ・90 日間経皮毒性試験 ・90 日間経皮毒性試験 ・0、内分泌かく乱物質スクリー ニングブログラム(EDSP)におい て優先リスト中の58 種類の農 薬有効成分の中には含まれていない。 ◇環境中運命についてく親 化合物のデータは完全である が、分解物についてはデータ が不足。 ◇ボリネーター及びや植物影響 響試験を含む生態の影響 については、データギャップが 確認された。					2011年5月          へ登録審査を開始するに         たっての本有効成分の使         状況及び防除管理情報         Regulations.gov         Supporting & Related_         Material Document         (FPU16)
	(FPU15)						2009 年 1 月         77/トシーhの記載事項要約           【Technical Fact Sheet】         (評価結果ではない)           Fipronil Technical Fact         ◇毒性症状           Sheet (orstadu)         (以RL のみ)           (URL のみ)         *腹腔内注射したマウスは、 直間代発作、顔面痙攣、i           部痙攣を示した。         ·ッフトおよびマウスに単回経 またに取入暴躍した場合           会性毒性の徴候は、活動 や歩行の変化、猫背の様相、震え、痙攣および痙雪 等。         ·6 週間の摂餌投与による ウスの毒性の臨床徴候は、 過活動、過敏性、異常な 行または姿勢、体の震え 痙攣、および死亡等。           ショ間のファル慢性毒性 験における毒性の徴候は 給餌および食物変換効率

	1				
					の低下、体重増加の減少、
					発作および発作に関連した
					死亡、甲状腺ホルモンの変
					化、肝臓および甲状腺の質
					量増加、腎臓への影響等。
					[th]
					・本有効成分を摂取した後
					に報告された臨床徴候およ
					び症状には、発汗、吐き気、
					嘔吐、頭痛、腹痛、めまい、
					激越、脱力感、および強直
					間代発作等。暴露の臨床徴
					間代先作寺。泰路のmm休暇 候は一般に可逆性であり、
					自然に消失する。
					<ul> <li>ロベロークターの。</li> <li>・ある症例報告では、50歳</li> </ul>
					の男性が圃場にフィプロニル製
					剤を5時間散布した後、頭
					痛、吐き気、めまい、脱力感
					を訴えた。症状は2時間後
					に発症し、自然に消失した
					と報告。著者らは、結膜炎
					や皮膚刺激の兆候は見ら
					れなかったが、曝露経路と
					して吸入または皮膚接触を
					示唆。
					◇RfD(Reference Dose)
					NOAEL(0.5ppm、
					0.019mg/kg/日)と不確実係
					数×100 に基づいて
					0.0002mg/kg/日。
					◇内分泌かく乱作用
					・ラット、ウサキ゛、マウス、イヌを対
					象とした短期及び長期毒性
					試験の結果からは、「いか
					なる内分泌かく乱作用も示
					唆していない」。
					・「甲状腺への直接的な影
					響ではなく、クリアランスの増加
					に起因する」と結論づけた。
					<ul> <li>・ラットを用いた2年間慢性毒</li> </ul>
					性試験では、最高用量
					(300ppm)で甲状腺、下垂
					体に関連した甲状腺腫瘍が
					観察された。結果はテットに
					観察された。相来はからに 特異的であると判断され
					特異的でのると判断された。
					/こ。 ◇発がん性
					◇ 死かん注 ・0、0.5、1.5、30.0、および
					*0、0.5、1.5、30.0、および 300.0ppm の用量で 2 年間
					に摂餌投与したところ、最高
					用量ではオス、メスともに甲状
					腺の良性および悪性濾胞
					性細胞腫瘍の発生率が増
					加。 
					・ラット(こフィフ゜ロニルテ・ススルフィル
					(光分解物)を0、0.5、2.0、
L					10.0ppm(オス:0、0.025、

			0.098、0.050mg/kg/日、メス:
			0、0.032、0.13、0.55mg/kg/
			日)で2年間摂餌投与した。
			10 ppm のオス及び 10 ppm
			のメスでは、発がん性の証拠
			はなく、臨床的な毒性の兆
			候が見られた。
			・ヒトのリンパ球、チャイニース・ハム
			スター V79 細胞、サルモネラ菌、
			マウス小核を用いた試験系で
			突然変異を起こさなかった。
			◇生殖毒性
			30 ppmの摂餌投与試験(オ
			スで:2.54 mg/kg/日、メス:
			2.74 mg/kg/日)では生殖へ
			の影響は認められなかった
			が、甲状腺及び肝臓重量の
			増加(オス及びメス)、下垂体
			重量の減少(メス)、甲状腺
			肥大症の発生率の増加(メ
			ス)を含む全身毒性が観察さ
			れた。生殖への影響が観察
			された最低用量は 300 ppm
			(オス:26.0 mg/kg/日、メス:
			28.4 mg/kg/日)であり、仔
			動物における特定できない
			臨床症状、産仔数の減少、
			体重の減少、交尾の減少、
			繁殖力の低下、着床後及び
			子孫の生存率の低下、身体
			的発達の遅れに基づく。
			・短期間の発達神経毒性試
			験では、授乳中の子犬の体
			重の有意な減少、およびオス
			の性発達遅延の兆候に基
			づき、LOAEL は
			0.90mg/kg/day。
			0.90mg/kg/day。 ◇動物代謝
			・経皮吸収はラットを用いた試
			験において1%未満
			・ヒト、ウサキ、、ラットの皮膚表皮
			を用いた <sup>14</sup> C-フィフ <sup>ロ</sup> ニルの in
			vitro 試験では、200g/L 溶
			液塗布の8時間後の浸透
			率は、0.08%(ラット)、0.07%
			(ウサギ)及び 0.01%(ヒト)で
			あった。0.2g/L の溶液塗布
			では、0.9%(ラット)、13.9%
			(ウサキ*)、0.9%(ヒト)の吸収
			率であった。別の in vitro 試
			験では、24 時間後にとトの皮
			膚表皮で 0.15~3.00%、ラット
			の皮膚表で1~35%)の浸
			透性であった。
			・犬と猫を対象とした <sup>14</sup> C-7ィ
			プロニルのスポットオン処理で
<u> </u>			ノロールのスホットオン処理で

		は、有効成分は主に皮膚表
		層に分布し、真皮や表皮下
		層(脂肪組織)では検出され
		なかった。
		<ul> <li>・ラット皮膚に<sup>14</sup>C-フィフ<sup>°</sup>ロニルテ<sup>°</sup></li> </ul>
		ススルフィル(光分解物)0.08~
		7.20mgを処理すると24 時
		間後に塗布した量の約 0.2
		~7.0%皮膚に浸透した。
		・標識したフィプロニルを 0.05、
		2.00、10.00ppm の用量でヤ
		ギの飼料経由で7日間投
		与したところ、吸収率は 15
		~33%であった。ラットを対象
		とした研究では、経口投与
		後の吸収率は 30~50%で
		あった。
		【分布】
		・本有効成分は哺乳類の体
		内に広く分布し、主に脂肪
		組織に検出される。単回経
		口投与されたラットでは、胃、
		消化管(GI)、脂肪、副腎で
		の濃度が最も高かった。中
		等度の濃度は肝臓、膵臓、
		甲状腺、卵巣に認められ
		た。低濃度は筋肉、脳、心
		臓、心臓の血液中に認めら
		nte.
		・犬と猫を対象としたスポットオ
		ン処理では、治療後2ヶ月
		後に皮脂腺、毛の周囲の上
		皮層、毛軸の露出部に <sup>14</sup> C-
		フィプロニルが検出され、毛と
		皮膚を覆う皮脂中に拡散し
		ていることが示唆された。ス
		ポットオン型のフィプロニル製品を
		犬に塗布し、毎日5分間、
		綿の手袋を使って犬を激し
		く撫でた。手袋に移行した残
		留物は、処理後24時間後
		に 589(±206)ppm でピーク
		を迎え、時間の経過とともに
		減少し(8日後には448±
		118ppm)、36 日後には検出
		されなくなった。
		【代謝】
		・ラットにおける全血中の半減
		期は、4mg/kgの経口投与
		で約 6.2~8.3 日であり、
		150mg/kg の経口投与では
		2.1~2.3 日。
		【排泄】
		・経口投与したラットでは、糞
		中に 45-75%、尿中に 5-
		25%のフィプロニルが排泄され
		た。親化合及びフィプロニルスル

				ホンは、糞及び尿共に検出さ
				れた。
				・授乳中のヤギにフィプロニルを
				7日間摂取させた場合に
				は、18~64%が糞中に、1~
				5%が乳中に排泄され、8~
				25%が体組織中に残存し
				た。
				・フィプロニルデススルフィルをヤキ゛
				に投与した場合には、糞中
				に 20~50%、尿中に 3~
				7%を排泄した。

### 略号(米国 EPA)

BEAD	Biological and Economic Analysis Division	FQPA	Food Quality Protection Act	NIOSH	National Institute for Occupational Safety and Health	
CDC	Centers for Disease Control	FWP	Final Work Plan	NOAEL	No-Observed-Adverse-Effect Level	
CTA	Comparative Thyroid Assay	HSPOC	Hazard and Science Policy Committee	OCSPP	Office of Chemical Safety and Pollution Prevention	
DCI	Data Call-In	HASPOC	Hazard and Science Policy Council	OPP	Office of Pesticide program	
DER	Data Evaluation Record	HED	Health Effects Division	PID	Proposed Interim Registration Decision	
DNT	Developmental NeuroToxicity	ID	Interim Registration Review Decision	PWP	Preliminary Work Plan	
DWA	Drinking Water exposure Assessment	IDS	Incident Data System	SF	Safety Factor	
EDWC	Estimated Drinking Water Concentration	LOAEL	Lowest-Observed-Adverse-Effect Level	SLUA	Screening Level Usage Analysis	
EEC	Estimated Environmental Concentration	NAWQA	National Water Quality Assessment	USGS	United States Geological Survey	
EFED	Environmental Fate and Effects Division			WOE	Weight of Evidence	

#### 引用 URL とその PDF ファイル(又はワードファイル、エクセルファイル)

FPU-1	Regulations.gov - Comment     Image: Comment.pdf
FPU-2	Regulations.gov - Comment     Port       Comment.pdf
FPU-3	Regulations.gov - Supporting & Related Material Document     FOR EPA-HQ-OPP-201 1-0448-0071.pdf
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FPU-5	Regulations.gov - Supporting & Related Material Document	
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FPU-6	Regulations.gov - Supporting & Related Material Document	<b>178日</b> 入
		EPA-HQ-OPP-201 1-0448-0073_cont
FPU-7	Regulations.gov - Supporting & Related Material Document	
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FPU-9	Regulations.gov - Supporting & Related Material Document	۲۵۲ ا
		EPA-HQ-OPP-201 1-0448-0043_cont
FPU-10	Regulations.gov - Supporting & Related Material Document	۲۵F ا
		EPA-HQ-OPP-201 1-0448-0045_cont
FPU-11	Regulations.gov - Supporting & Related Material Document	
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FPU-12	Regulations.gov - Supporting & Related Material Document	70F 入
		EPA-HQ-OPP-201 1-0448-0039_cont
FPU-13	Regulations.gov - Supporting & Related Material Document	۲۵۶ ب
		EPA-HQ-OPP-201 1-0448-0036_cont
FPU-14	Regulations.gov - Supporting & Related Material Document	
		EPA-HQ-OPP-201 1-0448-0003_cont
FPU-15	Regulations.gov – Supporting & Related Material Document	
		EPA-HQ-OPP-201 1-0448-0006_cont
FPU-16	Regulations.gov - Supporting & Related Material Document	778E
		EPA-HQ-OPP-201 1-0448-0008_cont
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### 9.1.1. 欧州 EFSA におけるフィプロニルの再評価概要と関係資料

評価 機関			① 事前のやり取りに関する文章		<ol> <li>② 追加要求され その内容</li> </ol>	れた試験項目及び に関する文章	③ ②の提出の	有無、再評価結果	<b>(4) ‡</b>	郭評価書	⑤ 規制[	内容/その他
EFSA									2021 年 1 月 [EU database] https://ec.europa.eu/food/ plant/pesticides/eu- pesticides- database/active- substances/?event=as.detai ls&as.id=1002	◇EU 登録データベース ・EU では農薬製品に使用さ れる有効成分(殺虫剤)とし て承認されていない。 ・猶予期間は2017年9月末 まで ◇エンドポイント ADI: 0.0002 mg/kg/日 ART: 0.0009 mg/kg		
									(URL のみ) 2021 年 1 月 [ECHA database]	AOEL: 0.0035 mg/kg/日 評価対象: フィプロニル、フィプロニ ルスルホン(MB46136) ◇ECHA データベース Hazard classification &		
									https://echa.europa.eu/de/ substance-information/- /substanceinfo/100.102.312 (URL のみ)	labelling:		
									[EU efforts for bee health] https://ec.europa.eu/food/ animals/live_animals/bees/ health_en (URL Ø矜)	◇ミツバチ保護に関する EU ウ ェブサイト		
									New Certified Reference Material for fipronil in egg https://ec.europa.eu/jrc/en /science-update/new- certified-reference- material-fipronil-egg (URL Ø#)	◇JRC(Joint Research Centre)のウェブサイト ・2017年に卵粉末中にフィブ ロールが検出されたことにより、残留分析の品質保証を 担当。		
									2020 年 4 月 【CERTIFICATION REPORT】 https://op.europa.eu/en/pu blication-detail/- /publication/41fff53-8508- 11ea-bf12- 01aa75ed71a1/language=en (FPE1)	◇JRC が発行した「卵粉末 に検出されるフィブロニル及びフ ィブロニルスルホンの標準分析方 法」		

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求された試験項目及び	③ ②の提出の有無、再評価結果	④ 再評価評価書	⑤規制	内容/その他
機関			その内容に関する文章				
EFSA	2019年6月 【SUMMARY REPORT】 Standing Committee on Plan Animals, Food and Feed Section Phytopharmaceutic - Residues <u>https://ec.europa.eu/food/is s/food/files/plant/docs/sc. to.20190613.ppr.sum.pdf</u> (FPE3)	ル、イマゾスルフロン、イソフ <sup>*</sup> ロツロ < ン、オルトスルファムロン、トリアスルフ ロン等の MRL に関する決定 事項。 < ◇すべての MRL を定量限				2019 年 10 月 [Official Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX32019R1792&from= EN (FPE2)	◇アミトロール、フィプロニル、フルビ ルスルホンメチル、イマン゙スルフロン、イ ソブロソロン、オルトスルファムロン、ト リアスルフロン等の MRL に関す る公告。 ◇フィプロニルの有効成分とし ての認可は 2017 年 9 月 30 日に失効。MRL は、検出限 界値(LOD)に設定。
	2018年7月 【Meeting Agenda】 https://ec.europa.eu/food/ sites/food/files/plant/docs /sc.phyto.20180719.ppl.age nda.pdf	◇ネオニコチノイド、フィブロニル についての一般裁判所の判 決					
	(FPE4) 2018年6月 【COMMISSION STAFF WORKING DOCUMENT】 <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/?uri=CEL</u> <u>EX%3A52018SC0302</u> (FPE6)	◇2013 年、欧州委員会は、 EFSA 127 による評価でミッ パチに高いリスクをもたらすことから、3 種類のネオニチノイ・ 系農薬とフィブロニルの使用を 制限。 ◇2018 年 2 月、EFSA は更 新された評価書 128 を公表 し、3 種類のネオニコチノイ・系 農薬のほとんどの用途が野 生パチとミッパチにリスクをもた らすことを確認。 ・3 種類のネオニコチノイト系農 薬の使用をさらに制限する 欧州委員会の提案は、2018 年 4 月 27 日に加盟国によ って承認。				2018年5月 【SCIENTIFIC REPORT】 <u>Occurrence of residues of</u> fipronil and other acaricides in chicken eggs and poultry muscle/fat (wiley.com) (FPE6)	◇養鶏場におけるフィブロニル の誤用が確認され、加盟国 と欧州委員会は、PAFF 委 員会において、特定のモニジリ ングプログラムを組織すること で合意。 ◇2017年9月1日から2017 年11月30日までに、5.439 サンプルの結果が提出され た。そのうち、742 サンプルは MRL 超過は主にフィブロニ ル。
	2017年12月 【Journal of Apicultural Research】 <u>https://ec.europa.eu/food/</u> <u>sites/food/files/plant/docs</u> / <u>gmo.rep_stud_mon-</u> <u>810_report=2017,ref-</u> <u>032.pdf</u> (FPE7)	○ こ年認。 ○ こ年認。 ◇ 非ハイブリッド、ハイブリッド又はトランスジェニックのトウモロン種子をフィブロニル、イミダクロブリドで処理または未処理の飼料を与えたミツバチの体液タンパク質と寿命を測定した。 ◇ シマリチのため、人気がしたい、 ◇ ジリ・チの寿命もは、供試いウモロン飼料の比較では同様だったが、ハチハン (beebread)よりも短く、ハチミッのみを与えたハチよりも長かった。 ◇トウモロンの品種に関係なく、パチジウロブリド処理では対照群と比較してリホフォリン量が25%以上低かった。				2017 年 12 月 [Factsheet] FIPRONIL IN EGGS <u>https://publications.jrc.ec.e</u> <u>uropa.eu/repository/bitstre</u> <u>am/JRC110632/jrc110632_f</u> <u>inal.pdf</u> (FPE8)	◇フィプロニル事件は、EU 加盟 国と欧州外において、殺虫 剤に汚染された卵と卵製品 が発見されたこもの。 ◇ブプロニルはイス、ネコのノミ、 ジニにたいする認可はある が、食用のニワトリ等には使 用禁止されている。 ◇ 適切な管理をするため に、JRCは、EU法に規定されているフィプロニルの定量に ついて公的検査機関の能 力を評価するための支援を 要請された。

評価	① 事前のや	り取りに関する文章	<ol> <li>追加要求された試験項目及び</li> </ol>	③ ②の提出の有無、再評価結果	④ 再評価評価書	<ol> <li>5 規制内容/その他</li> </ol>	
機関			その内容に関する文章				
EFSA	2017年11月 【SUMMARY REPORT】 https://ec.europa.eu/food/ sites/food/files/plant/docs /sc.phyto.20171121_ppr.su m.pdf (FPE9)	PAFF 委員会議事録 ◇2019~2021 年の EU 協 調複数年計画に関するモニタ リング草案を提示。 ◇動物由来の食品に関す る附属書 I にグリホサートとフィ フ <sup>*</sup> ロニルを追加。					
	2017 年 9 月 Conclusions from the Ministerial Conference on the follow up of the fipronil incident <u>https://ec.europa.eu/food/</u> sites/food/files/safety/doc <u>s/rasff.fipronil-</u> <u>incident_conclusions_201709</u> _pdf (FPEI0)	◇EU 閣僚会議議事録 2017 年夏のフィブロニルによる 汚染事件に関連して、9 月 26 日の閣僚会議での合意 事項。					
	2017 年 9 月 [SUMMARY REPORT] https://ec.europa.eu/food/ sites/food/files/animals/do cs/regroom_ani_ nutrit_20170911_sum.pdf (FPE11)	PAFF 委員会議事録 ◇ 産卵鶏農場でのフィプロニル の違法使用に関連した汚染 事件報告 ・フィプロニルによる違法な処理 を行った農場を特定。残留 量が EU-MRL である 0.005mg/kgを下回らない限 り、これらの農場からは出 荷停止。 ・2016 年から違法使用は行 われ、高濃度のフィプロニルが 残留する含む動物製品が 出荷されている可能性が高 い。 ・特に、産卵鶏の羽毛から 高濃度のフィプロニルが検出さ れた。					

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求された試験項目及び	③ ②の提出(	の有無、再評価結果	④ 再調	評価評価書	⑤ 規制	削内容/その他
機関			その内容に関する文章						
機関 EFSA	2017年8月 【SUMMARY REPORT】 https://ec.europa.eu/food/ sites/food/files/safety/doc s/reg_ com_toxic_20170830_sum.pd f (FPE12)	PAFF 委員会議事録 ◇産卵鶏農場におけるフィプ ロール含有製剤の違法使用に 関する意見交換・議論:状 況の評価と管理措置につい て						2017 年 7 月 【BfR Opinion】 https://www.bf.bund.de/c m/349/health-assessment- of-individual- measurements-of-fipronil- levels-detected-in-foods- of-animal-origin-in- belgium.pdf (FPE13)	BfR(ドイツ連邦リスク評価研究 所)からの報告 ◇ベルギー産の卵と卵製品 からフィ゙ロニルの高濃度残留 検出された旨の RASFF の 通知に基づいてリスク評価を 実施。 ◇分析結果は、鶏卵では 1kg あたり0.0031 ~1.2mg、 鶏肉では 1kg あたり0.0015 ~0.0156mg。 ◇ARFD はフットを用いた発生 毒性試験に基づき、 0.009mg/kg 体重。 ◇最も高い残留量(卵 1kg あたり1.2mg)のワーストケースと して試算し、欧州の消費デー ダを考慮すると、子供用の ARD を超えると判断した。 ◇必ずしも鶏卵の消費が具 体的な健康リスクをもたらすこ とを意味するものではない が、これらの汚染された鶏 卵を消費することにより潜 在的に子供の健康リスクが増
	2017年7月 【SCIENTIFIC REPORT】 https://efsa.onlinelibrary.wil ey.com/doi/epdf/10.2903/j. efsa.2017.4929 (FPE14)	◇2016年にJMPRは、12 の有効成分のリスク評価を実施。EFSAは、CODEXの MRL、毒性評価の提案に対するコメンを作成。 ・JMPR評価(2000年) ADI:0.0002mg/kg/day ARD:0.003mg/kg/day ・EFSA評価(2006年) ADI:0.002mg/kg/day ARD:0.009mg/kg/day		2017年4月 【APPLICATIONS FOR RENEWAL】 https://ec.europa.eu/food/ sites/food/files/plant/docs /pesticides_ppp_app- proc_air-3_sanco-2014- 10148.pdf (FPE15)	◇最終適用 2014 年 9 月 29 日 ◇今回のドシェ提出なし			2017 年 7 月 Information note <u>https://ec.europa.eu/newsr</u> <u>oom/sante/item-</u> <u>detail.cfm?item_id=127463</u> (URL のみ)	EU 委員会からの速報 ◇食品及び飼料に関する緊 急警報ンステム(RASFF)を 通じて、処理会社が産卵鶏 の養鶏場でワクモ (Dermanyssus gallinae)に 対する違法な薬剤を使用しているとの情報を得た。直 ちに事態の収拾に向けた措 置がとられ、現地での調査 が継続中。。
	2016年12月 【SUMMARY REPORT】 <u>https://ec.europa.eu/food/</u> <u>sites/food/files/plant/docs</u> <u>/sc.phyto_20161206.pppl_su</u> <u>m.pdf</u> (FPE16)	PAFF 委員会議事録 ◇フィブロニルのレビュー・現状と 次のステッブ ◇認可は 2017 年 9 月 30 日に失効。 ◇欧州委員会は、EFSA が これ以上のレビューを継続し ないことを決定。 ◇RMS:オーストリア		2016年11月 【Official Journal】 <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32016R2035&amp;from=</u> <u>EN</u> <b>(FPE17)</b>	◇フィブロニルの認可期間は、 2017 年9月30日から 2018 年7月31日まで延長 されたが、再評価用の資料 は提出されていない。 ◇従って、フィブロニルの認可 は、2017 年9月30日に失 効する。				

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求された試験項目及び	③ ②の提出の有無、再評価結果	④ 再評価評価書	⑤ 規制内	1容/その他
機関			その内容に関する文章				
EFSA	2016年10月 【SUMMARY REPORT】 https://ec.europa.eu/food/ sites/food/files/plant/docs /sc.phyto.20161006.pppl.su m.pdf	PAFF 委員会議事録 ◇欧州委員会は、AIR III 作 案計画の進捗状況について ドシI審査が遅れているた め、いくつかの有効成分で 認可延長が必要とする旨指 摘。					
	(FPE18)	(ク)イブロニルとマネブについては、申請書類が提出されていないため、期限が後ろ倒しになり、有効期限が延長される。					
	2016年9月 【SUMMARY REPORT】	PAFF 委員会議事録 ◇内分泌かく乱化学物質を 確認するための科学的基準					
	https://ec.europa.eu/food/ sites/food/files/plant/docs /sc_phyto_20160921_pppl_su	関する委員会の意見交換。 ◇例えば、毒性学的懸念が あり、関連する分析法が利					
	<u>m.pdf</u> (FPE19)	用可能な場合には、MRL は デフォルト値よりも低いレベルで 設定される可能性。(例: フィ プロニルの MRL0,005)					
	2016 年 6 月 【COMMISSION STAFF WORKING DOCUMENT】	◇内分泌かく乱性を検証するため、農薬用途324有効成分、殺生物用途98有効成分をスクリーニング。				2015年3月 【Office Journal】 <u>https://eur-</u>	◇代替品候補リストにフィフ <sup>*</sup> ロニ ル掲載。ADI が、それぞれの 物質/用途分類のケルーフ <sup>*</sup> 内 で認可されている他の有効
	https://ec.europa.eu/transp arency/regdoc/rep/10102/ 2016/EN/SWD-2016-211- F1-EN-MAIN-PART-6.PDF (FPE20)	◇フィフ <sup>`</sup> ロニルについては、 Option3 カテゴリーに分類され た。				lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32015R0408&from= EN (FPE21)	成分よりも有意に低い。
						2015年2月 【EC publication】 <u>https://ec.europa.eu/enviro</u>	◇ホオニコチノイト <sup>*</sup> 系殺虫剤とフィ プロ=ル系殺虫剤が鳥や魚に 影響を与え、餌の供給を減 らす可能性がある。
						nment/integration/research /newsalert/pdf/neonicotino id_and_fipronil_harm_fish_and _birds_402na5_en.pdf (FPE22)	

なりに関する文章	② 追加要求され	hた試験項目及び	③ ②の提出の	有無、再評価結果	④ 再評価評価書	5 規制内	容/その他
	その内容	客に関する文章					
			2014年1月 【REASONED OPINION】 <u>https://efsa.onlinelibrary.wil</u> ey.com/doi/pdf/10.2903/j.e fsa.2014.3543 (FPE24)	MRL 改訂 ◇前回の意見書で取り上げ られた動物加工食品中の MRL を再計算し、提案され たすべての MRL の安全性 に関する EFSA の結論を更 新。 ◇ドイツで認可されたジャガイ モに対する緊急認可と、欧 州でのキャヘツとケールに対す 認可が最近取り消されたこ とも考慮。 ◇必要とされるデータ不足は 認められず、消費者へのリス りは確認されなかった。		2014年10月 【Office Journal】 <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=OELEX:32014R1127&amp;from=</u> <u>EN</u> (FPE23)	◇既存 MRL について、77 ラナ科野菜の花と頭部、豚、 牛、羊、ヤギの脂肪と肝臓、 豚の腎臓、家禽の肝臓と卵 は引下げることを推奨。そ の他は、既存 MRL を引上 げるか、維持するよう勧告。 ◇トイツは 2012 年 2 月 10 日にコメツキ種の発生を理由 に、7イワニルを含む農薬契 品の一時的な認可を通告。 その結果、家禽の脂肪の MRL の引上げを求める要 請を通知。 ◇潜在的長期的健康リスクは 排除できないと結論付け、キ ャヘッとケールへの使用認可は 申請者により取り消された。
◇イタリア当局のプロジェクト 「APENET」で収集したネオニコ チノイト、類(チアメトキサム、クロチ アニジン、代ジクロフリト・)とフィ フロニルに関する科学的情報 を評価し、ハチへの影響に限 してこれらの物質の評価を 再検討。 ◇主にハチの健康状態、チアメ トキサム、クロチアニジン、代ジクロ フリト、フィブロニルを用いたトウモ ロン被覆種子の播種時の粉 塵飛散、粉塵に曝されたハチ の致死影響、帰巣行動と採 餌の影響を評価することを 目的とする。 ・全体的には、研究デザイン の不備、統計分析の弱点、 結果報告の不完全性など があり、すべての科学的情報について決定的な結論を 出すことはできなかった。し かし、粉塵にこさらされたハチ の致死影響、亜急性影響、クロチアニジンと病原体との 相互作用など、いくつかの 潜在的な、懸念事項が確認 され、ハチへの影響に関し て、チアメトキサム、クロチアニジン、 イジクロフリト、フィブロニルのリス ク評価を変更の必要性があ る。	2013 年 7 月 【Addendum to the Review report】 (FPE26)	ハチへのリスク評価に関するレ ビューの補遺 ◇イタリアの APENET プロジェク ドを受けて、欧州委員会は、 ミツハブ影響に関するフィブロニ ルのリスク評価を見直す。 ◇追加要求項目 ・ミツハブド以外の受粉媒介者 に対するリスク ・コロニーの生存、発達に対す る急性及び長期りリスク、なら びに植物・土壌代謝物に起 因する小F幼虫へのリスク。 ・種子処理作業中に放出さ れる粉塵への潜在的な曝 露、コロニーの生存と発達に対 する急性及び長期的リスク、 物塵に暴露された植生のク 繁殖リスク。 ・コロニーの生存と発達に対 する急性及び長期的リスク、 電子処理作業中に放出さ れる粉塵への潜在的な曝 露、コロニーの生存と発達に対 する急性及び長期的リスク、 電力に素が採食する状況での 繁殖リスク。 ・コロニーの生存と発達に対す る急性及び長期的リスク、 見生の甘露を採食することに よるハチの幼虫へのリスク。 ・溢液への潜在的な曝露、コ ロニーの生存と発達に対する 急性および長期的なリスク、 蜂で花粉、甘露、溢液中 の残留物への潜在的な暴 露。	2013年5月 【CONCLUSION ON PESTICIDE PEER REVIEW】 https://efsa.onlinelibrary.wil ey.com/doi/epdf/10.2903/j. efsa.2013.3158 (FPE27)	ビアレビュー結論 ◇イタリアのブロジェクト 「APENET」による科学的情 報の評価、その他のデータも 考慮。 ◇確認されたデータギャワブ ・すべての用途における植 物・土壌代謝物からの曝 露、パチへのリスクに対処する ための情報。 ・すべての用途に関連する ポリネークへのリスクに対処す るための情報。 ・種子処理作業中に放出さ れた粉塵が飛散した植生の 上でミツバチが採食した場合 のリスクに対処するための情 報。 ・汚染された花蜜や花粉を 摂取した場合のリスクに対処 するための情報。 ・定製の甘露を採食するミツ パチへのリスクに対処するための情報。 ・違液への曝露によるリスク に対処するための情報。 ・違液への曝露によるリスク に対処するための情報。 ・違液への曝露によるリスク に対処するための情報。 ・違液への曝露によるシスク に対処するための情報。 ・違液への曝露によるシスク に対処するための情報。		2013年8月 【Office Journal】 <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>-OELEX.32013R0781&amp;from=</u> <u>EN</u> (FPE25)	(小)にになったいたいたいたいたいたいたいたいたいたいたいたいたいたいたいたいたいたいたい
	◇イタリア当局のプロジュクト 「APENET」で収集したネオニコ テノイド類(テアントキサム, クロチ アニジン、イミダクロブリド)とフィ フロニルに関する科学的情報 を評価し、ハチへの影響に関 してこれらの物質の評価を 再検討。 ◇主にハチの健康状態、チアシ トキサム, クロチ7ニジン、イミダクロ ブリト、フィフロニルを用したたウモ ロコシ被覆種子の播種時の粉 塵飛散、粉塵に曝されたハチ の致死影響、帰巣行動と揉 餌の影響を評価することを 目的とする。 ・全体的には、研究デザイン の不備、統計分析の弱点、 結果報告の不完全性など があり、すべての科学的情 報について決定的な結論を 出すことはできなかった。し かし、粉塵にこちらなかった。し かし、動塵にこちらなたハチ 〜の致死影響、亜急性影 響、クロチ7ニジンと病原体との 相互作用など、いくつかの 潜在的な 懸念事項が確認 され、ハチへの影響に関し て、テアメトキ特ム、クロチ7ニジン、 イミダクコブリト、フィフにエルのリス ク評価を変更の必要性があ	<ul> <li>◇イタリア当局のプロジュクト</li> <li>△13年7月</li> <li>「APENET]で収集したれコニ ナ/ド類(Ŧアントキウム、クロテ ニシン、イミダウロブリト)とフ フロニルに関する科学的情報 を評価し、ハテへの影響に関し してこわらの物質の評価を 再検討。</li> <li>◇主にハチの健康状態、Ŧアン ドサム、クロチブニジン、イジ<sup>5</sup>/20 フリン、7/フロニルを用しいたいう年 ロシ被覆種子の播種時の粉 虚飛散、粉塵に唱されたハテ の致死影響、帰巣行動と採 餌の影響を評価することを 目的とする。</li> <li>・全体的には、研究デザ・ひ の不備、統計分析の弱点、 結果報告の不完全性など があり、すべての科学的情 報について決定的な結論を 出すことはできなかった。し かし、粉塵ににきらされたハテ への致死影響、亜急性影 響、クロチブニジンと病原体との 相互作用など、いくつかの 潜在的な 懸念事項が確認 され、ハテへの影響に関して、チブメトキ坊、クロテニジン、 (ジクロブ)ド、フィクロニルの以ス</li> </ul>	その内容に関する文章           ◇イ夘75当局のブロシ:ンウ ГАРЕ№ET」で収集したネオニコ テノイド類(テアントキ牡、フロテ アニジン(なシフロフ)ル、) とフ プロルに関する料学的情報 を評価し、ハテへの影響に関し してこれらの物質の評価を 再検討。 ◇シェに∧Fの健康状態、テアン トサセ、クロアニジン、(ジ? フリ゙、ファプロルを問したけた? Dix没種である「植業や植物の教 産飛散、物量に騙されたハテ の数死影響、帰巣行動と採 餌の影響を評価することを 目めとする。 ・2本体的には、研究デザ? のアニジン洗束的体結論を 出っこつ生存と発達に対す ふの形象と低した植物に生態へと調かけ、たいた Dix没種である「植業性のの別点」、 結果報告の不完全性など があり、オ ペての科学的情 報について決定的な結論を 出っこいて決定的な結論を してこれらの影響に関し て、テフントキリ、ハワアニジン (ジ?ロフリ)、ブィ?ロによの別(ス)         2013 年 7 月 (Addendum to the Review report]         ハチへのリスク酵価に関するレ ビューの補遺 ◇グリアの APENET ブロジ? ドを受けて、既外愛貴会は、 ワチと聞のの愛歌な君 このがするしたののと などして、村のの受粉媒介者 こたまないたかす の数死影響、無具行動と採 個の影響を評価することを 目的とする。 ・2本体的には、研究デザ? のプィーの生存と発達に対す る急性及び長期的リスク、 部屋について決定的な結論を 出すこことはできなかった。し かい、物量にときされたれず の数死影響、亜急性影 第、10077こジンと病原体との 相互作用など、いべつかの 器在的な 素ない、オへのの評価と関し て、テフントキリ、ハワアニン、 (ジ?ロフリル、フィブロによの0)(ス)         ハチへのリスク) パーク キロンの没た 常体のジス           第4日的なりない 第一位の 第一位の 第一位の 第一位の 第一位の 第一位の 第一位の 第一位の	その内容に関する文章         2014 年 1 月 [REASONED OPINION]           (イクリア当局のブロンか) [APENET]で取集したオロコ アイト型(デオ)パー型の(1) (オフロームを) マクロ)])         2013 年 7 月 [Addendum to the Review マクロ)]         Afへのリスク評価に関するは ビューの構造 マクロ)[25:0]         2013 年 5 月 [CONCLUSION ON PESTICIDE PEER REVIEW]           (イクリア当局のブロンか) [APENET]で取集したオロコ アイン型(デオ)パー型の)])         2013 年 7 月 [Addendum to the Review report]         Afへのリスク評価に関するは ビューの構造 マクリアの ADENET フロンロン ドを受けて、欧州 象合良は、 マクリアの ADENET フロンロン ドを受けて、欧州 象合良は、 マクリアの ADENET フロンロン ドを受けて、欧州 象合良は、 マクリアの ADENET フロンロン ドク型の (FPE28)         2013 年 5 月 [CONCLUSION ON PESTICIDE PEER REVIEW]           (FPE28)         (FPE28)         Afへのリスク評価を見見す の (ゴタローな) (FPE28)         2013 年 5 月 [CONCLUSION ON PESTICIDE PEER REVIEW]           (FPE28)         (FPE28)         Afon (FPE28)         2013 年 5 月 [CONCLUSION ON PESTICIDE PEER REVIEW]           (FPE28)         (FPE28)         (FPE28)         Afon (FPE28)         2013 年 5 月 [CONCLUSION ON PESTICIDE PEER REVIEW]           (FPE28)         (FPE28)         (FPE28)         (FPE27)         (FPE27)           (本) がり (TO) (TADE PEER TRATICATION (TADE PEER TRATICATION (T	・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・	O (クリア 三月の ) 2014 年 7 月 (PE20)         All 2 年 7 月 (PE20)         All 2 年 7 月 (PE20)         All 2 年 7 月 (PE20)         ML 2 年 (PE20)         ML 2 F (PE20)         ML 2	Operating of the control with the control with the control of the contro

		・種子コーティングは、専門的な
		種子処理施設でのみ実施。
		・土壌への浸透性を高め、
		流出を最小限に抑え、粉塵
		の排出を最小限に抑えるた
		めに、適切な種子処理装置
		の使用。
		・処理種子のラベルには、そ
		の種子がフィプロニルで処理さ
		れたことを表示し、認可に規
		定されている リスク軽減措置
		を明記。
		・ハチが広く採食に利用して
		いる地域や養蜂家が必要に
		応じて、実際の曝露を検証
		するために、モニタリングプログラ
		ムを開始。
		◇使用条件には、リスク軽減
		措置が含まれているものと
		し、申請者は、以下につい
		て 2015 年 3 月 30 日までに
		提出する。
		・ミツバチ以外の受粉媒介者
		に対するリスク。
		<ul> <li>コロニーの生存と発達に対す</li> </ul>
		る急性及び長期リスク、なら
		びに植物・土壌代謝物によ
		るハチの産卵へのリスク。
		・種子処理作業中に放出さ
		れる粉塵漂着物への潜在
		的曝露、コロニーの生存と発達
		に対する急性および長期的
		リスク、粉塵飛散に曝された
		植生でハチが採食する状況
		における幼虫に対するりス
		り。
		・コロニーの生存と発達に対す
		る急性および長期的リスク、
		昆虫の甘露を採食すること
		によるハチ幼虫へのリスク。
		(e)溢液への潜在的な曝露、
		コロニーの生存と発育に対す
		る急性および長期的リスク、
		幼虫へのリスク。
		(f) 残留性土壤代謝物
		(RPA 200766, MB 46136,
		MB 45950)を含む、圃場で
		発生した後作物や雑草の蜜
		や花粉、甘露、溢液中の残
		留物への潜在的な曝露。

<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求された試験項目及び その内容に関する文章	③ ②の提出の	有無、再評価結果	④ 再評価評価書	⑤ 規制内容/その他	
2012年5月 【RESONED OPINION】 <u>https://efsa.onlinelibrary.wil</u> <u>ey.com/doi/epdf/10.2903/j.</u> <u>efsa.2012.2707</u> (FPE29) 2011年12月	◇トイヤから、ジャガイモへの聚 急認可に伴う家禽脂肪中の MRLの引き上げを求める要請。 シゾャガイモへの使用は残留 データから十分に裏付けられ ており、MRLは0.01 mg/kg と提案。家畜飼料に関する 試験結果から、ジャガイモに使 用するには、家禽脂肪だけ でなく、反芻動物の肉、反芻 動物の脂肪、反芻動物の肉、反芻 動物の脂肪、反芻動物の肉、反芻 動物の脂肪、反芻動物の肉、下 職、豚の肉、豚の肝臓、豚 の腎臓、家禽の肉、および 牛乳について既存の MRL を修正する必要がある。 ◇リス?評価の結果、食品経 由での暴露は、ADIを超え る可能性がある ◇ジィがイモへの使用につい ては、動物性食品中の残留 物に起因する長期的な消費 者の健康リスクを排除できな いと結論付けた。		2012年5月 【RESONED OPINION】 <u>https://efsa.onlinelibrary.wil</u> ey.com/doi/pdf/10.2903/j.e fsa.2012.2688 (FPE30)	◇リスク評価結果 ・ADI:0.0002 mg/kg/day ・ARfD:0.009 mg/kg/day ◇土壌散布または種子処 理を用いた55種類の作物(3 作物グループ)で代謝試験実施。 ◇親化合物のフィブロニル及び のスルホン代謝物が評価対象 ◇親化合物のフィブロニル及び のスルホン代謝物が評価対象 ◇環識体を引いたたギスび 成場代謝試験では、フィブロニルとスルホン代謝物が、主要成 分であったので、食用動物 食品のリスク評価には、フィブロニルとそのスルホン代謝物の合計を用いる。 ◇家畜残留試験結果から 残留値は0.1 mg/kgを下 回っていたが、残留物は脂溶性であり、ADI が低い (0.0020 mg/kg/日)ことから、動物性食品中の残留物 影物性食品中の残留物 を考慮する必要がある。 ◇ドウモロシ飼料の暫定的 MRL については、関連する 認可を維持するために、さらなる追加試験が必要。		2011年5月	新規則 Regulation (EC)1
[Effects of coated maize seed on honey bees] (FPE31)	告(2011 年版) ◇トウモロンの種子コーティング に使用される有効成分が バに対して一定の毒性を有 する。コーティングごれた種子 からの粉塵の放出範囲は 制限されたが、パチ巣への被 害がいくつか検出された。 ◇圃場播種時に放出された 有効成分量はパチの致死量 を下回っていたが、成虫のの チの学習過程や記憶形成に 盈慮があることが判明した。					[Office Journal] <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32011R0540&amp;from=</u> <u>EN</u> (FPE32)	<ul> <li>1107/2009 での認可継</li> <li>純度:≥ 950 g/kg</li> <li>免行日:2007 年 10 月</li> <li>有効期限:2017 年 9 月</li> <li>日</li> <li>◇必要に応じてリスク軽調置を含めことが認可条件</li> <li>◇加盟国は、草食性鳥: び哺乳類、並びにミッパす に小君株に対する以入す</li> <li>を認可から1年以内にし 者が提出するよう要請す る。</li> </ul>
2011年5月 【Effects of coated maize seed on honey bees】 <u>https://www.reterurale.it/d</u> <u>ownloads/APENET_2010 Re</u> port_EN%206_11.pdf (FPE33)	<ul> <li>イタリア APENET プロジェクト報告(2010 年版)</li> <li>・モニタリングネットワーク</li> <li>・ミッハ・チーにおける種子処理での粉塵トリフト影響</li> <li>・種子処理した圃場での粉塵トリフトによる影響</li> <li>・種子処理の作物栽培上の有用性、作物中での移行</li> <li>・いつ液の影響</li> <li>・急性、亜急性影響</li> </ul>						

評価	① 事前のやり取りに	関する文章	<ol> <li>② 追加要求された試験項目及び</li> <li>2 の内容に関する文章</li> </ol>	③ ②の提出の4	ī無、再評価結果	④ 再評	価評価書	⑤ 規制内	1容/その他
機関			その内容に関する文章						
EFSA		ア APENET プロジェクト報 2009 年版)						2010年7月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32010R0750&from= EN (FPE35)	◇残留物の定義をフィブロニル 及びスルホン代謝物の合算で MRLを設定。
	(FPE34)					2010 年 3 月 Review report for the active substance (FPE37)	◇本レビュー報告書は、農薬 製品の上市に関する既存 有効成分レビューのための作 業計画の中で、フィプロニルの 再評価の結果として ANNEX I に含まれる可能 性を視野に入れて作成され た。 ◇委員会は、レビュー結果に 基づき、曝露された種に対 するリスクは許容可能である ことに同意する。EFSA によ る更なるレビューは必要ないと 考えられる。	(PPE36) 2010 年 3 月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32010L0021&from= EN (FPE36)	◇ミッパチのコロニーが大幅に 失われたことにより、加盟国 は予防措置を講じ、これら の有効成分を含む農薬製 品の上市を一時的に停止している。 ◇フィブロニルは、種子処理用 の殺虫剤としての使用のみ が認可されていが、加盟国 からの事故報告は不適切 使用が原因。 ◇将来の事故を避けるため に、クロチアニジン、チアメキサム、 フィブロニル、(ミゲ)ロブリドにつ いて、適切なリスク軽減手段 を含む追加規定を定める。 ◇加盟国は2010年10月 31日までに、クロチアニジン、チ アメトキサム、フィブロール、(ミゲ)ロ ブリドを含む農薬製品の認 可を修正または撤回する。
								2008 年 7 月 【Office Journal】 <u>https://eur-</u> lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32008R0839&from= <u>EN</u> ( <b>(FPE38)</b>	<ul> <li>◇MRL 更新</li> <li>◇残留物の定義をフィプロニル 及びスルホン代謝物の合算で</li> <li>MRL を設定</li> </ul>
								2008 年 1 月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32008R0149&from= EN (FPE39)	◇MRL 更新 ◇残留物の定義をフィプロニル 及びスルホン代謝物の合算で MRL を設定

評価機関	① 事前のやり取りに関する文章	② 追加要求された試験項目及び その内容に関する文章	③ ②の提出の有無、再評価結果	④ 再評	価評価書	⑤ 規制内	9容/その他
EFSA				2006 年 5 月	ヒ <sup>°</sup> アレヒ <sup>*</sup> ューレホ <sup>°</sup> ート	2007年8月	Annex I 改訂
EFSA				[Scientific Report]	◇フィプロニルは、第二段階の	[Office Journal]	◇RMS はフランスで、2004 年
				Conclusion on the peer	レビュープログラムの 52 物質に		2月10日に申請資料提出
				review of fipronil	含まれる。RMS フランスは、	https://eur-	之 <u>,</u> 10 日に平開夏和返出 済。
					2004年2月10日にEFSA	lex.europa.eu/legal-	///。 ◇純度:≥950 g/kg
				https://efsa.onlinelibrary.wil	に DARを提出し、その後レ	content/EN/TXT/PDF/?uri	施行日:2007年10月1日
				ey.com/doi/epdf/10.2903/j.	ビューを開始。	=CELEX:32007L0052&from=	有効期限:2017 年 9 月 30
				efsa.2006.65r	◇2005 年 2 月 9 日の評価	EN	日
				(FPE41)	会議で追加データの必要性	(FPE40)	□ ◇種子処理としての殺虫剤
				(FFE41)	去職 C追加7 - 500 安住 を合意。	(FFE40)	◇種子処理としての殺虫剤 としての使用のみが認可さ
					2005年6月、7月の加盟		れる。種子コーティングは、専
					○2003年0月、7月の加温 国の専門家会合で、残りの		的な種子処理施設でのみ
					問題点や申請者の追加デー		行う。これらの施設は、保
					の超点や中請有の追加す- 外を評価。		行う。これらの施設は、保管、輸送及び散布中の粉層
					9を評価。 ◇2006 年 2 月 7 日に加盟		官、制法及び取布中の粉磨 雲の放出を確実に排除する
					◆2006 年 2 月 7 日に加盗 国で専門家の協議の結果		芸の成田を確美に排除9. ために、利用可能な最善0
					国で専門家の協議の結果 について最終的な議論が行		7:00に、利用可能な (取書の) 技術を適用する。
					われ、結論に至った。		技術を週用9る。 ◇総合評価において、加盟
					1)れ、和画に主うた。 ◇代表的用途は、ヒマワリ、トウ		国は特に以下の点に注意
					モロシの種子処理。		払わなければならない。
					→毒性試験		<ul> <li>・懸念される光分解生成物</li> </ul>
					・短期試験では、中枢神経		の発生を回避するための
					系、肝臓、甲状腺に悪影響		販製品の包装。
					が認められ遺伝毒性や発		・土壌や気候条件が脆弱が
					が認められ遺伝毒性や光		地域に適用した場合、特に
					・甲状腺腫瘍の誘発効ニズム		地域に適用した場合、特に 親化合物よりも難分解性の
					はラットに特有のものであり、		高い代謝物による地下水
					thに外挿できない。		染の可能性。
					<ul> <li>生殖毒性、発生毒性はなく、神経系での病理組織学</li> </ul>		<ul> <li>・草食性鳥類や哺乳類、水</li> </ul>
							生生物、非標的節足動物、
					的所見は観察されていな		ミッパチの保護。
					い。		<ul> <li>・土壌への処理を確実にす</li> <li>ス液切た機器の体界し、#</li> </ul>
					•ADI:0.0002 mg/kg /日		る適切な機器の使用と、散
					•AOEL:0.0035 mg/kg /日•		布中の流出を最小限に抑 ること。
					ARfD:0.009 mg/kg		-
					<ul> <li>安全係数:100</li> <li>ヘ病物化調討</li> </ul>		◇認可の条件には、必要
					◇植物代謝試験		応じてリスク軽減措置を含め
					<ul> <li>・穀類、豆類、油糧種子、根</li> <li>・穀類、豆類、油糧種子、根</li> </ul>		ること。加盟国は、草食性
					茎及び塊茎の5作物につ		類及び哺乳類、並びにミッ
					いて実施。3作物ゲループに		チ、特にハチ群体に対するリ
					共通の代謝経路を定義し、		ク評価のための成績提出る
					関連代謝物(スルホン代謝物		要請すること。
					MB 46136)を同定。		
					◇光分解物 MB 46513 の 合株素株が感会されていた。		
					急性毒性が懸念されていた		
					が、種子処理用途では影響		
					がないと判断。光分解プロセ		
					スを防ぐために、処理された		
					種子を暗所で保管すること		
					のラベルの制限を提案。		
					◇家畜の摂取量は 0.1		
					mg/kg を大きく下回った		
					が、フィプロニルは脂溶性であ		
1					り、ADI が非常に低いた		1

	する必3 によるフ ルスルホン 費者に	産物への残留を考慮 要がある。種子処理 バフロール及びフィブロー - 残留物の暴露が消 高い慢性的なリスクを す可能性は低いと考 る。
	2004 年 4 月 Draft A (2005 年 1 月改訂) [DAR] https://www.efsa.europa.eu /sites/default/files/consult ation/consultation/59.zip (FPE42)	ssessment Report

#### 略号(欧州 EFSA)

AIR	Annex I Renewal	EFSA	European Food Safety Authority	JRC	Joint Research Centre	RASFF	Rapid Alert System for Food and Feed
BfR	Bundesinstitut fur Risikobewertung	EMS	Evaluating Member State	PAFF	Plants, Animals, Food and Feed	RMS	Rapporteur Member State
DAR	Draft Assessment Report	GAP	Good Agricultural Practice	PHI	Pre Harvest Interval	TTC	Threshold of Toxicological Concern

#### 引用 URL とその PDF ファイル (又はワードファイル、エクセルファイル)

FPE-1	https://op.europa.eu/en/publication-detail/-/publication/41ffff53-8508-11ea-bf12-01aa75ed71a1/language-en	KJNA30157ENN.e n.pdf
FPE-2	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1792&from=EN	CELEX_32019R17 92_EN_TXT (1).pt
FPE-3	https://ec.europa.eu/food/sites/food/files/plant/docs/sc.phyto_20190613_ppr_sum.pdf	sc_phyto_201906 13_ppr_sum.pdf
FPE-4	https://ec.europa.eu/food/sites/food/files/plant/docs/sc_phyto_20180719_ppl_agenda.pdf	sc_phyto_201807 19_ppl_agenda.pc
FPE-5	https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52018SC0302	CELEX 52018SC0302 EN
FPE-6	Occurrence of residues of fipronil and other acaricides in chicken eggs and poultry muscle/fat (wiley.com)	j.efsa.2018.5164. pdf

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FPE-7	https://ec.europa.eu/food/sites/food/files/plant/docs/gmo_rep-stud_mon-810_report-2017_ref-032.pdf	gmo_rep-stud_m	
		on-810_report-20	
FPE-8	https://publications.jrc.ec.europa.eu/repository/bitstream/JRC110632/jrc110632_final.pdf	PDF	
		jrc110632_final.p df	
FPE-9	https://ec.europa.eu/food/sites/food/files/plant/docs/sc.phyto.20171121_ppr.sum.pdf	PDE J~	
		sc_phyto_201711 21_ppr_sum.pdf	
FPE-10	https://ec.europa.eu/food/sites/food/files/safety/docs/rasff.fipronil-incident_conclusions_201709.pdf	PDF	
		rasff_fipronil-inci dent_conclusions_	
FPE-11	https://ec.europa.eu/food/sites/food/files/animals/docs/reg-com_ani-nutrit_20170911_sum.pdf	PDF	
		reg-com_ani-nutr it_20170911_sum	
FPE-12	https://ec.europa.eu/food/sites/food/files/safety/docs/reg-com_toxic_20170830_sum.pdf	PDF 2	
		reg-com_toxic_2 0170830_sum.pdf	
FPE-13	https://www.bfr.bund.de/cm/349/health-assessment-of-individual-measurements-of-fipronil-levels-detected-in- foods-of-animal-origin-in-belgium.pdf	PDF	
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FPE-14	https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4929	PDF	
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FPE-15	https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides.ppp_app-proc_air-3_sanco-2014-10148.pdf	J.efsa.2017.4929.	
FPE-15		j.efsa.2017.4929. pdf	
FPE-15 FPE-16		j.efsa.2017.4929. pdf	
	https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides.ppp.app-proc_air-3_sanco-2014-10148.pdf	j.efsa.2017.4929. pdf pesticides_ppp_a pep-proc_air-3_sar	
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Interaction     recritication(a) an       FPE-32     http://theorine.auropa.com/adv/pdf/10.2003/pha.2014.3142     FPE-2002/pha.2014.3142       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2014.3142     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2014.3142     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2014.3143     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2014.3143     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2014.3143     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2013.3145     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2013.3145     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2013.3145     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2013.3145     FPE-32       FPE-34     http://theorine.ibinary.witey.com/adv/pdf/10.2003/pha.2012.2727     FP	FPE-21	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R0408&from=EN	CELEX_32015R04
Image: CEE_S2014R11 Z7_EN_TXT, pdfFPE-24Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.0353Image: CEE_S2014.3543 .pdfFPE-27Mtsc//res-issuesce.logal-context/EN_TXT/PDF/Jur-CELES2019807B184rom-ENCEE_ES2.3018807 S1_EN_TXT.pdfFPE-27Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.0158Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-28Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.0158Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-27Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.0158Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-28Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.0128Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-29Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.012Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-29Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdfFPE-30Mtsc//refs.administrary.vilay.com/doi/odf/102003/sths2012.202Image: CEE_S2.3018807 S1_EN_TXT.pdf	FPE-22		neonicotinoid_an
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	FPE-29	https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2707	j.efsa.2012.2707. pdf
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		CELEX_32007L00						ļ
		52_EN_TXT.pdf						
FPE-41	https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2006.65r	PDF						
		2						
		j.efsa.2006.65r.p df						
FPE-42	https://www.efsa.europa.eu/sites/default/files/consultation/consultation/59.zip	POF	PDF	POF	PDF	PDF	PDF	
		Fipronil_DAR_01_	Fipronil_DAR_02_	Fipronil_DAR_03_	Fipronil_DAR_04_	Fipronil_DAR_05_	Fipronil_DAR_06_	
		Vol 1_public.pdf	Vol 2_public.pdf	Vol 3_B1-B5_publ	Vol 3_B6_part_1_	Vol 3_B6_part_2_	Vol 3_B6_part_3_	
		POF	PDF	PDF	PDF	POF	POF	
		Fipronil_DAR_07_	Fipronil_DAR_08_	Fipronil_DAR_09_	Fipronil_DAR_10_	Fipronil_DAR_11_	Fipronil_DAR_12_	
		Vol 3_B6_part_4_	Vol 3_B6_part_5_	Vol 3_B7_part_1_	Vol 3_B7_part_2	Vol 3_B8_public.p	Vol 3_B9_public.p	
L								

### 9.1.2. フェンメディファム

【人の健康影響評価についての要約】

- ▶ 米国
  - ✓ EPA レビューページ(別紙1)

https://www.regulations.gov/docket?D=EPA-HQ-OPP-2014-0546

▶ 初年度登録 1970 年

年月	評価資料	食品経由での人健康影響エンドポイント ・追加要求
2020年3月	Interim Registration Review Decision	♦ EDSP (Endocrine Disruptor
		Screening Program:内分泌かく乱物質
	[PHU1]	スクリーニングプログラム)に関連したとト健康
	POF A	影響評価を実施していないため、今後の ESA
	EPA-HQ-OPP-201	(The Endangered Species Act : 絶滅
	4-0546-0025.pdf	危惧種保護法)での評価結果により登録審
		査が最終決定される。
		◇本登録審査のために想定される追加データ
		要求はない。
2019年9月	Proposed Interim Registration	◇ラベル変更要求
	Review Decision	・除草剤耐性対応
		・非標的生物影響
	[PHU2]	・スプレードリフト対応
	20F	◇ポリネーター影響データを要求する可能性あ
	EPA-HQ-OPP-201 4-0546-0020.pdf	Ŋ
	4-0546-0020.pdi	◇EDSP スクリーニングの対象ではあるが、優
		先リストの対象ではない。PID(Proposed
		Interim Decision:暫定決定案)完了時
		までには本有効成分に対する決定を行う。
2015年9月	Final Work Plan	◇PWP(Preliminary Work Plan)以降
		の更新事項
		・とト健康評価については、作業者暴露評価
	2	及び累積リスク評価が未了
	EPA-HQ-OPP-201 4-0546-0012.pdf	・テンサイの登録を維持するためにはミジンコ急
		性毒性、淡水魚急性毒性、淡水無脊椎動
		物慢性毒性、魚類 ELS(Early Life
		Stage)に関する試験が必要
2015年3月	Combined Preliminary Work Plan and	◇バイエル社からフェンメディファムとデスメディフ

	Summary of Draft Human Risk	アム混合製剤の自主的登録取下げ(2013
	Assessment	年11月)。テンサイにおいては製剤登録が失
		効したが、原体ラベルを維持するためには、追
	[PHU8]	加データが必要。
	PDF 人	◇ヒト健康影響評価については、作業者暴露
	EPA-HQ-OPP-201	評価、収穫後処理における評価及び累積リス
	4-0546-0007.pdf	ク評価が未了
		◇データギャップ
		・ミツバチを含むポリネーター影響試験
		・底質生物影響試験
		・土壌・水中の分析バリデーション試験
2015年3月	Scoping Document and Draft Human	◇現行の毒性データ要件では、21/28 日間
	Health Risk Assessment in Support	の経皮、亜急性吸入、免疫毒性、神経毒性
	of Registration Review.	(急性及び亜急性)の試験が必要とされて
		いるが、HASPOC(Hazard and Science
	[PHU9]	Policy Council : は、WOE(Weight of
	PDF	Evidence)アプローチに基づき、これらの試
	EPA-HQ-OPP-201	験は必要ないと判断した。
	4-0546-0006.pdf	◇亜急性(ラット、マウス、イヌ)および慢性
		(ラット)試験で、赤血球数、ヘモグロビン濃
		度、ヘマトクリットの減少から溶血性貧血をもた
		らしたため、主要標的は赤血球と判断した。
		◇EPA 発がん性リスク評価ガイドライン草案
		(1999 年 7 月)に従い、HED 発がん評価
		検討委員会(CARC)は、「ヒトに対して発が
		ん性があるとは考えられない」カテゴリーに分類
		した。
		◇ウサギへの出生前暴露およびラットへの出生
		前/出生後暴露による感受性の増加を示す証
		拠はなく、 FQPA のデフォルト値の 10 倍の安
		全係数は、1倍に引き下げられた。
		◇毒性データベースに不足はなく、ラベルの変
		更及び追加要求はない。
2015年3月	Tier I Drinking Water Assessment for	◇親化合物と分解物 MHPC(methyl-3-
	the Registration Review of	hydroxyphenylcarbamate)でのモデル
	Phenmedipham and its Degradate,	計算 Tier I 評価。
	MHPC, for use in Human Health Risk	・ウィスコンシン州砂質土壌シナリオでは、
	Assessment.	MHPC について最高値の地下水 EDWC
	Health Risk Assessment in Support of Registration Review. [PHU9] EPA-HQ-OPP-201 4-0546-0006.pdf Tier I Drinking Water Assessment for the Registration Review of Phenmedipham and its Degradate, MHPC, for use in Human Health Risk	<ul> <li>◇現行の毒性データ要件では、21/28日間の経皮、亜急性吸入、免疫毒性、神経毒性(急性及び亜急性)の試験が必要とされているが、HASPOC(Hazard and Science Policy Council:は、WOE(Weight of Evidence)アプローチに基づき、これらの試験は必要ないと判断した。</li> <li>◇亜急性(ラット、マウス、イヌ)および慢性(ラット)試験で、赤血球数、ヘモゲロビン濃度、ヘマトクリットの減少から溶血性貧血をもたらしたため、主要標的は赤血球と判断した。</li> <li>◇EPA発がん性リスク評価ガイドライン草案(1999年7月)に従い、HED発がん評価検討委員会(CARC)は、「Eトに対して発がん評価検討委員会(CARC)は、「Eトに対して発がん性があるとは考えられない」カテゴリーに分類した。</li> <li>◇ウサギへの出生前暴露およびラットへの出生前/出生後暴露による感受性の増加を示す証拠はなく、FQPAのデフォルト値の10倍の安全係数は、1倍に引き下げられた。</li> <li>◇毒性データベースに不足はなく、ラベルの変更及び追加要求はない。</li> <li>◇親化合物と分解物 MHPC(methyl-3-hydroxyphenylcarbamate)でのモデル計算Tier I 評価。</li> <li>・ウィスコンシン州砂質土壌シナリオでは、</li> </ul>

		(Estimated Drinking Water
	[PHU10]	Concentration)が得られた。親化合物の
	PDF	濃度は、6つの PRZM-GW (The
	2	
	EPA-HQ-OPP-201 4-0546-0013.pdf	Pesticide Root Zone Model
	+ 05+0 0013.pu	Groundwater) シナリオすべてで実質的にゼ
		ロであった。
		・米国では地表水と地下水のモニタリングデー
		タで検出された報告はない。
		・表層水: USGS(United States
		Geological Survey : 米国地質調査所の
		NAWQA (National Water-Quality
		Assessment) データベースでは、これらの化
		合物の検出報告なし。カリフォルニア州データベ
		ースでの検出報告なし。
		・地下水:USGSのNAWQAデータ検索での
		検出報告なし。
2015年3月	Registration Review - Preliminary	◇製剤における生態影響及び環境動態、飲
	Problem Formulation for Ecological	料水リスクに係るデータギャップ
	Risk and Environmental Fate,	・ミツバチ成虫慢性毒性
	Endangered Species, and Drinking	・ミツバチ幼虫急性毒性
	Water Assessments	・ミツバチ幼虫慢性毒性
		・花粉・花蜜野外残留試験
	[PHU11]	・ポリネーター野外影響試験
	PDF 2	・底質における2種の淡水甲殻類での亜急性
	EPA-HQ-OPP-201	影響
	4-0546-0008.pdf	・汽水又は海水底質における1種の淡水又は
		汽水甲殻類の亜急性影響
		・土壌及び水中での分析バリデーション
2015年2月	Tier I Review of Human Incidents	◇現在、IDS(the OPP Incident Data
	[PHU12]	System)、SENSOR-Pesticides
		(Sentinel Event Notification System
	2	for Occupational Risk)のいずれにも本有
	EPA-HQ-OPP-201 4-0546-0005.pdf	効成分の症例は報告されていない。現時点で
		は、さらなる調査を必要とするような懸念はな
		い。事故情報の監視を継続し、懸念が生じた
		場合には、リスクアセスメントに追加分析が含ま
		れる予定。
L	1	

2005年3月	Reregistration Eligibility Decision	◇本有効成分については必要なラベル修正に 基づき、再登録の対象となると判断した。
	【PHU15】	
		<ul> <li>◇EPA は、ラベル修正及び製品に係るデータ</li> <li>要求のための DCI (データーコールイン) 通知</li> </ul>
	r	
	phenmedipham_r ed.pdf	を発行する予定である。
1987年3月	Fact Sheet	ファクトシート
	【PHU16】 91024L35.pdf	
	Guidance for the registration of	◇データギャップ要求
	pesticide products containing	【物化性】
	Phenmedipham	・すべての物化性試験
		【残留性】
	[PHU17]	・植物、動物代謝
	PDF	·残留分析法
	9100AV8F.pdf	・保存安定性
		・作物残留試験
		【毒性】
		・ラット急性吸入
		・眼刺激性
		・皮膚感作性
		・亜急性経皮
		・発がん性(動物種2種)
		·催奇形性(動物種2種)
		·変異原性
		•代謝
		【環境動態】
		・加水分解性
		·水中光分解性
		·嫌気的土壌中分解
		・土壌移行性(リーチング、吸脱着性)
		・土壌中分解性
		·後作残留性
		·魚類濃縮性

		【生態影響】 ・鳥類急性毒性
1973年8月	REQUIRED FOR REGISTRATION OF PESTICIDES FOR SPECIALTY AND SMALL ACREAGE CROPS AND OTHER MINOR USES] [PHU18] 9100FM9J.pdf	<ul> <li>◇マイナー栽培作物の登録取得に関するデー タ要求</li> <li>・ホウレンソウ使用について本有効成分の記載 あり</li> </ul>

- ▶ 欧州
  - ✓ EUデータベース(別紙1)
     https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/activesubstances/?event=as.details&as\_id=973
  - ✓ ECHAデータベース(別紙1) <u>https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/80412</u>

年月	評価資料	食品経由での人健康影響エンドポイント
		/追加要求
2021年3月	REASOND OPINION	◇申請者 UPL Europe Ltd.は、セロリにおけ
		る本有効成分の既存の最大残留基準値
	(PHE1)	(MRL)を 0.15mg/kg に引き上げる申請
	PDF R	を行った。提出された残留試験は提案の基準
	j.efsa.2021.6482.	値 0.15mg/kg に十分なものである。
	pdf	◇今回の MRL 申請は、本有効成分の再評
		価に関する EU のピアレビューが完了する前に
		提出されたため、最初の認可時のリスク評価の
		結論に従った。本評価では、(EC)No
		1107/2009 に基づいて特定されたデータギャ
		ップは考慮していない。従って、毒性学的参照
		値(TRV)、テンサイ以外の根菜類に対する
		リスク評価残留物の定義および関連する代謝
		物の毒性に関する結論を出すことができなかっ
		te.
2021年2月	REASOND OPINION	◇NEU GAP に基づいた 7 試験により、イチゴ
		の MRL は 0.7mg/kg。 完全なデータセットに
	(PHE2)	するためには追加1試験が必要。
	PDF	◇今回の MRL 申請は、再評価の EU 農薬ピ
	j.efsa.2021.6436.	アレビューが終了する前に提出されたため、最
	pdf	初に承認された際の毒性に関する結論に基づ
		いて実施。
		◇毒性プロファイルに関する様々なデータギャッ
		プを指摘。フェンメディファムとその代謝物の毒物
		学的プロファイルに関する様々なデータギャップ
		が確認され、果実類の残留を定義し、関連す
		る代謝物の毒性について結論を出ための

(TRVs)
って、本評
が完了す
,理由で遅
る前に失効
忍期間を延
に関する評
)延長が必
1日
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- 
乱性の試
で審査が遅
前に失効す
期間を延長
1日
で審査が遅
る前に失効
忍期間を延
1日
るコメント
対するコメ
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Renewal Assessm	ent Report	◇RMS : フィンランド、共同 RMS : デンマーク
(PHE8)		◇2017 年 9 月に改訂。専門家会議での議 論を経て、2017 年 10 月に再改訂。
《FILO》 《原体》		○発がん性:カテゴリー2、生殖毒性:カテゴ
PDF	PDF	
Phenmedipham_ dRAR_01_VOL_1_	Phenmedipham_ dRAR_02_VOL_2_	◇内分泌かく乱作用の可能性を除外すること はできなかった。
POF	PDF 2	毒性·代謝:dRAR_08
Phenmedipham_ dRAR_04_VOL_3_	Phenmedipham_ dRAR_05_VOL_3_	代謝概要 P.8~P.10 (P.229~P.236 にマ
		ウス骨髄へのアイソトープ化合物分布試験あ り)
PDF	PDF	9) 急性毒性概要 P.42~P.44
Phenmedipham_	Phenmedipham_	短期毒性概要:P.75~P.79
dRAR_07_VOL_3_	dRAR_08_VOL_3_	遺伝毒性概要:P.157~163
		長期毒性・発がん性概要 : P.239~P.246
PDF	PDF 2	生殖毒性概要:P.344~P.347
Phenmedipham_ dRAR_09_VOL_3_	Phenmedipham_ dRAR_10_VOL_3_	
Phenmedipham_ dRAR_11_VOL_3_		
《製剤》		
NZCHJ//	PDF	
Phenmedipham_	Phenmedipham_	
dRAR_13_VOL_3_	dRAR_14_VOL_3_	
POF	PDF J~	
Phenmedipham_ dRAR_16_VOL_3_	Phenmedipham_ dRAR_17_VOL_3_	
Phenmedipham_	Phenmedipham_	
dRAR_18_VOL_3_	dRAR_19_VOL_3_	

	PDF	
	<u>}</u>	
	Phenmedipham_ dRAR_List of stud	
2017年5月	Office Journal	
		遅れているため、更新の決定がなされる前に失
	(PHE9)	   効する可能性がある。従って、承認期間を延
	PDF	長する必要がある。
	CELEX_32017R08	◇延長期限:2018年7月31日
	41_EN_TXT.pdf	
2015年11月	Office Journal	◇MRLの改訂
	(PHE10)	
	PDF	
	CELEX_32015R20	
	75_EN_TXT.pdf	
2014年7月	REASOND OPINION	既存 MRL のレビュー
		◇ADI : 0.03mg/kg/日
	(PHE11)	◇植物代謝
	PDF	・テンサイとイチゴの葉面散布試験を実施し、
	j.efsa.2014.3807.	評価対象は親化合物のみと定義したが、根菜
	pdf	類と塊茎野類については十分な情報が得られ
		ていない。従って、根菜類と塊茎類の代表的な
		作物での追加代謝研究が必要。
		・主要作物の代謝と貯蔵安定性に関するデー
		タギャップを考慮すると、MRLとリスク評価値は
		暫定的なものと考える。
		・輪作作物における代謝試験からは残留物定
		義を設定できなかった。
		◇家畜代謝
		ヤギ、ニワトリの代謝試験結果から、残留物を
		定義できなかったため反芻動物での追加の代
		謝試験が必要。
		◇ARfD 設定の必要性はない。
		◇上記の評価に基づき、本有効成分の
		AnnexIVへの掲載を推奨しない。暫定的な
		MRL または既存の EU の MRL は、以下のデ
		ータによって確認される必要がある。

		<ul> <li>・根菜類及び塊茎類での植物代謝試験</li> <li>・葉菜類の植物代謝試験</li> <li>・酸性作物での保存安定性試験</li> <li>・作物残留試験4試験(イタリア南部の屋外GAP)</li> <li>・テンサイ(根)での作物残留試験3試験(欧州南部の屋外GAP)</li> <li>・輪作作物での追加植物代謝試験</li> <li>・反芻動物での追加家畜代謝試験(P.22~P.23)</li> </ul>
2014年7月	Application Dossier         (PHE12)         (原体》)         D_P-D_1_01_M-5         D_P-D_2_01_M-5         03004_000320628         D_P-D_2_01_M-5         03004_000320628         D_P-D_2_01_M-5         03007_000320628         D_P-Document_         A_01_M-502989_(         D_P-Document_         A_01_M-503018_0(         D_P-E_1_01_M-5         03011_000320629         D_P-LCA_Section         J_P-LCA_Section         J_O_P-LCA_Section         J_O_P-LCA_Section	◇Bayer CropScience AG (バイエル社) と UPL のタスクフォースによる申請用ドシエ提 出。 ◇RMSフィンランド、共同RMSデンマーク 毒性・代謝概要: D_P-MCA Section5_01 (《原体》の20個 目のPDFファイル)

D_P-LCA_Section _7_01_M-504164_	D_P-LCA_Section _8_01_M-504404_		
D_P-LCA_Section _10_01_M-50367(	D_P-MCA_Sectio n_1_01_M-50337!		
D_P-MCA_Sectio n_2_01_M-503024	D_P-MCA_Sectio n_3_01_M-503033		
D_P-MCA_Sectio n_4_01_M-50515:	D_P-MCA_Sectio n_5_01_M-50303;		
D_P-MCA_Sectio n_6_01_M-504547	D_P-MCA_Sectio n_7_01_M-50304:		
D_P-MCA_Sectio n_8_01_M-50477(	D_P-MCA_Sectio n_9_01_M-50477:		
D_P-MCA_Sectio n_10_01_M-50304	D_P-N_2_01_M-5 04396_000320629		
D_P-N_3_01_M-5 03142_000320629	D_P-N_4_01_M-5 03146_000320629		
D_P-N_5_01_M-5 03151_000320630	D_P-OCA_01_M- 505138_00032063		
 《製剤》			

D_P-D_1_01_M-5 03004_000320655	D_P-D_2_01_M-5 03007_000320655	
D_P-Document_ C_01_M-475768_(	D_P-Document_ C_02_M-475747_(	
D_P-Document_ C_03_M-475742_(	D_P-Document_ C_04_M-475736_(	
D_P-Document_ C_05_M-475781_(	D_P-Document_ C_06_M-475891_(	
D_P-Document_ C_07_M-475805_(	D_P-Document_ C_08_M-483300_(	
D_P-Document_ C_09_M-475820_(	D_P-Document_ C_10_M-475817_(	
D_P-Document_ C_11_M-475788_(	D_P-Document_ C_12_M-475894_(	
D_P-Document_ C_13_M-475897_(	D_P-Document_ C_14_M-475902_(	
D_P-Document_ C_15_M-475901_(	D_P-Document_ C_16_M-475900_(	

D_P-Document_ C_17_M-475899_(	D_P-Document_ C_18_M-475839_(	
D_P-Document_ C_19_M-502996_(	D_P-Document_I _01_M-503168_0(	
D_P-LCP_Section _2_01_M-503683_	D_P-LCP_Section _4_01_M-503690_	
D_P-LCP_Section _5_01_M-504407_	D_P-LCP_Section _7_01_M-504168_	
D_P-LCP_Section _9_01_M-504408_	D_P-LCP_Section _10_01_M-50456:	
D_P-LCP_Section _12_01_M-503702	D_P-MCP_Sectio n_1_01_M-50304	
D_P-MCP_Sectio n_2_01_M-503055	D_P-MCP_Sectio n_3_01_M-503066	
D_P-MCP_Sectio n_4_01_M-503072	D_P-MCP_Sectio n_5_01_M-50415	
D_P-MCP_Sectio n_6_01_M-503074	D_P-MCP_Sectio n_7_01_M-503076	

	PDF	
	J         J           D_P-MCP_Sectio         D_P-MCP_Sectio           n_8_01_M-503084         n_9_01_M-504773	
	POF         Pof           D_P-MCP_Sectio         D_P-MCP_Sectio           n_10_01_M-50457         n_11_01_M-50308	
	PDF         PDF           D_P-MCP_Sectio         D_P-OCP_01_M-5           n_12_01_M-50314         05140_000320656	
2014年6月	REASOND OPINION	◇エンドポイント
		ADI : 0.03mg/kg/日
	(PHE13)	ARfD : 不要
	PDF 2	◇レタスでの代謝試験はなく、英国はテンサイ
	j.efsa.2014.3738	での代謝試験を外挿することを提案。
	(1).pdf	EFSA は、テンサイからの外挿の提案は受入れ
		られないとの見解を示す。
		◇GAP に従った作物残留試験は十分だが、
		葉菜類におけるデータギャップが解決されていな
		いと、MRL設定提案はできない。
		<ul> <li>◇レタスは生食されるため、調理加工試験は</li> <li>必要ない。レタスは輪作で栽培することができる</li> </ul>
		し安ない。レダスは##1FC秋石9ることができる ため、追加の輪作の代謝試験が必要。
		(P.11)
2011年5月	Office Journal	◇Annex I リスト収載
		•原体純度:970g/kg
	(PHE15)	·認可日:2005年3月1日
	PDF 2	・有効期限:2015年2月28日
	CELEX_32011R05	・除草剤としての使用
	40_EN_TXT.pdf	
2008年1月	Office Journal	◇MRL改訂
	(PHE16) CELEX_32008R01 49_EN_TXT.pdf	
2004年4月	Office Journal	◇RMS フィンランドは 2000 年 1 月 5 日に

	(PHE17)	DAR を提出。2001 年 11 月 13 日にフェン メディファムに関するタスクフォースからサマリード シエを受領。
2004年2月	Review report for the active substance (PHE18) EC Review Report Phenmedi	レビュー報告書 Annex I 掲載のための評価 ◇RMS フィンランドは 2000 年 1 月 5 日に DAR を提出。2001 年 11 月 13 日にフェン メディファムに関するタスクフォースからサマリード シエを受領。 ◇短期的には以下に注意を払う必要がある。 ・水生生物の保護 ◇現段階では、追加試験は必要ないが、いく つかのエンドポイントでは、追加試験の作成又 は提出が必要となる場合がある。 ・原体不純物を確認するためのバリデーション 試験 ・原体中のトルエンの分析方法又は環境影響 がないことの証明
2003年10月	DAR Addendum 3 (PHE19) PMP_addendum_ 3_A.pdf 3_B.pdf	<ul> <li>DAR 補遺 3&lt;</li> <li>◇追加提出データ(人健康影響関係)</li> <li>・代謝物 desmedipham のコリンエステラーゼ</li> <li>に対する影響についての理由書(P.7)</li> <li>・テンサイ、飼料用ビートのMRL設定に関する</li> <li>理由書(P.7)</li> </ul>
2002年10月	DAR Addendum 2 (PHE20) PMP_addendum_ 2_A.pdf 2_B.pdf	<ul> <li>DAR 補遺 2</li> <li>◇追加提出データ</li> <li>・物化性</li> <li>・分析方法</li> <li>・作物残留試験</li> </ul>
2001年11月	DAR Addendum 1	DAR 補遺 1 ◇追加提出データ(ヒト健康影響関係) ・ラット慢性毒性試験、発がん性試験の病理

	(PHE21)	PMP_Addendum_ 1_B.pdf	学的評価 ・皮膚透過性試験 ・ヒトおよびラットの表皮からの吸収 ・作業者暴露評価 ・農業従事者のリエントリー暴露評価 (P.5)
1999 年 12 月	DAR (PHE22) 《Vol.1》 Append_1.pdf	Append_2.pdf	Draft Assessment Report (DAR)
	ContVol1.pdf	Cover.pdf	
	Institut.pdf	Level 1.pdf	
	Level 2.pdf	Level 3.pdf	
	Level 4.pdf		
	《Vol.2》 》 Volume2.pdf		
	«Vol.3»		

Append_1.pdf	Append_2.pdf	
B1-B4.pdf	B5_tox.pdf	
B6_resid.pdf	B7_fate.pdf	
B8_ecoto.pdf	B9_class.pdf	
ContVol3.pdf	Cover.pdf	

# 別紙1.データベース

## ▶ 米国

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# EU データベース

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	Active substance Phenmedipham									
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	Phenmedipham			09 [2		Approved				
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# ECHA データベース

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	nonised classi al Information		x VI of Regulat	ion (EC) No	1272/2006 (CLP Regulatio	n)			
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ara	essification (Tabi	affectuat			Labelling		Specific Concentration Bailts, H Factors, Acute	Tostilly Notes	
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## 9.1.2. 米国 EPA におけるフェンメディファムの再評価概要と関係資料

5	① 事前のやり	取りに関する文章	② 追加要求され	れた試験項目及び	<ol> <li>②の提出の有無、再評価結果</li> </ol>	④ 再評価評価	書	⑤ 規制内	容/その他
5			その内容	に関する文章					
							Dock https://doc	gistration Review ket] s://www.regulations.gov sket?D=EPA-HQ-OPP- I-0546	EPA レビューヘーン
							(URI	Lのみ)	
	2019 年 9 月 【PID】 Proposed Interim Registration Review Decision https://www.regulations.gov	◇ラベル変更要求 ・除草剤耐性対応 ・非標的生物影響 ・スフ'レート'リフト対応 ◇ボ'リネ-ター影響データを要求 する可能性あり ◇EDSP スクリーニングの対象で	/document?D=EPA-HQ- OPP-2014-0546-0025	◇ハテへの影響に懸念があ るが、使用バターンに基づく暴 露の可能性は限定的。 ◇現時点で、登録審査に必 要な追加データはない。					
	/document?D=EPA-HQ- OPP-2014-0546-0020 (PHU2)	はあるが、優先リストの対象 ではない。PID 完了までに は本有効成分に対する決 定を行う。	(PHU1)						
	2019 年 8 月 [MEMORANDUM] EFED Response to Public Comments Submitted on the Draft Risk Assessment (DRA) https://www.regulations.gov /document?D=EPA-HQ- <u>OPP-2014-0546-0022</u> (PHU4)	ルマコメに対する回答 ◇ホリネーター追加試験及び 生態影響評価に関するバイ エル社からのバブリックコメントに 対する回答	2019 年 8 月 【MEMORANDUM】 Addendum to Draft Risk Assessment for Registration Review <u>https://www.regulations.gov</u> /document?D=EPA-HQ- OPP-2014-0546-0021 (PHU3) 2018 年 12 月 【MEMORANDUM】	◇DRAの更新 ・底生無脊椎動物およびホリ ネーターに対する潜在的リスク 評価 ・データト*マワブストの更新 ミツハ*方幼忠急性毒性 シハ*天成虫慢性毒性 海産注違影響試験 ジアノハ*クテリア影響試験 嫌気的水中代謝試験 土壌/水分析ハ*リテーション試験 灸前回の評価と同様に、曝 露評価と推定環境濃度					
	2015年9月	◆PWP 以降の更新事項	Image: Construction of the system       Draft Risk Assessment for Registration Review       https://www.regulations.gov       /document?D=EPA-HQ_       OPP-2014-0546-0016       (PHU5)       2016 # 5 月	(UEC)を親化合物のみで実施。 ◆生態影響と環境運命のデータヘースはほぼ十分。 ◆嫌気性水中代謝試験(OCSPP 8354400)をデータ ギャップとして追加。 ◇ハイエルクロプサイエンス、UPL					
	(Final Work Plan] https://www.regulations.gov /document?D=EPA+HO- OPP-2014-0546-0012 (PHU7)	(・)11 公共の登録を維持するためにはミジンコ急性毒性、淡水魚急性毒性、淡水無脊椎動物慢性毒性、魚類ELSに関する試験が必要	[Data Call in] https://www.regulations.gov /document?D=EPA-HQ- OPP-2014-0546-0014 (PHU6)	か舎録保持者 ◇データギャップ ・底質生物影響(淡水/海水) ・ボリネ-ター野外試験 ミジンコ急性毒性 ・魚類急性毒性(淡水/海水) ・シンコ優性毒性 ・魚類色は毒性(淡水/海水) ・ジンコ優性毒性 ・気が、行気虫急性毒性 ・ざが、右塞残留試験 ・ボリネ-ター半野外試験 ・分析パリデーション					

ī	<ol> <li>事前のやり</li> </ol>	り取りに関する文章	<ol> <li>追加要求さ</li> </ol>	された試験項目及び	③ ②の提出の?	有無、再評価結果	④ 再評	価評価書	⑤ 規制	内容/その他
]			その内容	に関する文章						
	2015 年 3 月 [PWP, Oraft HRA] Combined Preliminary Work Plan and Summary of Draft Human Risk Assessment https://www.regulations.gov /document?D=EPA-HQ- OPP-2014-0546-0007 (PHU8)	<ul> <li>◇∩、イエルからフェンボ、イファムと デスメデ、イフィム混合製剤の自 主的登録取下げ(2013年11 月)。</li> <li>◇テンサイにおいては製剤登場が 学校、効したが、原体ラル を維持するためには、以下 の追加データが必要。</li> <li>・ミツハ・チを含むホリネーター影響 試験</li> <li>・店賃生物影響試験</li> <li>・土壌・水中の分析パリデーション試験</li> </ul>		◇製剤における生態影響及び環境勤態、飲料水リスクに 係るデータギャワブ ・ミッハチ成虫慢性毒性 ・ミッハチが虫虫慢性毒性 ・ミッハチが虫虫慢性毒性 ・ミッハチが虫虫慢性毒性 ・ボやか・花蜜野外氏智試験 ・底質における2種の淡水 甲殻類での亜急性影響 ・汽水又は海水尾質におけ る1種の淡水又は汽水甲殻 類への亜急性影響 ・土壌及び水中での分析バリ デーション	2015 年 3 月 [Scoping Document and Draft HHRA] Scoping Document and Draft Human Health Risk Assessment in Support of Registration Review. https://www.regulations.gov /document?D=EPA-HQ- OPP-2014-0546-0006 (PHU9)	◇現行の毒性データ要件で は、21/28 日間の経皮、亜 急性吸入、免疫毒性、神経 毒性(急性および亜急性) の試験が必要とされている が、HASPOCは、WOE 77'ロ - デに基づき、これらの試験 は必要ないと判断した。 ◇亜急性(ラット、マウス、43) 及び慢性(ラット)試験で、赤 血球数、ペチプルビン濃度、ペマ トクリットの減少から溶血性貧 血をもたらしたため、主要標 的は赤血球と判断した。 ◇EPA 発がん性リスク評価か パライン草案(1999 年 7 月) に従い、HED 発がん評回検 討委員会(CARC)は、「ヒトに 対して発がん性リカ評価か」 パライン草案(1999 年 7 月) に従い、HED 発がん性リスク評価が ポライン草案(1999 年 7 月) に従い、HED 発がん性リスク評価が ポライン草案(1999 年 7 月) に従い、HED 発がん性リスク評価が ポライン草案(1999 年 7 月) に従い、HED 発がん性があるとは 考えられない」カテコ`リーに分 類した。 ◇うりトへの出生前暴露及 びうりトへの出生前暴露及 びうりトへの出生前場器及 びうりトへの出生前場器及 びうりトへの出生前場器及 びうりトへの出生意があるとは 考えられない」カテコ`リーに分 類した。 ◇売かのの生気を係数 は、1倍に引き下げられた。 ◇毒性データヘースに不足は なく、ラヘルの変更及び追加 要求はない。			2015 年 3 月 [MEMORANDUM] Tier I Drinking Water Assessment for the Registration Review of Phenmedipham and its Degradate, MHPC, for use in Human Health Risk Assessment. https://www.regulations.gov /document?D=EPA-HQ- <u>OPP-2014-0546-0013</u> (PHU10)	◇親化合物と分解物 MH (methyl-3- hydroxphenylcarbamate でのモデル計算 Tier I 評 (◇カイスンジン州砂質ナリジ は、MHPC I ついて最高 の地下水 EDWC が得ら. た。親化合物の濃度は、 つの PRZM-GW ジナリオす てで実質的にど口であっ (シス国では地表水と地 のモニタリングデータが供出事 例はない。 ◇表層水:米国地質調督 (USGS)の NAWQA デー -スでは、これらの化合報 検出報告なし。別フルコ データヘ - スでの検出報告 し。 ◇地下水: USGS の NAWQA データ検索での根 報告なし。
-	2014年9月 【MEMORANDUM】 BEAD Chemical Profile (BCP) for Registration Review <u>https://www.regulations.gov</u> /document?D=EPA-HQ- <u>OPP-2014-0546-0003</u> (PHU13) 2014年6月 [Screening Level Usage	◇適用はホウレンりク、テンサイで あるが、ホウレンソウへの使用 は少なく、大部分のテンサイに 使用されていることから、テン サイの雑草防除に重要な役 割を果たしている可能性が 高い。 ◇ SLUA (Screening Level Usage Analysis )での 2004			2005年3月 【RED】 Duraitation [[iiikiita	◆本有効成分については必要な7~ル修正に基づき、再 発行の→1分したる」と判断			2015年2月 【MEMORAMDUM】 Tier I Review of Human Incidents https://www.regulations.gov /document?D=EPA-HQ- OPP-2014-0546-0005 (PHU12)	◇事故報告例なし
	Analysis] https://www.regulations.gov /document?D=EPA-HQ- OPP-2014-0546-0004 (PHU14)	年から 2012 年までのホウレン ソウ及びテンサイにおける本農 薬使用量			Reregistration Eligibility Decision <u>https://archive.epa.gov/pes</u> <u>ticides/reregistration/web/</u> <u>pdf/phenmedipham_red.pdf</u> (PHU15)	登録の対象となると判断した。 ◇EPAは、ラベル修正及び製 品に係るデー9要求のための デー9-コールイン(DCI)通知を 発行する予定で。				

評価	① 事前のや	り取りに関する文章	<ol> <li>追加要求さ</li> </ol>	れた試験項目及び	<ol> <li>③ ②の提出の有無、再評価結果</li> </ol>	④ 再調	評価評価書	⑤ 規制	内容/その他
機関			その内容	に関する文章					
EPA	1973 年 8 月 【THE DEVELOPMENT OF DATA REQUIRED FOR REGISTRATION OF PESTICIDES FOR SPECIALTY AND	◇マイナー栽培作物の登録取 得に関するデー9要求 ・ホウレンソウ使用についての7 ェンメディファム記載	1987 年 3 月 【Fact Sheet】 Document Display NEPIS   US EPA (PHU18)	\$779ŀ>−ŀ					
	SMALL ACREAGE CROPS AND OTHER MINOR USES] Document Display   NEPIS   US EPA (PHU18)		[GUIDANCE] Guidance for the registration of pesticide products containing Phenmedipham Document Display   NEPIS   US EPA (PHU17)	<ul> <li>◇データギャッブ</li> <li>「物化性]</li> <li>・すべての物化性試験</li> <li>「残留性]</li> <li>・植物、動物代謝</li> <li>・残留分析法</li> <li>・保存安定性</li> <li>・作物残留試験</li> <li>【毒性]</li> <li>・フト急性吸入</li> <li>・眼刺激性</li> <li>・皮膚感作性</li> <li>・密急性経疫</li> <li>・発がんぜ(動物種2種)</li> <li>・催奇形性(動物種2種)</li> <li>・確奇形性(動物種2種)</li> <li>・変異原性</li> <li>・代謝</li> <li>【環境動態]</li> <li>・加水分解性</li> <li>・波令分解性</li> <li>・遠線気的土壌中分解</li> <li>・土壤移行性(リーチング、吸脱着性)</li> <li>・土壤や分解性</li> <li>・後作残留性</li> <li>・食病類濃縮性</li> <li>【生態影響]</li> </ul>					

#### 略号(米国 EPA)

BEAD	Biological and Economic Analysis Division	FQPA	Food Quality Protection Act	NIOSH	National Institute for Occupational Safety and Health	
CDC	Centers for Disease Control	FWP	Final Work Plan	NOAEL	No-Observed-Adverse-Effect Level	
CTA	Comparative Thyroid Assay	HSPOC	Hazard and Science Policy Committee	OCSPP	Office of Chemical Safety and Pollution Prevention	
DCI	Data Call-In	HASPOC	Hazard and Science Policy Council	OPP	Office of Pesticide program	
DER	Data Evaluation Record	HED	Health Effects Division	PID	Proposed Interim Registration Decision	
DNT	Developmental NeuroToxicity	ID	Interim Registration Review Decision	PWP	Preliminary Work Plan	
DWA	Drinking Water exposure Assessment	IDS	Incident Data System	SF	Safety Factor	
EDWC	Estimated Drinking Water Concentration	LOAEL	Lowest-Observed-Adverse-Effect Level	SLUA	Screening Level Usage Analysis	
EEC	Estimated Environmental Concentration	NAWQA	National Water Quality Assessment	USGS	United States Geological Survey	
EFED	Environmental Fate and Effects Division			WOE	Weight of Evidence	

## 引用 URL とその PDF ファイル(又はワードファイル、エクセルファイル)

PHU-1	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0025	EPA-HQ-OPP-201 4-0546-0025.pdf
PHU-2	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0020	EPA-HQ-OPP-201 4-0546-0020.pdf
PHU-3	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0021	EPA-HQ-OPP-201 4-0546-0021.pdf
PHU-4	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0022	EPA-HQ-OPP-201 4-0546-0022.pdf
PHU-5	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0016	EPA-HQ-OPP-201 4-0546-0016.pdf
PHU-6	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0014	EPA-HQ-OPP-201 4-0546-0014.pdf
PHU-7	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0012	EPA-HQ-OPP-201 4-0546-0012.pdf
PHU-8	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0007	EPA-HQ-OPP-201 4-0546-0007.pdf
PHU-6 PHU-7	https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0014 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0012	EPA-HQ-OPP-201 4-0546-0016.pdf EPA-HQ-OPP-201 4-0546-0014.pdf EPA-HQ-OPP-201 4-0546-0012.pdf EPA-HQ-OPP-201

PHU-9 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0006	EPA-HQ-OPP-201 4-0546-0006.pdf
PHU-10 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0013	EPA-HQ-OPP-201 4-0546-0013.pdf
PHU-11 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0008	EPA-HQ-OPP-201 4-0546-0008.pdf
PHU-12 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0005	EPA-HQ-OPP-201 4-0546-0005.pdf
PHU-13 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0003	EPA-HQ-OPP-201 4-0546-0003.pdf
PHU-14 https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0546-0004	EPA-HQ-OPP-201 4-0546-0004.pdf
PHU-15 https://archive.epa.gov/pesticides/reregistration/web/pdf/phenmedipham.red.pdf	phenmedipham_r ed.pdf
PHU-16 Document Display   NEPIS   US EPA	91024L35.pdf
PHU-17 Document Display   NEPIS   US EPA	9100AV8F.pdf
PHU-18 Document Display   NEPIS   US EPA	9100FM9J.pdf

## 9.1.2. 欧州 EFSA におけるフェンメディファムの再評価概要と関係資料

平価	① 事前のやり	取りに関する文章	<ol> <li>追加要求され</li> </ol>	た試験項目及び	<ol> <li>②の提出の</li> </ol>	有無、再評価結果	④ 再評価	「評価書	5 規制内容/その他	
幾関			その内容に関する文章							
FSA					2021年3月 【REASOND OPINION】 https://efsa.onlinelibrary.wil ey.com/doi/10.2903/j.efsa.2 021.6482 【PHE1】	◇UPL Europe Ltd.が、レタス の MRLを0.15mg/kg に引き 上げる申請 ◇本申請は、本有効成分の 再評価が完了する前に実 施されたものであるため、 毒性評価は、最初の申請時 の評価結果で実施。			2021 年 2 月 [EU database] https://ec.europa.eu/food/ plant/pesticides/eu- pesticides- database/active- substances/?event=as.detai ls&as.id=973 (URL Ø\$)	EU データベース ・承認日:2005 年 3 月 1 E ・有効期限:2021 年 7 月 3 日. ・ADI 0.03 mg/kg 日 ・ARfD 該当なし ・AOEL 0.13 mg/kg/日 ・EU レヘルでの再評価中。
									2021 # 2 月 [ECHA database] https://echa.europa.eu/info rmation-on-chemicals/cl- inventory-database/- /discli/details/80412 (URL のみ)	ECHA 7 <sup>-</sup> -9^7
	2020年6月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32020R0869&from= EN (PHE3)	◇審査が申請者の管理の 及ばない理由で遅れている ため、更新の決定がなされ る前に失効する可能性が高い。そのため、承認期間を 延長する。さらに、内分泌か く乱作用に関する評価を実施するためにも承認期間の 延長が必要。 ◇承認延長日:2021年7月 31日			2021年2月 【REASOND OPINION】 <u>https://www.efsa.europa.eu</u> /en/efsajournal/pub/6436 (PHE2)	◇追加試験が実施されていないため、イチゴへのMRLは、0.7 mg/kg。 ◇今回のMRL申請は、再評価のEU農薬ビアレビューが終了する前に提出されたため、毒性評価は、最初の申請時の評価結果で実施。 ◇今回の評価は、再評価に 関連したデータギャップを考慮していないことに留意。				
	2019 年 8 月 【Letter of Task Force to EFSA】 (URLなし) (PHE4)	◇申請者9スクフォース(Bayer Crop Science、UPL Europe Ltd)は、EU2018/605 に沿っ た内分泌かく乱性の試験計 画を提案。								
	2019 年 5 月 【Office Journal】 https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32019R0707&from= EN (PHE5)	◇申請者の管理の及ばない理由で審査が遅れているため更新の決定がなされる前に失効する可能性が高い。そのため、承認期間を延長する必要がある。◇承認延長日:2020年7月31日								

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求さ	れた試験項目及び	③ ②の提出の	<b>有無、再評価結果</b>	④ 再	評価評価書	<ol> <li>5 規制内容/その他</li> </ol>	
機関			その内容	に関する文章						
	2018年6月 [Office Journal] <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32018R0917&amp;from=</u> <u>EN</u> (PHE6)	◇申請者の管理の及ばない理由で審査が遅れているため、更新の決定がなされる前に失効する可能性が高い。そのため、承認期間を延長する必要がある。 ◇承認延長日:2019年7月31日			2017年12月 【Peer Review Report】 (URLなし) (PHE7)	<ul> <li>◇ 再評価報告書(RAR)</li> <li>(PHE8)に対するコメント</li> <li>◇報告表</li> <li>◇ 農薬ビアレビュー会議報告書</li> <li>◇評価表</li> <li>◇追加情報評価に対するコメント</li> <li>◇EFSA CONCLUSION(案)</li> <li>Iに対するコメント</li> </ul>				
EFSA	2017年5月 [Office Journa]] https://eur lex.europa.eu/legal- content/EN/TXT/PDE/?uri =CELEX:32017R0841&from= EN (PHE9)	◇申請者の管理の及ばな い理由により審査が遅れて いるため、更新の決定がな される前に失効する可能性 がある。従って、承認期間を 延長する必要がある。 ◇延長期限:2018年7月31日	2014年7月 【REASOND OPINION】 https://efsa.onlinelibrary.wil ey.com/doi/epdf/10.2903/j. efsa.2014.3807 (PHE11)	既存 MRL のレビュー ◇ADI:0.03 mg/kg/日 ◇植物代謝 ・テンサイとイテュ゙の葉面散布試 酸で、評価対象は親化合物 のみと定義したが、根菜類 と塊茎野類については十分 な情報が得られていない。 従って、代表的な作物での。 追加代謝研究が必要。 ・主要作物の代謝と貯蔵安 定性データギャッフを考慮する と、MRL とリスク評価値は暫 定的なもの。 ・輪作作物における代謝試 酸からは残留物定義を設定 できない。 ◇家畜代謝 ヤギ、コフトリの代謝試験結果 から、残留物を定義できな いため反芻動物での代謝試 酸が必要。 ◇ARD 設定は必要ない。 ◇上記の評価に基づき、本 有効成分の AnnexIVへの 掲載を推奨しない。暫定的 な MRL または既存の EU の MRL は、以下のデータによって確認される必要があ る。 ・根菜類及び塊茎類での植 物代謝試験 ・葉菜類の植物代謝試験 ・葉菜類の植物代謝試験 ・葉菜類の植物代謝試験 ・葉菜類の植物代謝試験 ・葉菜類の植物代謝試験 ・葉菜類の植物代謝試験 ・花り洗留和定義類での植 物代謝試験 ・方ンサイ(根)での作物残留 試験 3 試験(欧州南部の屋 外 GAP) ・論作作物での追加家畜代 謝試験			2017年12月 [RAR] Renewal Assessment Report (URLなし) (PHE8)	再評価評価書(ドラフト) ◇RMS: アンシマーウ ◇2017 年 9 月に改訂。 専門家会議での議論を経 て、2017 年 10 月に再改 訂。 ◇発がん性:カテゴリー 2 の 生殖毒性:カテゴリー 2 ◇内分泌かく乱作用の可能 性を除外することはできな かった。	2015年11月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32015R2075&from= EN (PHE10)	◇MRL の改訂

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求さ	れた試験項目及び	③ ②の提出の	の有無、再評価結果	④ ₮	評価評価書	⑤ 規制	内容/その他
機関			その内容	に関する文章				1		
EFSA	2014年7月 【Application Dossier】 (URLなし) (PHE12)	◇ Bayer CropScience AG (ハ <sup>*</sup> イル社)と UPL Europe Ltd.のタスクォースによる申請 用ドシェ提出。 ◇ RMS フィンラント <sup>*</sup> 、共同 RMS テ <sup>*</sup> ンマーク			2014年6月 【REASOND OPINION】 https://efsa.onlinelibrary.wil ey.com/doi/pdf/10.2903/j.e fsa.2014.3738 (PHE13)	◇毒性試験結果は十分で、 ・ADI:0.03mg/kg/日 ・ARD:不要 ◇b93代謝試験は実施され ていないため、英国はテンサイ での代謝試験を外挿するこ とを提案したが、EFSA は受 入れられないとの見解を示 す。 ◇GAPに従った作物残留試 験結果は十分だが、薬菜類 におけるデータギャップが解決 されないため、MRL 設定提 案はできない。 ◇b93は輪作で表培するこ とがあるため輪作での追加 代謝試験が必要。			2012年9月 [Office Journal] https://eur- lex.europa.eu/LexUriServ/L exUriServ.do?uri=OJ:L:2012; 252:0026:0032:EN:PDF (PHE14)	◇農薬製品上市に関する 有効成分の更新手続きの 実施に必要な規定を定める ガイダンス文書。
						「「「四」日本海大な、大学文。			2011年5月	◇Annex I リスト掲載
									【Office Journal】	・原体純度:970 g/kg
									https://eur- lex.europa.eu/legal-	<ul> <li>・認可日:2005年3月1日</li> <li>・有効期限:2015年2月28</li> </ul>
									content/EN/TXT/PDF/?uri =CELEX:32011R0540&from= EN	・除草剤としての使用
									(PHE15)	
									2008 年 1 月 【Office Journal】	◇MRL 改訂
									https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32008R0149&from= EN	
							2004年2月	レビュー報告書、Annex I 掲載	(PHE16) 2004年4月	◇RMS フィンランドは 2000 年
							【Review report for the active substance】 (URLなし) (PHE18)	のための評価 ◇RMS 74/5/5/12 2000 年 1月5日にDARを提出。 2001年11月13日に32.07 オースからサマリードシェを受領。 ◇短期的には水生生物の 保護に注意を払う必要があ る。 ◇現段階で、追加試験は必 要ないが、いくつかのエンドボ インたでは、追加資料提出が	[Office Journal] https://eurolex.europa.eu/L exUriServ/LexUriServ.do?ur i=O.j1.2004:120:0026:0029: EN:PDF (PHE17)	1月5日にDARを提出。 2001年11月13日にフェンメ ディファムに関するタスクフォース からサマリードシェを受領。
								いては、量加減行使用が 必要となる場合がある。 ・原体不純物を確認するためのパリテーション試験 ・原体中のトルエンの分析方 法又は環境影響がないこと の証明		

評価	① 事前のやり取りに関する文章	② 追加要求。	された試験項目及び	③ ②の提出の	D有無、再評価結果	④ 再評価評価書	⑤ 規制	内容/その他
機関		その内容に関する文章						
EFSA		2003年10月 [DAR] Addendum 3 (URLなし) (PHE19) 2002年10月 [DAR] Addendum 2 (URLなし)	DAR 補遺 3 ◇追加提出データ(人健康影 響関係) ・代謝物 desmedipham のコリ ンエステラーゼ'に対する影響に ついての理由書 ・テンサイ、飼料用ピートのMRL 設定に関する理由書 DAR 補遺 2 ◇追加提出データ ・物化性 ・分析方法 ・作物残留試験					
	1999 年 12 月 【DAR】 (URLなし) (PHE21)	(PHE20) 2001年11月 [DAR] Addendum 1 (URLなし) (PHE22)	DAR 補遺 1 今追加提出データ(人健康影 響関係) ・ラット慢性毒性試験、発がん 性試験の病理学的評価 ・皮膚透過性試験 ・比およびラットの表皮からの 吸収 ・作業者暴露評価 ・農業従事者のリェントリー暴 露評価					

### 略号(欧州 EFSA)

AIR	Annex I Renewal	EFS	European Food Safety Authority	JRC	Joint Research Centre	RASFF	Rapid Alert System for Food and Feed
BfR	Bundesinstitut fur Risikobewertung	EMS	Evaluating Member State	PAFF	Plants, Animals, Food and Feed	RMS	Rapporteur Member State
DAR	Draft Assessment Report	GAF	Good Agricultural Practice	PHI	Pre Harvest Interval	TTC	Threshold of Toxicological Concern
ECHA	European Chemicals Agency	JMF	Joint FAO/WHO Meeting on Pesticide Residues	RAR	Renewal Assessment Report		

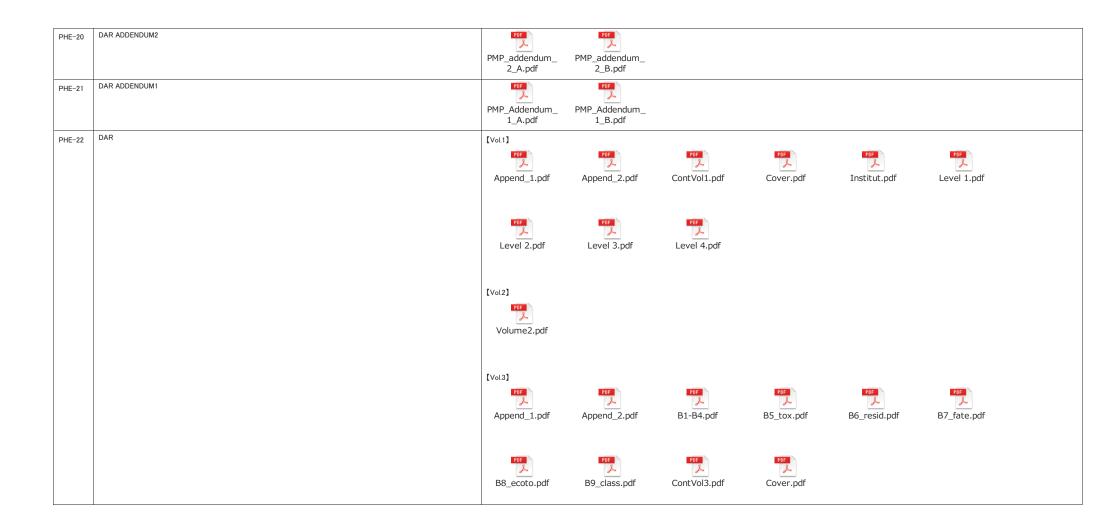
## 引用 URL とその PDF ファイル(又はワードファイル、エクセルファイル)

PHE-1	https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2021.6482	j.efsa.2021.6482. pdf
PHE-2	https://www.efsa.europa.eu/en/efsajournal/pub/6436	j.efsa.2021.6436. pdf
PHE-3	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0869&from=EN	CELEX_32020R08 69_EN_TXT.pdf

PHE-4	PDF 2		
	etter of Task ce Phenmedipl		
PHE-5 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R0707&from=EN	PDF		
	.EX_32019R07 _EN_TXT.pdf		
PHE-6 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0917&from=EN	PDF		
	.EX_32018R09 _EN_TXT.pdf		
PHE-7	PDF		
	EFSA Peer iew Report_2(		
PHE-8	<b>本</b> ]		
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	PDF	PDF	
	enmedipham_ Phenmedipham_ Phe	enmedipham_	
	AR_09_VOL_3_ dRAR_10_VOL_3_ dRA	AR_11_VOL_3_	
	钊】		
	POF 2	POF スレート	POF 2-
		enmedipham_ Phenmedipham_ Phenmedipham_ AR_16_VOL_3_ dRAR_17_VOL_3_ dRAR_18_VOL_3_	Phenmedipham_ dRAR_19_VOL_3_
	PDF		
	enmedipham_		
	AR_List of stud		
PHE-9 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0841&from=EN			
	.EX_32017R08 _EN_TXT.pdf		
PHE-10 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2075&from=EN	PDF 		
	.EX_32015R20 _EN_TXT.pdf		
PHE-11 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3807	PDF		
	sa.2014.3807. pdf		

	【压化】					
PHE-12	【原体】 D_P-D_1_01_M-5 03004_000320628	D_P-D_2_01_M-5 03007_000320628	D_P-Document_ A_01_M-502989_(	D_P-Document_ B_01_M-502992_(	D_P-Document_F _01_M-503018_0(	D_P-E_1_01_M-5 03011_000320625
	D_P-E_2_01_M-5 03007_000320625	D_P-LCA_Section _2_01_M-504161_	D_P-LCA_Section _3_01_M-503667_	D_P-LCA_Section _4_01_M-504981_	D_P-LCA_Section _5_01_M-504163_	D_P-LCA_Section _6_01_M-504572_
	D_P-LCA_Section _7_01_M-504164_	D_P-LCA_Section _8_01_M-504404_	D_P-LCA_Section _10_01_M-50367(		D_P-MCA_Sectio n_2_01_M-503024	D_P-MCA_Sectio n_3_01_M-503033
	D_P-MCA_Sectio n_4_01_M-50515:	D_P-MCA_Sectio n_5_01_M-50303;	D_P-MCA_Sectio n_6_01_M-50454;	D_P-MCA_Sectio n_7_01_M-50304:	D_P-MCA_Sectio n_8_01_M-50477(	D_P-MCA_Sectio n_9_01_M-504771
	D_P-MCA_Sectio n_10_01_M-50304				D_P-N_5_01_M-5 03151_00032063(	
	【製剤】 D_P-D_1_01_M-5 03004_000320655		D_P-Document_ C_01_M-475768_(	D_P-Document_ C_02_M-475747_(	D_P-Document_ C_03_M-475742_(	D_P-Document_ C_04_M-475736_(
	D_P-Document_ C_05_M-475781_(	D_P-Document_ C_06_M-475891_(	D_P-Document_ C_07_M-475805_(	D_P-Document_ C_08_M-483300_(	D_P-Document_ C_09_M-475820_(	D_P-Document_ C_10_M-475817_(
	D_P-Document_ C_11_M-475788_(	D_P-Document_ C_12_M-475894_(	D_P-Document_ C_13_M-475897_(	D_P-Document_ C_14_M-475902_(	D_P-Document_ C_15_M-475901_(	D_P-Document_ C_15_M-475901_(
	D_P-Document_ C_16_M-475900_(	D_P-Document_ C_17_M-475899_(	D_P-Document_ C_18_M-475839_(	D_P-Document_ C_19_M-502996_(	D_P-Document_I _01_M-503168_0(	D_P-LCP_Section _2_01_M-503683_

		PDF 2	PDF	PDF	Por	PDF 2	ere J
		D_P-LCP_Section _4_01_M-503690_	D_P-LCP_Section _5_01_M-504407_	D_P-LCP_Section _7_01_M-504168_	D_P-LCP_Section _9_01_M-504408_	D_P-LCP_Section _10_01_M-504561	D_P-LCP_Section _12_01_M-503702
		D_P-MCP_Sectio n_1_01_M-503049	D_P-MCP_Sectio n_2_01_M-50305!	D_P-MCP_Sectio n_3_01_M-503066	D_P-MCP_Sectio n_4_01_M-503072	D_P-MCP_Sectio n_5_01_M-50415	D_P-MCP_Sectio n_6_01_M-50307 <sup>2</sup>
		D_P-MCP_Sectio n_7_01_M-50307ť	D_P-MCP_Sectio n_8_01_M-503084	D_P-MCP_Sectio n_9_01_M-50477:	D_P-MCP_Sectio n_10_01_M-5045;	D_P-MCP_Sectio n_11_01_M-5030&	D_P-MCP_Sectio n_12_01_M-50314
		D_P-OCP_01_M-5 05140_000320656					
PHE-13	https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2014.3738	j.efsa.2014.3738 (1).pdf					
PHE-14	https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:252:0026:0032:EN:PDF	CELEX_32012R08 44_EN_TXT.pdf					
PHE-15	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0540&from=EN	CELEX_32011R05 40_EN_TXT.pdf					
PHE-16	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0149&from=EN	CELEX_32008R01 49_EN_TXT.pdf					
PHE-17	https://eurolex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:120:0026:0029:EN:PDF	LexUriServ.pdf					
PHE-18		EC Review Report Phenmedij					
PHE-19	DAR ADDENDUM3	PMP_addendum_ 3_A.pdf	PMP_addendum_ 3_B.pdf				

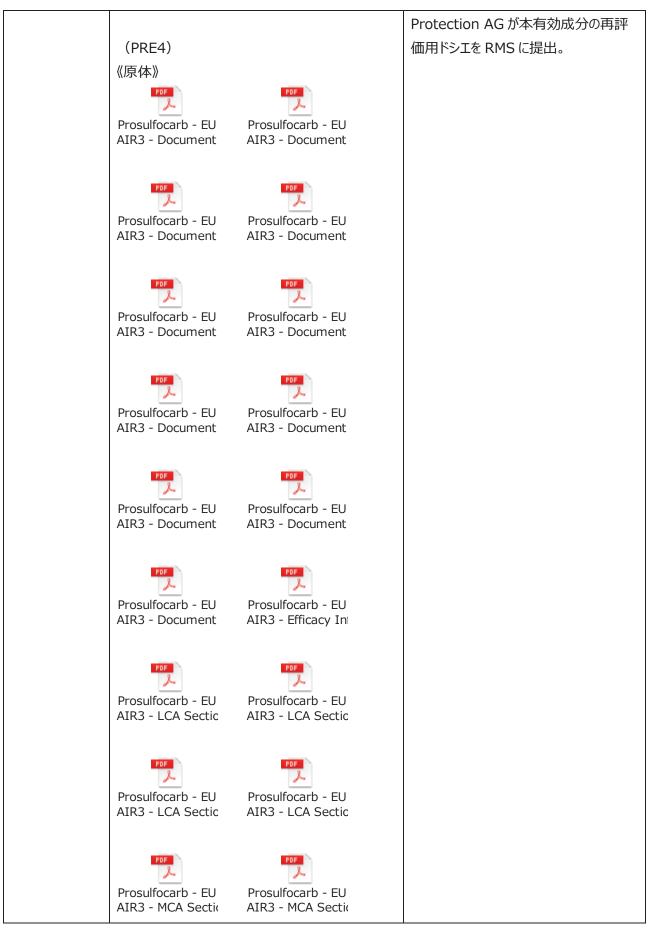


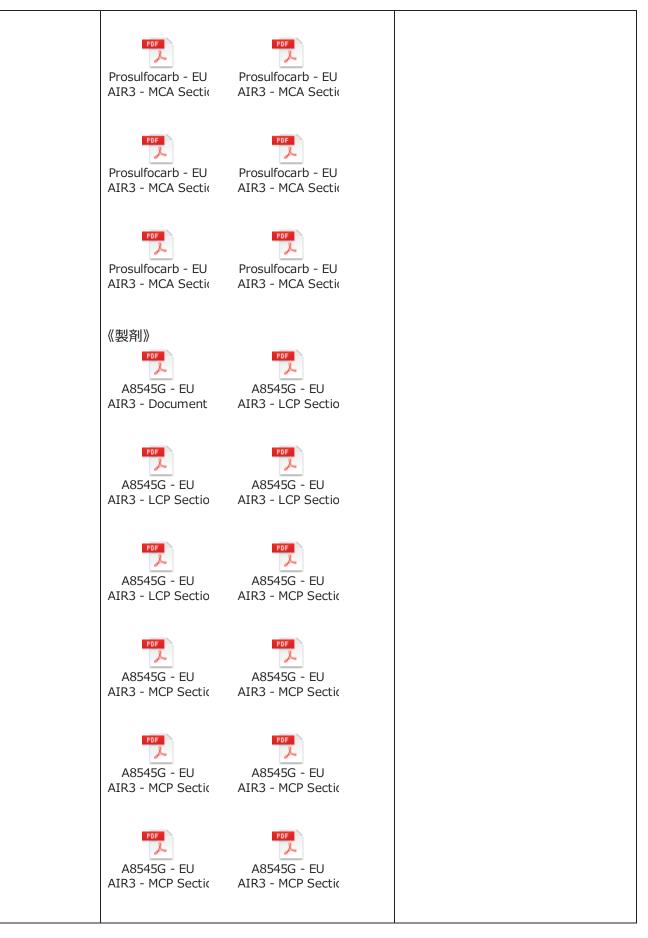
9.1.3. プロスルフォカルブ

【人の健康影響評価についての要約】

- ▶ 米国
- ✓ PubChem データベース(別紙1)
   <u>Prosulfocarb | C14H21NOS PubChem (nih.gov)</u>
- ✓ 米国登録なし。FDA (Food and Drug Administration: 米国食品医薬品局)は、本化合物の食品、加 工品のモニターを実施しているが検出例なし。
- ▶ 欧州
- ✓ EUデータベース(別紙1)
   <a href="https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as\_id=711">https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as\_id=711</a>
- ✓ ECHA データベース(別紙1)
   https://echa.europa.eu/substance-information/-/substanceinfo/100.162.812

年月	評価	食品経由での人健康影響エンドポイント /追加要求
2020年10月	Office Journal	◇有効成分の認可期間延長
		・有効期限:2021 年 10 月 31 日
	(PRE1)	
	PDF 2	
	CELEX_32020R15 11_EN_TXT.pdf	
2019年9月	Office Journal	◇有効成分の認可期間延長
		・有効期限:2020 年 10 月 31 日
	(PRE2)	
	CELEX_32019R15 89_EN_TXT.pdf	
2018年9月	Office Journal	◇有効成分の認可期間延長
		・有効期限:2019 年 10 月 31 日
	(PRE3)	
	POF 2-	
	CELEX_32018R12 62_EN_TXT.pdf	
2016年4月	DOSSIER	◇タスクフォースの Syngenta Crop





	A8545G - EU AR3 - MCP Sectic - Document OCP	
	《公表用》 Sanitized - A8545G - EU AIR: A8545G - EU AIR:	
	Sanitized - Sanitized - A8545G - EU AIR: A8545G - EU AIR:	
	Sanitized - Sanitized - A8545G - EU AIR: Prosulfocarb - EU	
	Sanitized - Prosulfocarb - EU Sanitized - Prosulfocarb - EU	
	Sanitized - Sanitized - Prosulfocarb - EU Prosulfocarb - EU	
	Sanitized - Prosulfocarb - EU Sanitized - Prosulfocarb - EU	
	Sanitized - Sanitized - Prosulfocarb - EU Prosulfocarb - EU	
	Sanitized - Prosulfocarb - EU	
2015年11月	APPLICATION FOR APPROVAL RENEWAL	◇タスクフォースと Globachem を代表し て Syngenta Crop Protection AG

	(PRE5)	が本有効成分の再評価申請を提出。
2013年8月	Office Journal (PRE6) CELEX_32013R07 77_EN_TXT.pdf	◇MRL 設定
2013年3月	REASOND OPINION (PRE7) j.efsa.2013.3133. pdf	<ul> <li>◇EMS (Evaluating Member State) ベルギーは既存の MRL を定量 限界 0.05mg/kg から 1.5mg/kg に 引き上げることを提案。評価報告書を作 成し、提出。</li> <li>◇EFSA は本有効成分の植物代謝につ いては十分に検討されていないため、フェ ンネルの MRL を設定するにはデータが不 十分であり、加工食品での残留に関する データギャップが確認された。従って、</li> <li>EFSA はフェンネルにおける MRL の修正 を提案しない。 (P.15~P.16)</li> </ul>
2011年8月	REASOND OPINION (PRE9) j.efsa.2011.2346. pdf	<ul> <li>◇ADI: 0.005mg/kg/日、ARfD:</li> <li>0.1mg/kg。評価対象化合物はプロス ルフォカルブ。</li> <li>◇植物代謝試験を根菜類/塊茎類、穀 類、豆類及び菜種類で実施。親化合物</li> <li>に関連する代謝物は検出されず、親化</li> <li>合物が残留。特にセリ科作物では、早い</li> <li>生育段階でのTRR(Total</li> <li>Radioactive Residue)の特徴付けと</li> <li>定量化の情報が必要。評価対象化合</li> <li>物をプロスルフォカルブと暫定的に定義。</li> <li>◇以下の追加データが必要。</li> <li>・栽培期間が短いセリ科植物等での植物</li> <li>代謝試験</li> <li>・加工食品(特に栽培期間が短いニンジ</li> </ul>

2011年5月	Office Journal (PRE10) CELEX_32011R05 40_EN_TXT.pdf	<ul> <li>ン)での残留物の加水分解試験</li> <li>・イチゴの欧州北部 GAP (Good Agricultural Practice)での作物残 留 8 試験</li> <li>・生ハーブの欧州南部 GAP での作物残 留 3 試験及び欧州北部 GAP での作物残 留 3 試験及び欧州北部 GAP での作物 残留 4 試験</li> <li>・種子香辛料、果実、ベリー類の分析法</li> <li>◇各国の認可に影響を与える可能性の ある以下のデータギャップを確認。</li> <li>・種子香辛料の欧州南部 GAP での作 物残留 4 試験。</li> <li>・牧草の欧州北部 GAP での作物残留 4 試験。</li> <li>・上記のデータギャップが対処されない場 合、加盟国は国レベルで関連する認可を 撤回または修正することが推奨される。</li> <li>(P.22~P.23)</li> <li>登録即新</li> <li>◇登録認可期間:2008 年 11 月 1 日~2018 年 10 月 31 日</li> <li>◇適用:除草剤</li> <li>◇加盟国は以下の点に特に注意を払う こと。</li> <li>・作業者への安全性及び適切な個人用 保護具の適用の規定</li> <li>・水生生物の保護および認可条件に適 切な緩衝地帯などのリスク軽減策が含ま れていること</li> <li>・非標的植物の保護及び必要に応じて 認可条件に圃場内に無散布緩衝地帯 などのリスク緩和策が含まれていること</li> </ul>
2010年7日		(P.65)
2010年7月	Office Journal	MRL 更新 ◇ニンジンとセレリアックの MRL 更新
	(PRE11)	◇_ノンノンセレリアックの MRL 更新
2009年11月	REASOND OPINION	ニンジン及びセレリアックの既存 MRL の改 訂

	(PRE12)	・ニンジン: 1.5mg/kg
	PDF J	・セレリアック:01mg/kg
	j.efsa.2009.1373.	の暫定 MRL 設定
	pdf	◇根菜類/塊茎類、穀類、豆類/菜種類
		での植物代謝試験において、代謝経路
		は類似し、親化合物及び関連代謝物も
		検出されなかったため、評価対象は親化
		合物と結論付けた。
		◇代表的な代謝試験では、PHI の短い
		生育初期のニンジンにおいて残留が大き
		いことを考慮すると、より早い生育段階で
		の TRR の特徴と定量化について情報が
		必要。
		◇後作物あるいは家畜に関する残留につ
		いては、問題ない。
		◇加工食品中の残留試験については必
		要ない。
		◇追加試験
		・生育期間の短い根菜類における植物
		代謝試験
		・パン焼き、醸造、煮沸、低温殺菌、滅
		第二日の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本の日本
		(P.14~P.15)
2008年7月	Office Journal	(F.14 °F.15) MRL 更新
2008年7月		
	(PRE14)	
	2	
	CELEX_32008R08 39_EN_TXT.pdf	
2008年1月	Office Journal	MRL 更新
	(PRE15)	
	×	
	CELEX_32008R01	
	49_EN_TXT.pdf	
2007年12月	Office Journal	DAR レビュー結果
		◇2005年4月20日にRMS
	(PRE16)	(Rapporteur Member State)スウ

	2	ェーデンが DAR (Draft Assessment
	CELEX_32007L00	Report)提出。2007年10月9日
	76_EN_TXT.pdf	に欧州委員会のレビュー報告書の形式で
		最終的にまとめられた。
		·発効日:2008年11月1日
		·有効期限:2018年10月31日
		・最低純度:970g/kg
		・適用:除草剤
		◇加盟国は特に以下の点に注意しなけ
		ればならない。
		・作業者の安全性及び適切な個人用保
		護具の適用
		・水生生物の保護。認可条件に緩衝地
		帯などのリスク軽減策が含まれていること
		・非標的植物の保護:認可条件に、必
		要に応じて圃場内にバッファーゾーン等の
		リスク軽減策が含まれていること
		(P.4)
2007年10月	EC Review Report	EC レビュー報告書
		◇再評価結果
	(PRE17)	・ADI:0.005mg/kg/日
	PDF 8	•ARfD : 0.1mg/kg
	ProsulfocarbRevi	・AOEL : 0.007mg/kg/日
	ew.pdf	・申請者 : Syngenta Crop
		Protection AG
		・RMS:スウェーデン
		◇加盟国は以下に特に注意を払うこと。
		・作業者の安全を確保するため適切な保
		護手段を含める
		・水生生物の保護。必要に応じてバッファ
		ーゾーンなどのリスク軽減策を適用すること
		・非標的植物の保護。圃場でのバッファー
		ゾーンの様なリスク緩和策を適用すること
		・現段階で追加要求データはない。
		(P.3~P.4)
2007年7月	Scientific Report	◇本有効成分は再評価プログラムの第3
		段階パートAの79物質に含まれる。
	(PRE18)	◇RMSスウェーデンは、DARを2005年4
	1	

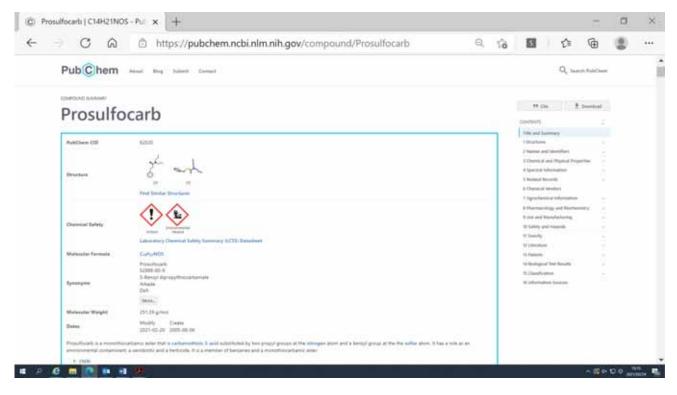
<b></b>		1
	PDE	月20日にEFSAへ提出。
	j.efsa.2007.111r.	◇植物由来の食品・飼料中のプロスルフ
	pdf	オカルブの残留量及び土壌・水・大気中
		の残留量はGC-MSDにより測定可能。
		◇急性経口毒性は中程度、急性経皮・
		吸入毒性は低い。ウサギでは皮膚や眼で
		わずかな刺激が見られ、マウスでは皮膚
		感作性が見られた。短期毒性試験では、
		ラットとイヌで肝臓と腎臓が標的臓器とさ
		れた。また、イヌでは溶血性貧血が観察さ
		れ、骨髄や脾臓に関連する病理組織学
		的所見が見られた。遺伝毒性はない。長
		期毒性試験では、主に食餌量の減少と
		体重の減少が認められた。また、生殖器
		官への影響は認められなかった。催奇形
		性を示すいくつかの証拠があったが、基準
		値に対して十分な安全マージンが設定さ
		れた。ADIは0.005mg/kg/日、AOEL
		は0.007mg/kg/日、ARfDは
		0.1mg/kg。
		◇小麦、ジャガイモにおいて、速やかに代
		謝され、親化合物に関連する代謝物は
		確認されなかった。評価対象は親化合物
		のみ。
		◇小麦とジャガイモのMRLはLOQで設
		定。輪作作物、加工食品、動物由来食
		品には残留しない。
		(P.30~P.32)
2007年7月	Final addendum to DAR	◇RMS スウェーデンから申請者、EFSA、
		EU 加盟国に提供された評価の概要。
	(PRE19)	
	PDF A	
	Final addendum	
	to the Draft Asse	
2007年4月	[PEER REVIEW REPORT	◇DAR に対するコメント
		◇報告書のリスト
	(PRE20)	◇PRAPeR (Pesticide Risk
		Assessment Peer Review Unit)

	PDF		専門家会議報告書
	EFSA Peer Review Report on		◇評価結果一覧表
2006年12月	DAR		◇Annex I 掲載用のリスク評価に用いられる情報、試験、研究のリスト
	(PRE21)		
2006年4月	DAR (PRE22)		RMS スウェーデンが提出した既存有効成 分の DAR(レビュープログラム第 3 段階 (パート A))
	Prosulfocarb_DA R_01_Vol 1_public	Prosulfocarb_DA R_02_Vol 2_public	2005年4月付け、2006年3月付け 改訂版。
	Prosulfocarb_DA R_03_Vol 3_B1-B!	Prosulfocarb_DA R_04_Vol 3_B6_p	
	Prosulfocarb_DA R_05_Vol 3_B6_p;	Prosulfocarb_DA R_06_Vol 3_B7_p	
	Prosulfocarb_DA R_07_Vol 3_B8_pi	Prosulfocarb_DA R_08_Vol 3_B9_p	

別紙1.データベース

## ▶ 米国

PubChem データベース



## ▶ 欧州

## EU データベース

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Prosulfo	carb			Chil legistation	aperintando (2) Inc. Una activita Inc. Una activita		Approved			
Prosulfo Heat courts Betwee under Authenhante Maximum Ru Cesenfortier	icarb Is Is Ing Ing Ing	preparing Direction,	INVITABLE (* )		populative la generative constraint generative constraint investment		Approved			
Prosulfo Heat courts Bates under Aufternatio Maximum Ra	icarb Is Is Ing Ing Ing	preparations Disactions.)	honartec <sup>(2)</sup>	Oid legislation			Approved			
Prosulfo Heat courts Betwee under Authenhante Maximum Ru Cesenfortier	icarb Iss Ing Internation Internation	Inspecting Direction I Inspectation Date of approval	BANAMEC <sup>12</sup> ) Ban dala benerita Ban dala benerita Provinsion	Did legislation Explorition of approved	en-manager		Approved			

# ECHA データベース

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	IC Substance Infocard See a problem or here freed				
	Prosulfacarb	-			
	Total same ) the motion (	10			
	Industance identity         It abard classification & labelling         Properties of concern           KC / List no.: 534-872-9         Image: state classification in provided by compares to industry of data solved in industry of data solved in industry of data solved in industry of activity of data solved in industry of				
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	Key datasets				
	And traffic Middle and Color State State State				

## 9.1.3. 米国 EPA おけるプロスルフォカルブの再評価概要と関係資料

評価	① 事前のやり取り	に関する文章	<ol> <li>追加要求され</li> </ol>	れた試験項目及び	③ ②の提出の4	<b>「無、再評価結果</b>	④ 再評	価評価書	⑤ 規制内	9容/その他
機関			その内容	に関する文章						
EPA									【PubChem データベース】	◇米国登録なし
									Prosulfocarb C14H21NOS	◇FDA は、本化合物の食
									– PubChem (nih.gov)	品、加工品のモニターを実
										施しているが検出例なし

## 9.1.3. 欧州 EFSA におけるプロスルフォカルブの再評価概要と関係資料

5	① 事前のやり取りに関する文章	② 追加要求された試験項目及び その内容に関する文章	<ol> <li>②の提出の有無、再評価結果</li> </ol>	④ 再評価評価書	⑤ 規制内	9容/その他
A					2021 年 3 月	EU データベース
1					[EU database]	•有効期限:2021 年 10 月
						31日.
					https://ec.europa.eu/food/	•ADI 0.005 mg/kg/日
					plant/pesticides/eu-	•ARfD 0.1mg/kg
					pesticides-	・AOEL 0.007 mg/kg/日
					database/active-	・評価対象:Prosulfocarb
					substances/?event=as.detai	
					ls&as_id=711	
					(URL のみ)	
					2021年3月	ECHA データベース
					[ECHA database]	
					https://echa.europa.eu/sub	
					stance-information/-	
					/substanceinfo/100.162.812	
					(URL のみ)	
					2020年10月	◇有効成分の認可期間
					[Office Journal]	長:2021 年 10 月 31 日ま
					https://eur-	
					lex.europa.eu/lega⊢	
					content/EN/TXT/PDF/?uri	
					=CELEX:32020R1511&from=	
					EN	
					(PRE1)	
					2019年9月	◇有効成分の認可期間
					[Office Journal]	長:2020年10月31日ま
					https://eur-	
					lex.europa.eu/legal-	
					content/EN/TXT/PDF/?uri	
					=CELEX:32019R1589&from=	
					EN	
					(PRE2)	
2016 年					2018年9月	◇有効成分の認可期間
[DOSSIE	ER】 Crop Protection AG が本有 効成分の再評価用ドシェを				[Office Journal]	長:2019 年 10 月 31 日ま
(URLな					https://eur-	
(PRE4)					lex.europa.eu/legal-	
					content/EN/TXT/PDF/?uri	
					=CELEX:32018R1262&from=	
					EN	
					(PRE3)	

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	③ ②の提出の	の有無、再評価結果	④ 再評価評価書	⑤ 規制	別内容/その他
機関		その内容に関する文章					
EFSA	2015 年 11 月 【APPLICATION FOR APPROVAL RENEWAL】 (URL なし) (PRE5)		2013年3月 【REASOND OPINION】 <u>https://efsa.onlinelibrary.wil</u> <u>ey.com/doi/epdf/10.2903/j.</u> <u>efsa.2013.3133</u> ( <b>PRE7</b> )	◆EMS ベルギーは評価報告 書を提出し、既存 MRL の定 量限界 0.05mg/kg から 1.5mg/kg に引き上げること を提案。 ◇植物代謝について十分に 検討されていなく、フェンネルの MRL を設定するにはデータ が不十分である。加工食品 での残留に関するデータギャッ ブが確認された。従って、 EFSA はフェンネルにおける本 有効成分の MRL の修正を 提案しない。		2013年8月 [Office Journal] <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32013R0777</u> ( <b>PRE6</b> )	◇MRL 設定
						2013 年 8 月 【Office Journal】 <u>https://eur-</u> <u>lexcuropa.eu/LexUriServ/L</u> <u>exUriServ.do?uri=OJ:L:2012:</u> <u>252:0026:0032:EN:PDF</u> (PRE-8)	◇農薬製品上市に関する 有効成分の更新手続きの 実施に必要な規定を定める がイダンス文書
			2011 # 8 月 [REASOND OPINION] https://doi.org/10.2903/j.ef sa.2011.2346 (PRE9)	◇ADI:0.005mg/kg/日、 ARD:0.1mg/kg, 評価対象 化合物はブロスルフカルブ。 ◇植物代謝試験を根菜類/ 境茎類, 穀類, 豆類, 菜種 類で実施。親化合物に関連 する代謝物は検出されなか った。切利作物の早い生育 段階でのTRRの特徴付け と定量性の情報が必要。評 価対象化合物をブロスルフオカ ルブと暫定的に定義。 ◇追加要求データ ・裁培期間が短いり利植物 等での植物代謝試験 ・加工食品(特にニンジン)での残留物の加水分解試験 ・欧州北部 GAPでのハーブ・の作物残留 3 試験 ・欧州北部 GAPでのハーブ・の作物残留 3 試験 ・欧州南部 GAP での水う数 州北部 GAP での水う数 《データギャップを確認 ・欧州南部 GAP での種子 香辛料の作物残留 4 試験。 ・欧州市部 GAP での牧車 の残留 4 試験 ・欧州南部 GAP での教車		2011年5月 【Office Journal】 <u>https://eur- lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32011R0540&amp;from=</u> <u>EN</u> (PRE10)	登録更新

評価	① 事前のやり取りに関する文章	<ol> <li>追加要求された試験項目及び</li> </ol>	③ ②の提出	の有無、再評価結果	④ 再評価評価書	⑤ 規制	内容/その他
機関		その内容に関する文章					
EFSA			2009年11月 [REASOND OPINION] https://efsa.onlinelibrary.wil ey.com/doi/pdf/10.2903/j.e fsa.2009.1373 (PRE12)	ニシジン及びセレリアックの既存 MRLの改訂 -ニンジン:1.5mg/kg ・セレリアック:01mg/kg の暫定 MRL設定 令根菜類/塊茎類、穀類、 豆類/菜種類での植物代謝 試験において、代謝経路は 類似し、親化合物及び関連 代謝物も検出されなかった ため、評価対象は親化合物 と結論付けた。 令代表的な代謝試験では、 PHIの短い生育初期のニンジ ンにおいて残留が大きいこと を考慮すると、より早い生育 段階でのTRRの特徴と定量 化について情報が必要。 令後作物あるいは家畜に関 する残留については、問題 ない。 今加工食品中の残留試験 については必要ない。 令追加試験 ・生育期間の短い根菜類に おける植物代謝試験 ・小焼き、醸造、煮沸、低 温殺菌、減菌のモデル条件 下での安定性試験		2010年7月 [Office Journal] https://eur- lex.europa.eu/LexUriServ/L exUriServ.do?uri=O.J:L:2010; 220:0001:0056:EN:PDF (PRE11)	MRL 更新 ◇ニンジンとセレリアックの MRLを 更新
						2008年12月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32008R1272&from= en (PRE13) 2008年7月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri	<ul> <li>ラヘル及び包装容器に関する分類</li> <li>・急性毒性:H302</li> <li>・皮膚感作性:H317</li> <li>・水生生物慢性影響:H411</li> <li>・GHS07, GHS09, 警告</li> <li>MRL 更新</li> </ul>
						content/EN/TXT/PDF/?uri =CELEX:32008R0839&from= EN (PRE14)	

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	③ ②の提出の	の有無、再評価結果	④ 再評価評価書	⑤ 規制	制内容/その他
機関		その内容に関する文章					
EFSA						2008 年 1 月 【Office Journal】	MRL 更新
						https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32008R0149&from=	
						EN (PRE15)	
			2007 年 10 月 【EC Review Report】	EC レビュー報告書 ◇再評価結果 ・ADI:0.005mg/kg/日		2007 年 12 月 【Office Journal】	DAR レビュー結果 ◇2005 年 4 月 20 日に RMS スウェーデンが DAR 提出。2007
			http://search.fytoweb.be/N L/doc/ProsulfocarbReview. pdf	・ARfD:0.1mg/kg ・AOEL:0.007mg/kg/日 ・申請者:シンジェンタ		<u>https://eur-</u> <u>lex.europa.eu/legal-</u> content/EN/TXT/PDF/?uri	年 10 月 9 日に欧州委員会 のレビュー報告書の形式で最 終的にまとめられた。
			(PRE17)	・RMS:スウェーテ <sup>・</sup> ン ◇加盟国は以下に特に注 意を払うこと。		=CELEX:32007L0076&from= EN (PRE16)	・発効日:2008 年 11 月 1 日 ・有効期限:2018 年 10 月 31 日
				・作業者の安全を確保する ため適切な保護手段を含め		(FRE10)	・最低純度:970g/kg ・適用:除草剤
				る ・水生生物の保護。必要に 応じてパッファーゾーンなどのリス ク軽減策を適用すること			◇加盟国は特に以下の点 に注意しなければならな い。 ・作業者の安全性及び適切
				・非標的植物の保護。圃場 でのバッファーゾーンの様なリスク			な個人用保護具の適用 ・水生生物の保護。認可条
				緩和策を適用すること ・現段階で追加要求データは ない。			件に緩衝地帯などのリスク軽 減策が含まれていること ・非標的植物の保護:認可
							条件に、必要に応じて圃場 内にバッファーゾーン等のリスク軽 減策が含まれていること

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	③ ②の提出(	の有無、再評価結果	④ 再	評価評価書	⑤ 規制	内容/その他
機関		その内容に関する文章						
EFSA			2007年7月 [Scientific Report] https://efsa.onlinelibrary.wil ey.com/doi/pdf/10.2903/j.e fsa.2007.111r (PRE18)	◇本有効成分は再評価プロ グラムの第3段階パートAの79 物質に含まれる。 ◇RMSスウェーデンは、DARを 2005年4月20日にEFSAへ 提出。 ◇急性経口毒性は中程度、 急性経皮・吸入毒性は低 い。ウサギでは皮膚や眼でわ ずかな刺激が見られ、マウス では皮膚感作性が見られ た。短期毒性試験では、ラウ とイズで肝臓と腎臓が標的臓 器とされた。イスでは溶血性 質血が複聚され、骨髄や脾 臓に関連する病理組織学 的所見が見られた。遺伝毒 性はない。長期毒性試験で は、主に食類毒性試験で は、主に食類毒性試験で は、主に食類毒性試験で は、シーに、骨髄や脾 臓に関連する病理組織学 的所見が見られた。遺伝毒 性はない。長期毒性試験で は、シーに食類素性試験で し、一般では溶血が この多いた。こ 素た、生殖器官への影響は認 められなかった。しかし、催 奇形性を示すいくつかの証 拠があり、十分な安全マージ ンが設定された。ADI: 0.005mg/kg/日、ACEL: 0.007mg/kg/日、ACEL: 0.007mg/kg/日、ARFD: 0.1mg/kg。 ◇小麦、ジャガイモにおいて、 代謝は速やかで、親化合物 のみ。 ◇小麦とジャガイモのMRLは LOQで設定。輪作作物、加 工食品、動物由来食品には	2007年7月 【Final addendum to DAR】 (URLなし) (PRE19)	◇RMS スウェーテ`から申請 者、EFSA、EU 加盟国に提 供された評価の概要。		
			2007 年 4 月 【PEER REVIEW REPORT】 PDF のみ ( <b>PRE-20)</b>	残留しない。 ◇DAR に対するコメント ◇報告書のリスト ◇PRAPeR専門家会議報 告書 ◇評価結果一覧表	2006年12月 【DAR】 http://search.fytoweb.be/N L/doc/ProsulfocarbEssenti al.pdf (PRE21)	◇Annex I 掲載用のリスク評 価に用いられる情報、試 験、研究のリスト		
					(PRE21) 2006年4月 [DAR] https://www.efsa.europa.eu /sites/default/files/consult ation/consultation/92.zip (PRE22)	RMS スウェーデンが提供する、 既存有効成分の DAR(レビュ ープログラム第 3 段階(パート A)) 2005 年 4 月付け、2006 年 3 月付け改訂版。		

略号(欧州 EFSA)

AIR	Annex I Renewal	EFSA	European Food Safety Authority	JRC	Joint Research Centre	RASFF	Rapid Alert System for Food and Feed
BfR	Bundesinstitut fur Risikobewertung	EMS	Evaluating Member State	PAFF	Plants, Animals, Food and Feed	RMS	Rapporteur Member State
DAR	Draft Assessment Report	GAP	Good Agricultural Practice	PHI	Pre Harvest Interval	TTC	Threshold of Toxicological Concern
ECHA	European Chemicals Agency	JMPR	Joint FAO/WHO Meeting on Pesticide Residues	RAR	Renewal Assessment Report		

### 引用 URL とその PDF ファイル(又はワードファイル、エクセルファイル)

PRE-1         https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R1511&from=EN	CELEX_32020R15 11_EN_TXT.pdf					
PRE-2 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1589&from=EN	CELEX_32019R15 89_EN_TXT.pdf					
PRE-3         https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX.32018R1262&from=EN	CELEX_32018R12 62_EN_TXT.pdf					
PRE-4	《原体》 Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document
	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Document	Prosulfocarb - EU AIR3 - Efficacy Int
	Prosulfocarb - EU AIR3 - LCA Sectic	Prosulfocarb - EU AIR3 - LCA Sectio	Prosulfocarb - EU AIR3 - LCA Sectic	Prosulfocarb - EU AIR3 - LCA Sectio	Prosulfocarb - EU AIR3 - MCA Sectio	Prosulfocarb - EU AIR3 - MCA Sectio
	Prosulfocarb - EU AIR3 - MCA Section	Prosulfocarb - EU AIR3 - MCA Sectio	Prosulfocarb - EU AIR3 - MCA Sectic	Prosulfocarb - EU AIR3 - MCA Sectio	Prosulfocarb - EU AIR3 - MCA Sectio	Prosulfocarb - EU AIR3 - MCA Sectio
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		A8545G - EU AIR3 - Document	A8545G - EU AIR3 - LCP Sectio	A8545G - EU AIR3 - LCP Sectio	A8545G - EU AIR3 - LCP Sectio	A8545G - EU AIR3 - LCP Sectio	A8545G - EU AIR3 - MCP Sectic
		PDF 2	PDF 2	POF 2	PDF	PDF	POF
		A8545G - EU AIR3 - MCP Sectic	A8545G - EU AIR3 - MCP Sectic	A8545G - EU AIR3 - MCP Sectic	A8545G - EU AIR3 - MCP Sectic	A8545G - EU AIR3 - MCP Sectic	A8545G - EU AIR3 - MCP Sectic
		A8545G - EU AIR3 - MCP Sectic	A8545G- EU AIR3 - Document OCP				
		《公表用》					
		Sanitized - A8545G - EU AIR:	Sanitized - A8545G - EU AIR:	Sanitized - A8545G - EU AIR:	Sanitized - A8545G - EU AIR:	Sanitized - A8545G - EU AIR:	Sanitized - Prosulfocarb - EU
		Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU
		Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU	Sanitized - Prosulfocarb - EU			
PRE-5		Application for approval renewal					
PRE-6	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R0777	CELEX_32013R07 77_EN_TXT.pdf					
PRE-7	https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3133	j.efsa.2013.3133. pdf					
PRE-8	https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:252:0026:0032:EN:PDF	CELEX_32012R08 44_EN_TXT.pdf					
PRE-9	https://doi.org/10.2903/j.efsa.2011.2346	j.efsa.2011.2346. pdf					
PRE-10	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0540&from=EN	CELEX_32011R05 40_EN_TXT.pdf					

PRE-11	https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:220:0001:0056:EN:PDF	CELEX_32010R07 50_EN_TXT.pdf						
PRE-12	https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2009.1373	j.efsa.2009.1373.						
PRE-13	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R1272&from=en	CELEX_32008R12 72_EN_TXT.pdf						
PRE-14	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0839&from=EN	CELEX_32008R08 39_EN_TXT.pdf						
PRE-15	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0149&from=EN	CELEX_32008R01 49_EN_TXT.pdf						
PRE-16	https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32007L0076&from=EN	CELEX_32007L00 76_EN_TXT.pdf						
PRE-17	http://search.fytoweb.be/NL/doc/ProsulfocarbReview.pdf	ProsulfocarbRevi ew.pdf						
PRE-18	https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2007.111r	j.efsa.2007.111r. pdf						
PRE-19		Final addendum to the Draft Asses						
PRE-20		EFSA Peer Review Report on						
PRE-21	http://search.fytoweb.be/NL/doc/ProsulfocarbEssential.pdf	ProsulfocarbEsse ntial.pdf						
PRE-22	https://www.efsa.europa.eu/sites/default/files/consultation/consultation/92.zip		Prosulfocarb_DA R_02_Vol 2_public	Prosulfocarb_DA R_03_Vol 3_B1-B!	Prosulfocarb_DA R_04_Vol 3_B6_pi	Prosulfocarb_DA R_05_Vol 3_B6_p;	Prosulfocarb_DA R_06_Vol 3_B7_pi	

PDF 人	PEF 人
Prosulfocarb_DA	Prosulfocarb_DA
R_07_Vol 3_B8_p	R_08_Vol 3_B9_p

# 9.1.4. ピメトロジン

【人の健康影響評価についての要約】

- ▶ 米国
- ✓ EPA レビューページ(別紙1)

Regulations.gov - Docket Folder Summary

年月	評価資料	食品経由での人健康影響エンドポイント
		・追加要求
2020年7月	Interim Registration Review Decision	◇ヒト健康影響及び生態影響リスク評価
		の結論は、PID(Proposed Interim
	(PYU1)	Registration Review Decision) $\mathcal{E}$
	EPA-HQ-OPP-201 3-0368-0060_cont	ID (Interim Registration Review
		Decision)の間で変更されなかった。ヒ
		ト健康影響に関する追加データ不要
		◇EPA は、PID に対して提出された意見
		を検討した結果、以下のように決定した。
		・ジャガイモ及びペカンの単回最大散布率
		及び年間最大散布率低減を要求しな
		ί, ۱°
		・観葉植物の屋内散布の年間最大散布
		量を、1 エーカーあたり 3.125 ポンドの有
		効成分で維持する。
		・PID に記載されている空中散布を禁止
		しない。
2018年12月	Proposed Interim Registration Review	◇ヒト健康影響リスク評価では、飲料水
	Decision	用途の地下水に含まれるピメトロジン残
		留物による急性、慢性及び発がん性の可
	(PYU2)	能性が示唆される。
		・急性及び慢性のヒト健康へのエンドポイ
	EPA-HQ-OPP-201	ントは、ラット発達神経毒性試験で観察
	3-0368-0036_cont	された脳形態学的変化である。
		・発がん性のエンドポイントイは、マウスのオ
		スにおけるヘパトーマおよび/または肝細胞
		・飲料水中の残留物暴露は、リスク評価
		において主な原因であった。食品への暴
		露だけでは、急性、慢性、または発がん性

*個の合きや フリフ りは 仕じ キンロ・ギー 金切り し
が懸念されるリスクは生じないが、飲料水
を食事暴露の計算に含めると、懸念レベ
ル(LOC=急性母集団調整線量
(aPAD)または慢性母集団調整線量
(cPAD)の100%を超えていた。
◇以下の緩和策を提案する。
・コンテナ栽培の観葉植物を除くすべての
用途について、軟弱土壌で栽培された作
物への使用禁止
・コンテナ栽培の観葉植物(屋外栽培)
について、深度が浅い地下水の場合、水
管理の実施または集水システムから 900
フィート以内での使用禁止。
・ジャガイモ、ペカン及び花卉類への処理
量低減
・職業リスクを軽減するため、空中散布の
禁止。
◇非標的生物に対するリスクを軽減する
ためのラベル変更
◇耐容摂取量の再評価に基づく適用変
更
◇DCI について追加データ要求はない。
ただし、ミツバチに対する潜在的リスクを考
慮し、ポリネーターに関する追加要求を行
う可能性がある。
◇疫学データ
OPP (The Office of Pesticide
program) の IDS(Incident Data
System)及び CDC(Centers for
Disease Control) /NIOSH
(National Institute for
Occupational Safety and Health)
$\mathcal{O}$ SENSOR (Sentinel Event
Notification System for
Occupational Risks ) -Pesticides
データベースを用いて検討。2012年1
月1日から2017年6月7日までの間
に、1 件の軽度の症例 IDS に報告され

		ていた。1998 年から 2013 年の間に SENSOR-Pesticides で 5 件の事例が 確認された。この事例はすべて複数の有 効成分が関与していた。本有効成分につ いて報告された事例の頻度と重症度が低 いことを考えると、現時点での懸念はな い。
2018年12月	Addendum to the Draft Human Health Risk Assessment for Registration Review (PYU3) EPA-HQ-OPP-201 3-0368-0032_cont	<ul> <li>◇懸念されるリスクは、主に飲料水への残留。そのための緩和策を提案。</li> <li>・最大散布量を低減</li> <li>・空中散布を禁止するか、保護マスクの 要件を追加する</li> <li>◇緩和策での急性および慢性の食品経 由(飲料水を含む)暴露評価では、懸 念すべきリスクはない。</li> </ul>
	Update to the Drinking Water Assessment for registration Review of Pymetrozine dated August 24,2017 (PYU4) EPA-HQ-OPP-201 3-0368-0030_cont	◇飲料水経由でのリスク評価(2017 年8月)の更新
2018年11月	Syngenta & EPA Registration Review Risk Management Discussion (PYU5) EPA-HQ-OPP-201 3-0368-0035_cont	◇シンジェンタ社は、保護マスクに関する 追加記載、散布ドリフトに関する記載の 標準し、軟弱土壌に対する散布量低減 と散布制限に関する議論に合意
	Response to Public Comments on the Drinking Water Assessment and Preliminary Ecological Risk Assessment	◇飲料水経由のリスク評価及び生態影 響に関する予備的リスク評価のパブコメに 対するコメント

	(PYU6) EPA-HQ-OPP-201 3-0368-0031_cont Response to OPP's Pymetrozine Mitigation Proposals (PYU7) EPA-HQ-OPP-201 3-0368-0034_cont	◇ADAMA は特定されたリスクを軽減す るために、空中散布用途禁止、軟弱土 壌への散布制限及び一部の作物への散 布量を低減ことに合意。
2017年12月	Draft Human Health Risk Assessment for Registration Review (PYU9) EPA-HQ-OPP-201 3-0368-0017_cont	<ul> <li>◇FQPA (Food Quality Protection Act) に基づきとトの健康影響リスク評価 に係る毒性データベースは十分。</li> <li>◇免疫毒性試験と亜急性吸入毒性試 験は実施していないが、HED はこれらの 試験は必要ないと判断。</li> <li>◇哺乳類では、標的臓器は肝臓。さらに 急性、亜慢性及び発達神経毒性試験で 神経影響がみられた。</li> <li>◇ラット及びウサギにおける発生毒性試 験あるいは発達神経毒性試験において、 出生前の感受性の増加が認められた。ラ ットの生殖毒性試験では認められなかった。</li> <li>◇毒性および暴露データを評価した結 果、FQPA 安全係数 (SF)をX10と 設定した。</li> <li>◇HED は、本有効成分を「とト発がん性 物質の可能性が高い」と分類し、マウス (オス、メス)及びラット(メス)における 複合肝腫瘍(良性肝腫及び/またはがん 腫)について、発がん性リスクの定量化を 求める。</li> <li>◇食品経由暴露評価に基づき、急性、</li> </ul>

	Acute, Chronic, and Cancer Dietary Exposure and Risk Assessments in Support of the Registration Review Risk Assessment (PYU10)	慢性及び食品経由の発がん性リスクは、 一般的に懸念レベルを超過している。その 原因は食品経由ではなく飲料水に含まれ る残留物である。
	EPA-HQ-OPP-201 3-0368-0018_cont	
2017年8月	Drinking Water Assessment for Registration Review (PYU11) EPA-HQ-OPP-201 3-0368-0028_cont	<ul> <li>◇EFED (Environmental Fate and Effects Division) は、食品経由リスク 評価において、HED が使用している地下 水の最高濃度 404µg/L 及びその後の 平均値を 367µg/L とすることを推奨す る</li> </ul>
2017年7月	Tier I Update Review of Human Incidents and Epidemiology for Draft Risk Assessment (PYU12) EPA-HQ-OPP-201 3-0368-0019_cont	◇疫学データ 2012年1月から2017年6月のIDS及び SENSOR-Pesticideのデータベースにお いて、本有効成分による事故は報告され ていない。
2014年5月	GENERIC DATA CALL-IN NOTICE (PYU14) EPA-HQ-OPP-201 3-0368-0014_cont	<ul> <li>◇ 公開された PWP (Preliminary Work Plan) で概説されたデータギャップ についての追加要求であり、FWP (Final Work Plan) で満たされている と指摘されたデータギャップは不要。</li> <li>◇追加要求データ</li> <li>・好気的水中代謝試験</li> <li>・発芽影響</li> <li>・植物体影響</li> <li>・鳥類経口急性毒性</li> <li>・分析バリデーション</li> <li>・ミッバチ半野外影響</li> </ul>
2013年12月	Final Work Plan	◇エビデンスの重み付け(WOE)に基づき、免疫毒性試験は不要と結論付けた。

	(PYU15)	パブコメ結果から、加水分解試験及び土
	POF 2	壌分解試験は不要となった。
	EPA-HQ-OPP-201	◇データギャップ更新
	3-0368-0012.pdf	·好気的水中代謝
		・土壌、水中の分析バリデーション
		・鳥類経口急性毒性
		·陸生植物影響(発芽)
		・陸生植物影響
		・ミツバチ影響半野外試験
2013年12月	EFED Response to Public Comments on	◇パブコメへの回答
	Registration Review	シンジェンタ社からのコメントに対する
		EFEDの回答。主に環境動態、飲料水
	(PYU16)	経由の暴露、生態影響関連。
	POF	
	EPA-HQ-OPP-201	
	3-0368-0013_cont	
2013年6月	Preliminary Work Plan	◇要求される可能性のあるデータギャップ
		・加水分解性
	(PYU17)	・好気的水中代謝
		・土壌、水中の分析バリデーション
	EPA-HQ-OPP-201	・鳥類経口急性毒性
	3-0368-0008.pdf	・陸生植物影響(発芽)
		・陸生植物影響
		・ミツバチ影響半野外試験
		・免疫毒性
2013年5月	Human Health Assessment Scoping	◇亜急性吸入毒性及び免疫毒性を除
	Document in Support of Registration	き、毒性データベースは十分。
	Review	◇HEDの HASPOC は、 亜急性吸入毒
		性試験は不要と判断した。
	(PYU19)	◇ラット及びマウスの経口投与試験によ
	PDF 人	り、肝臓が主な標的臓器で、肝臓腫で陽
	EPA-HQ-OPP-201 3-0368-0003.pdf	性であると考えられる。
	5 0500-0003.pdi	◇発がん性物質リスク評価ガイドラインに
		基づき、CARC(Cancer
		Assessment Review Committee)
		は、「ヒト発がん性物質の可能性が高い」
		と分類。提出された試験からは、変異原

		性及び遺伝毒性は認められない。
		☆ラット及びウサギの発生毒性試験、ラッ
		トの2世代生殖毒性試験では、胎内、
		出生前/後の暴露による感受性の増加は
		認められなかった。
		◇ラットの発達神経毒性試験では、母体
		毒性を生じない投与量で仔の脳の形態
		変化が認められたが、懸念の程度は低
		い。
		◇急性、亜急性、発達神経毒性で神経
		影響は認められなかった。
		◇ラット発達神経毒性試験のエンドポイン
		トを用いて、仔で認められた脳形態学的
		変化、用量選択、定量的感受性を考慮
		して、FQPA ファクターをX10、不確実係
		数を×1000 とした。
		◇唯一の免疫毒性試験がデータギャッ
		プ。
2013年2月	Review of Human Incidents	◇IDS 及び NIOSH SENSOR
		Pesticides の両方のデータベースを用い
	(PYU21)	た事故例の頻度と重症度が低いため、現
	PDF	時点では、懸念はないと推定。◇事故情
	EPA-HQ-OPP-201	報を継続的に監視し 懸念が生じた場合
	3-0368-0007.pdf	には、リスク評価を含めた追加分析を実
		施する。
		◇AHS (The Agricultural Health
		Study)も特定の農薬のとト健康影響
		」 に関する潜在的な情報源であるが、本有
		効成分は対象となっていない。
2010年8月	federal Register	◇NRDC (The Natural Resource
		Council) から提出された反対意見請
	(PYU24)	願への対応として以前のリスク評価を更
		新。
		↓ ◇リスク評価に安全係数 X1,000 を適
	2010-19423 (1).pdf	→ ハハヨー 山に文工 所致 ハコ,000 とし 用したが、許容値が安全であるという結論
		を変更しなかった。従って、NRDCの請願
		と安美しながった。 は却下された。
2000年8月		暫定登録用のファクトシート
2000年8月	FACT SHEET	首に豆球用のファットンート

	1	1
		◇ヒト、鳥類、水生生物、哺乳類及びハ
	(PYU25)	チに対する急性毒性が低いと判断。原体
	PDF	の急性毒性カテゴリーは III 及び IV。経
	fs_PC-101103_01	皮吸収率は1%と推定された。
	-Aug-00.pdf	◇変異原性はない。
		◇神経毒性を示したが、その頻度と程度
		は低かった。親への影響レベルにおいて仔
		動物に発生毒性影響を示した。
		◇地下水を汚染する危険性はないと予
		想される。
		◇同じ使用パターンで使用されている有
		機リン殺虫剤(OP)の代替品と考えてい
		వె.
		◇2 種動物(ラット、マウス)、両性(マ
		ウス)において 2 つのタイフ°(liver
		benign hepatoma 及び/又は がん
		種)の腫瘍が発生したことから、ヒトに対
		する発がん性物質として分類した。
		◇使用場所が限定され、使用率が低く、
		曝露量が少ないため、ヒトへのリスクは懸
		念レベル以下である。
1999年9月	NOTICE OF PESTICIDE	条件付登録認可
		◇データギャップ(提出期限)
	(PYU26)	・分析法(6ヶ月)
	PDF	・保存安定性、腐食性(15ヵ月)
	000100-00913-19	<ul> <li>・発達神経毒性(2年)</li> </ul>
	990930.pdf	・鳥類繁殖性(2 年)
		・飲料水モニタリングデータ(3年)

- ▶ 欧州
- ✓ EUデータベース(別紙1)
   <u>https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as\_id=1405</u>
- ✓ ECHAデータベース(別紙1)
   <a href="https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details/73456">https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-//discli/details/73456</a>

年月	評価資料	食品経由での人健康影響エンドポイント /追加要求
2021年2月	Office Journal	◇MRL 設定削除
	(PYE1) CELEX_32021R01 55_EN_TXT.pdf	
2018年10月	Office Journal	◇ピメトロジンの認可は、2019 年 6 月 30
		日に失効する。
	(PYE2)	◇経緯概要
	L	・申請者は認可更新申請、補足書類を提出
	CELEX_32018R15 01_EN_TXT.pdf	した。RMSは、この申請は完全であると判断
		し、RAR を作成し、2013 年 6 月 28 日に
		EFSA 及び欧州委員会に提出。
		・EFSAは、該当するすべての地下水シナリオ
		において。関連する代謝物 CGA371075 あ
		るいは他の代謝物が基準値 0.1µg/L を超え る地下水ばく露が生じる可能性が高いと結論
		付けた。
		・リスク評価のための作物残留の代謝物の毒
		性プロファイルは確認できず、代謝物 M3MF
		への曝露による水生生物へのリスク評価は、最
		終的に決定することはできないと結論付けた。
		・EFSAは、異なる種及び年齢で内分泌器官
		に悪影響を及ぼすと結論づけた。しかし、潜在
		的な内分泌かく乱特性に関する科学的評価
		は EFSA が最終的に決定することができなかっ
		た。

2018年6月	Office Journal (PYE3) CELEX_32018R09 17_EN_TXT.pdf	<ul> <li>・欧州委員会は意見を提出するよう申請者に 求め、申請者はコメントを提出し、その内容は 慎重に検討された。しかし、懸念を取り除くこと はできず、有効成分の認可を更新しないことと した。</li> <li>認可期間延長</li> <li>・失効: 2018年6月30日</li> <li>・有効期限2019年6月30日</li> </ul>
2018年6月	Final Renewal Report (PYE4) Pymetrozine_EC Final Renewal Rep	<ul> <li>最終再評価報告書</li> <li>◇エンドポイント</li> <li>・ADI: 0.03mg/kg/日</li> <li>・ARfD: 0.1mg/kg</li> <li>・AOEL: 0.03mg/kg/日</li> <li>・AOEL: 0.1mg/kg</li> <li>◇該当するすべての地下水シナリオで、毒性</li> <li>関連代謝物 CGA371075 及び他の代謝物</li> <li>は基準値 0.1µg/L を超える曝露の可能性があり、人健康影響評価ができない</li> <li>◇RAR 記載の GAP では FOCUS シナリオで</li> <li>CGA371075 が 0.1µg/L 以下になる可能</li> <li>性があるが、この GAP は代表的な使用方法</li> <li>ではなく、嫌気性土壌条件が発生する地域に</li> <li>おける菜種類のリスク評価を最終化できないと</li> <li>結論づけた。</li> <li>・嫌気性土壌代謝物 CGA180777 及び</li> <li>GS23199 の土壌生物、水生生物、地下水</li> <li>④への影響の可能性</li> <li>・嫌気性土壌代謝物 CGA249257 の土壌</li> <li>生物、地下水水質への影響の可能性</li> <li>◇内分泌かく乱作用性について異なる生物種</li> <li>と生育ステージで内分泌器官に有害な影響を</li> <li>ちらしたが、潜在的な内分泌かく乱特性に関</li> <li>する科学的評価は最終的に決定できない。</li> <li>◇提出された情報に基づいて行われた評価の</li> <li>結論として、本有効成分の認可は抹消される</li> </ul>

		べき。 (P.3~P.4)
2017年5月	Office Journal	認可期間延長
		·有効期限:2018年6月30日
	(PYE9)	
	PDF	
	CELEX_32017R08	
	41_EN_TXT.pdf	
2016年12月	STATEMENT OF EFSA	♦ EFSA は、 Tthe draft technical
		guidance on assessment of negligible
	(PYE10)	exposure of an active substance in a
	P0F	plant protection product under
	j.efsa.2017.4678.	realistic conditions of use.」に従って、申
	pdf	請者シンジェンタが提案したジャガイモ、ナタネ
		の代表的な使用方法について、無視できる暴
		露を考慮した評価を実施。
		◇EFSA は、本有効成分の再評価のためのピ
		アレビューの結論として、発がん性カテゴリー2、
		生殖毒性カテゴリー2 に分類することを提案。
		◇この 2 つの分類は、規則(EU) No
		1107/2009 の AnnexII に定められた認可
		基準に適合しないことを意味する(特に、内
		分泌かく乱特性に関する第3段落で設定され
		た基準を満たしているため、AnnexIII point
		3.6.5に相当)。
		ある)。
		◇植物代謝試験での代謝物の毒物学的デー
		タがないため、これらの残留レベルが上記ガイダ
		ンス案、すなわち「リスクを著しく増加させず、安
		全に無視できるほど小さいレベル」を満たすかど
		うかは不明であり、ハザードについても考慮する
		必要がある。
		◇地下水シナリオにおいて、代謝物
		CGA371075 が飲料水基準値を超えて地下
		水に暴露される可能性があり、重要な懸念事
		項として特定。
		◇従って無視できるレベルとして判断することは
		できない。(P.3)
2016年12月	Peer Review Report	◇無視できる暴露に関する申請者の提出書

	(PYE11)	類に対するコメント
	PDF 2	◇無視できる暴露に関する Draft EFSA
	Pymetrozine_EFS A Peer Review Re	STATEMENT に対するコメント
2016年8月	List of information (PYE12) Pymetrozine_RA R_list of studies_N	有効成分の認可更新のための評価用に RMS (報告担当加盟国)が 2016 年 8 月 11 日 付の RAR 確認した情報、試験等のリスト
2016年5月	Request for revised assessment of	◇2015 年 6 月 : Syngenta は無視できる
	negligible exposure	暴露量に関する資料を EU 委員会に提出。
		◇2016 年 5 月:EU 委員会は Syngenta
	(PYE13)	に対し、以下のガイダンス文書を考慮して改訂
		するよう要求。
	Pymetronzine_Re quest of the appli	•Technical guidance on the
		interpretation of points 3.6.3. to
		3.6.5, and 3.8.2 of Annex II to
		Regulation (EC) No 1107/2009, in particular regarding the assessment
		of negligible exposure to an active
		substance in a plant protection
		product under realistic conditions of use"; SANCO-2014-12096.
		•EFSA Guidance on the assessment
		of exposure of operators, workers,
		residents and bystanders in risk
		assessment for plant protection
		products (EFSA Journal 2014;
		12(10):3874).
		◇主な変更点
		・技術的に可能な限りばく露を低減するための
		緩和策の検討
		・急性 AOEL の設定と、操作者、作業者、傍 観者、居住者の関連暴露量の算出
2016年4月	Office Journal	認可期間延長
		·有効期限:2017 年 6 月 30 日
	(PYE14)	

	PDF	
	2	
	CELEX_32016R05	
	49_EN_TXT.pdf	
2015年10月	Office Journal	認可期間延長
		·有効期限:2016年6月30日
	(PYE15)	
	PDF	
	CELEX_32015R18	
	85_EN_TXT.pdf	
2015年7月	Scientific Report	JMPR でのレビュー結果まとめ
		⇔ADI
	(PYE16)	・JMPR:0.03mg/kg/日
	PDF	•EFSA : 0.03mg/kg/日
	j.efsa.2015.4208.	根拠:イヌ90日間、1年間反復経口投与
	pdf	試験及びラット2年間反復経口投与試験
		⇔ARfD
		JMPR: 0.1mg/kg
		•EFSA : 0.1mg/kg
		根拠 : ウサギ生殖毒性試験及びラット 28 日
		間強制経口投与試験
		◇EU 評価では、暫定的な残留基準に含まれ
		る植物代謝物(GS23199,
		CGA294849, CGA266591,
		CGA128632)の毒性プロファイル評価が最
		終的に決まらなかった。JMPR は、一部の代謝
		物が遺伝毒性の可能性を指摘し、TTC
		(Threshold of Toxicological
		- Concern:毒性学的懸念の閾値)法を用
		いて関連性を判断した。
		・Ia17:TTC(0.0025 µg/kg)を下回る
		推定慢性暴露量
		•CGA215525:Ames 試験陰性、
		Cramer class IIIのTTC(慢性暴露 1.5
		μg/kg、急性暴露 5μg/kg)未満の暴露
		・CGA294849, 慢性暴露および単回暴露
		の TTC を超える暴露
		・CGA300407 : in vitro 及び in vivo 遺
		伝毒性試験陽性との知見

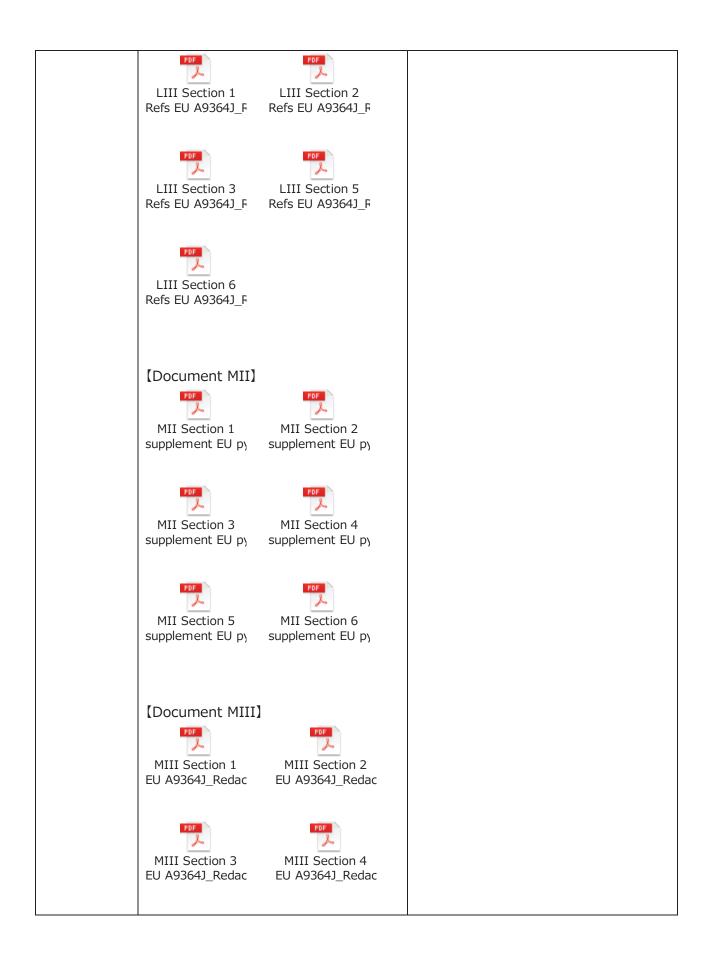
		<ul> <li>◇JMPRは、CGA294849、CGA300407</li> <li>の毒性評価の結論が得られなかったため、残</li> <li>留物定義の評価について結論を保留。</li> <li>CGA294849は遺伝毒性の可能性があり、</li> <li>推定曝露量はTTC値を超えていた。</li> <li>CGA300407は、in vitro、in vivo遺伝</li> <li>毒性試験で陽性の結果が報告された。</li> <li>◇EUの残留物定義、MRLは、遺伝毒性に</li> <li>関する警告を考慮して、早急に再考する必要がある。(P.121~P.123)</li> </ul>
2014年9月	PEER REVIEW REPORT (PYE17) EFSA Peer Review Report on	<ul> <li>◇ピアレビュー報告書</li> <li>・評価書に対するコメント</li> <li>・報告書の表</li> <li>・ピアレビュー会議報告書</li> <li>・評価リスト</li> <li>・追加評価に対するコメント</li> <li>・EFSA Conclusion へのコメント</li> </ul>
2014年8月	CONCLUSION ON PESTICIDE PEER REVIEW (PYE18) i.efsa.2014.3817. pdf	<ul> <li>ピアレビュー結論</li> <li>◇データギャップ</li> <li>・物化性、分析法:なし</li> <li>・哺乳類毒性:不純物の毒性影響で1つの</li> <li>データギャップ</li> <li>◇発がん性カテゴリー2及び生殖毒性カテゴリ</li> <li>-2への分類で、内分泌かく乱性に関す重要な懸念事項が特定された。</li> <li>◇本有効成分の内分泌系への影響についての情報が限定的であるため、OECD</li> <li>Conceptual Framework で現在示されているレベル2試験について、データギャップとして認識される。</li> <li>◇地下水中の代謝物は評価・規制の対象となる。</li> <li>◇作物残留及び消費者ばく露の観点から、輪作作物の作物残留試験、トマト及びナスの作物残留試験がデータギャップとなる。</li> <li>◇環境動態についてのデータは十分。ただし、嫌気性土壌代謝物 CGA19077、</li> <li>GS23199、CGA249257についての地下水</li> </ul>

		経由でのばく露評価は実施されていないため、
		これら3代謝物に関する地下水経由の影響
		は不明。
		◇評価した地下水シナリオにおいて, 関連代
		謝物 CGA371075 による地下水基準値
		0.1 μg/L を超えるばく露の可能性があること
		が重大な懸念事項。
		◇生態影響
		・水生生物への影響についてデータギャップあ
		り。
		・地下水中代謝物 M3MF, 地表水中及び
		土壌代謝物 CGA180777, GS23199,
		CGA249257 についてデータギャップあり
		・散布から開花前の期間までの影響を明らか
		にするため、ナタネでの減衰に関するデータが必
		要。
		・潜在的な内分泌かく乱作用について、さらな
		る生態影響試験が必要になる可能性あり
	OECD Conceptual Framework for	上記で引用されている OECD Conceptual
	Testing and Assessment of Endocrine	Framework
	Disrupters	
	(PYE19)	
	PDF	
	OECD Conceptual	
	Framework for Te	
2013年8月	REASONED OPINION	アザロール、セロリ、フェンネルの MRL 改訂
		◇エンドポイント
	(PYE20)	・ADI:0.03mg/kg/日
	PDF	•ARfD:0.1mg/kg
	j.efsa.2013.3348.	◇植物代謝
	pdf	葉面散布後の果実、果菜類,根茎類、塊根
		類、豆類、菜種類、穀類の代謝試験に基づ
		き、残留物定義は親化合物と定義。
		◇作物残留
		アザロール(0.7mg/kg)、セロリ
		(0.03mg/kg)、フェンネル
		(0.03mg/kg) の MRL 提案は、残留物の

		<u>にたった。またに思えて、 カギュップがたてたみ</u>
		保存安定性に関するデータギャップがあるため
		◇提案されているアザロール、セロリ、フェンネル
		への使用は、消費者の暴露が毒性学的参照
		値を超えることはなく、消費者の健康リスクを引
		き起こす可能性は低いと結論付けた。
		(P.14~P.15)
2013年5月	RAR	再評価 評価書
		RMS:ドイツ
	(PYE21)	Co-RMS : ベルギー
	PDF L	
	Pymetrozine_RA Pymetrozine_RA	
	R_01_Volume1_2 R_02_Volume2_2	
	PDF PDF	
	Pymetrozine_RA Pymetrozine_RA	
	R_03_Volume3CA R_04_Volume3CA	
	PDF	
	Pymetrozine_RA Pymetrozine_RA	
	R_05_Volume3CA R_06_Volume3CA	
	PDF	
	Pymetrozine_RAPymetrozine_RAR_07_Volume3CAR_08_Volume3CA	
	PDF	
	ト	
	Pymetrozine_RAPymetrozine_RAR_09_Volume3CAR_10_Volume3CA	
	PDF	
	スープ	
	Pymetrozine_RA Pymetrozine_RA R_11_Volume3CA R_13_LoEP_2013-	
	N_II_VOIGHESCA N_IS_LUEF_2013.	
	2	
	Pymetrozine_RA	
	R_list of studies_v	
2012年10月	REASOND OPINION	lamb`s lettuce と豆類(さや付き)の

		MRL改訂
	(PYE22)	◇エンドポイント
	PDF 人	・ADI:0.03mg/kg/日
	j.efsa.2012.2939.	•ARfD:0.1mg/kg
	pdf	◇果実、根茎類、菜種類、穀類の代謝試験
		に基づき、残留物定義は親化合物と定義。
		◇作物残留試験データの試料の保存安定性
		において、
		分解を考慮した補正係数を適用することでの
		提案は受け入れられない。従って、
		MRL提案は十分でないと結論付けた。
		(P.12)
2012年10月	REASOND OPINION	MRL 改訂
		◇エンドポイント
	(PYE23)	・ADI:0.03mg/kg/日
	PDF 2	•ARfD:0.1mg/kg
	j.efsa.2012.2919.	◇植物代謝
	pdf	トマト、ジャガイモ、綿、米の葉面散布した植
		物代謝試験では代謝経路がよく特定され、代
		謝パターンが類似していることから残留定義を
		確立。一次作物と二次作物の代謝パターンは
		類似しており、輪作作物での重大な影響はな
		いと結論付けた。
		◇家畜代謝
		乳用反芻動物、肉用反芻動物、豚の代謝試
		験の結果から豚にも外挿可能。組織中の残
		留物定義、本リスク評価時にはピメトロジンと
		6-ヒドロキシルメチルピメトロジンの合計と定
		義。
		◇MRL 値について一部の MRL 値は更なる
		検討が必要なため、AnnexIIへの掲載を推
		受しない。特に、いくつかの暫定的な MRL お
		よび既存の EU MRL については、追加の作物
		残留試験等が必要。
		(P.41~P.43)
2012年2月	Dossier	◇申請者 Syngenta が 2010 年 12 月 7
		◇ 中語 日 ラ パラには が こうこう 中 エン・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・
	(PYE24)	○本有効成分の登録期限を2015年12月

(Document A)	31 日まで延長。	
Document A EU Docume pymetrozine_Red pymetroz		
Document C EU Document pymetrozine_Red represent		
Document D2 EU Documer pymetrozine_Red pymetroz		
Document E1 EU Documer pymetrozine_Red pymetroz		
Document F EU Docume pymetrozine_Red pymetroz		
(Document LII)		
LII Section 3 supplement Refs Refs supple		
LII Section 5 Refs supplement Refs supple		
[Document LIII]		



	MIII Section 5 EU A9364J_Redac	
	Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Constraint of the second system         Image: Constraint of the second system       Image: Consecond system <t< td=""><td></td></t<>	
	(Document O) Document O Part 1 EU pymetrozine Document O Part 2 EU pymetrozine	
	Document O Part 3 EU pymetrozine 4 EU A9364J_Red	
	[Efficacy Statement]	
2011年5月	Office Journal (PYE25) httpseur-lex. europa.pdf	有効成分の認可リスト ・純度 950g/kg ・発行日:2001年11月1日 ・有効期限:2015年12月31日 ・殺虫剤用途
2010年11月	Office Journal (PYE26) CELEX_32010L00 77_EN_TXT.pdf	Annex I 掲載期限 ・純度:950g/kg ・発効日:2001年11月1日 ・有効期限:2015年12月31日 ・殺虫剤用途
2010年11月	REASOND OPINION	lamb`s lettuce と豆類(さや付き)の MRL改訂

	(PYE27)	◇エンドポイント
		• • • • •
	r	・ADI:0.03mg/kg/日
	j.efsa.2010.1881. pdf	•ARfD:0.1mg/kg
	pui	
		穀類、果実、果菜類、菜種類、根茎類、塊
		茎類の代謝試験において類似した代謝経路
		が確認されたため、残留物定義は親化合物と
		定義。葉菜類の追加代謝試験は必要ない。
		(P.13)
2007年12月	Office Journal	MRL 改訂
	(PYE28)	
	P0F 2	
	CELEX_32007L00	
	73_EN_TXT.pdf	
2002年7月	Final Renewal Report	最終再評価報告書
		◇エンドポイント
	(PYE29)	・ADI : 0.03mg/kg/日
	PDF 2	•ARfD : 0.1mg/kg
	Pymetrozine_EC	・AOEL:0.03mg/kg/日
	Review Report_2(	•AAOEL : 0.1mg/kg
		◇該当する地下水シナリオで、毒性関連代謝
		物 CGA371075 及び他の代謝物は基準値
		0.1µg/Lを超える可能性があり、人健康影
		響評価に影響がないことは証明できない。
		◇RAR 記載の GAP では FOCUS(FOrum
		for the Co-ordination of pesticide fate
		models and their Use) シナリオで
		CGA371075 が 0.1µg/L 以下になる可能
		性があると示されているが、この GAP は代表
		的な使用方法ではなく、嫌気性の土壌条件が
		発生しうる地域におけるナタネのリスク評価を最
		終化できない。
		◇内分泌かく乱作用性:
		◇ F357 施が、400F77314・ 異なる生物種と生育ステージで内分泌器官に
		有害な影響をもたらしたが、潜在的な内分泌
		内害な影響をりこうりにか、 個任時な内力 版 かく乱特性に関する科学的評価は最終的に
		決定できない。

		<ul> <li>◇提出された情報に基づいて行われた評価の</li> <li>結論として、本有効成分の認可は</li> <li>抹消されるべき。</li> <li>(P.3~P.4)</li> </ul>
2001年10月	Office Journal	Annex I 収載
		・原体純度:950g/kg
	(PYE30)	·発行日:2001年11月1日
	CELEX_32001L00 87_EN_TXT.pdf	·有効期限:2011 年 10 月 31 日

別紙1.データベース

- ▶ 米国
- ✓ EPA レビューページ

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	Welcome to the new Regulations gov. Check out the latest value.						
Regulations.gov			I	SUPP	087		
NONRULEMAKING DOCKET							
Pymetrozine Regist	ration Review						
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Created by the Environmen	tal Protection Agency						
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## EU データベース

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	Described Mills Date	Category	Protocologia	Risk Assessment					
		7004							
		Remarks	Non-sense of approaching (St. 2010)11 Workshow of approximations by 32 April 21 May people of grace, 50 January 2010						

# ECHA データベース

Summary of Cla	ssification and	d Labelling							
Harmonised clas General Informatio		ex VI of Regul	ation (EC) No	o 1272/2008 (CLP Regulatio	m)				6
Index Number	SC/ Unit Hol. @	CAS Number	ei l		International Ches	must Meetification			
613-202-00-4		122312-09-0	pursetrui	ne (350); (7) 4,5-drivbo-5-meth ne (350) Aydro-6-methyl 4 (3 pyridylmeth					
ATP Inserted / Updab CLP Classification (Ta Cla Haland Class and Cal	ole 3) milliotation		aid Italament	Laboling Tappionettry raced Dataset	Pictopoles, Spar West	Specific Concentration Boolty, N Estimates (		Nature	
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## 9.1.4. 米国 EPA におけるピ 外ロジンの再評価概要と関係資料

評価	① 事前のやり取りに関する文章		前のやり取りに関する文章 ② 追加要求された試験項目及び		③         ②の提出の有無、再評価結果         ④         再評価語		i評価書	⑤ 規制	<ol> <li>5 規制内容/その他</li> </ol>		
幾関			その内容	に関する文章							
EPA									【Registration Review Docket】 Regulations.gov - Docket	EPA レビューページ	
									Folder Summary		
	2018年12月	◇懸念されるリスクは、主に	2018年12月	◇ヒト健康影響リスク評価で	2020年7月	◇ヒト健康影響及び生態影			(URL のみ)		
	[MEMORANDUM]	飲料水への残留。そのため	[PID]	は、飲料水用途の地下水に	[Interim Registration	響リスク評価の結論は、PIDと					
	Addendum to the Draft	の緩和策を提案。	Proposed Interim	含まれるピパロジン残留物に	Review Decision】	ID の間で変更されなかっ					
	Human Health Risk Assessment for	<ul> <li>・最大散布量を低減</li> <li>・空中散布を禁止するか、</li> </ul>	Registration Review Decision	よる急性、慢性及び発がん 性の可能性が示唆される。	https://beta.regulations.gov /document/EPA-HQ-OPP-	た。とト健康影響に関する追 加データ不要					
	Registration Review	保護マスクの要件を追加する	Decision	・急性及び慢性の比健康へ	2013-0368-0060	かりつい ◇EPA は、PID に対して提					
		◇緩和策での急性及び慢	https://beta.regulations.gov	のエントポイントは、ラット発達神	(PYU1)	出された意見を検討した結					
	https://beta.regulations.gov	性の食品経由(飲料水を含	/document/EPA-HQ-OPP-	経毒性試験で観察された脳		果、以下のように決定した。					
	/document/EPA-HQ-OPP-	む)暴露評価では、懸念す	2013-0368-0036	形態学的変化である。		・ジャガイモ及びペカンの単回					
	2013-0368-0032	べきリスクはない。	(PYU2)	発がん性のエントホイントイ		最大散布率及び年間最大					
	(PYU3)			は、マウスのオスにおけるヘパト -マ及び/または肝細胞がん		散布率低減を要求しない。				1	
	2018 年 12 月	飲料水経由でのリスク評価		マ及ひ/または肝細胞かん である。		・観葉植物の屋内散布の年 間最大散布量を、1 エーカーあ				1	
	[MEMORANDUM]	(2017年8月)の更新		<ul> <li>・飲料水中の残留物暴露</li> </ul>		間最大1011重を、11-1-00 たり 3.125 ポンドの有効成分				1	
	Update to the Drinking			は、リスク評価において主な		で維持する。					
	Water Assessment for			原因であった。食品への暴		・PID に記載されている空					
	registration Review of			露だけでは、急性、慢性、ま		中散布を禁止しない。					
	Pymetrozine dated August			たは発がん性が懸念される							
	24,2017			リスクは生じないが、飲料水 を食事暴露の計算に含める							
	https://beta.regulations.gov			と、懸念レベル(LOC=急性							
	/document/EPA-HQ-OPP-			母集団調整線量(aPAD)ま							
	2013-0368-0030			たは慢性母集団調整線量							
	(PYU4)			(cPAD)の 100%を超えてい							
				た。							
				◇以下の緩和策を提案す る。							
				□ ・コンテナ栽培の観葉植物を除							
				くすべての用途について、						1	
				軟弱土壌で栽培された作物						1	
				への使用禁止							
				・コンテナ栽培の観葉植物(屋							
				外栽培)について、深度が						1	
				浅い地下水の場合、水管理 の実施または集水システム						1	
				の美施または集水システム から 900 フィート以内での							
				使用禁止。							
				・ジャガイモ、ヘカン及び花卉類							
				への処理量低減						1	
				・職業リスクを軽減するため、						1	
				空中散布の禁止。							
				◇非標的生物に対するリスク を軽減するためのラヘル変更							
				を 控減 9 る 7 と の の 7 へ ル 変 更 ◇ 耐容 摂取 量の 再評価に							
				基づく適用変更						1	
				◆DCI について追加データ要							
				求はない。ただし、ミツバチに							

	5	対する潜在的リスクを考慮			
	U	し、ポリネーターに関する追加			
	3	要求を行う可能性がある。			
		◇疫学データ			
		OPP の IDS 及び			
		CDC/NIOSH の SENSOR-			
	F	Pesticides データベースを用い			
	-	て検討。2012年1月1日			
	7	から 2017 年 6 月 7 日まで			
		の間に、1 件の軽度の症例			
		IDS に報告されていた。			
	1	1998 年から 2013 年の間			
	1	に SENSOR-Pesticides で			
	5	5 件の事例が確認された。			
		この事例はすべて複数の有			
	3	効成分が関与していた。本			
		有効成分について報告され			
		た事例の頻度と重症度が低			
		いことを考えると、現時点で			
		の懸念はない。			

評価	① 事前のやり取りに関する文章	<ol> <li>② 追加要求された試験項目及び</li> </ol>	<ol> <li>②の提出の有無、再評価結果</li> </ol>	④ 再評価評価書	⑤ 規制内容/その他
機関		その内容に関する文章			
EPA	2018 年 11 月 【Response】 Syngenta & EPA Registration Review Risk Management Discussion <u>https://beta.regulations.gov</u> /document/EPA-HQ-OPP- 2013-0368-0035 (PYU5)				
	2018 年 10 月 (MEMORANDUM] Response to Public Comments on the Drinking Water Assessment and Preliminary Ecological Risk Assessment <u>https://beta.regulations.gov</u> /document/EPA-HQ-OPP- 2013-0368-0031 (PYU6)				
	2018 年 10 月 [Response] Response to OPP's Pymetrozine Mitigation Proposals https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0368-0034 (PYU7)				
	2017 年 12 月 【Response】 Preliminary Ecological Risk Assessment for the Registration Review <u>https://beta.regulations.gov</u> /document/EPA-HQ-OPP- 2013-0368-0016 (PYU8)				

評価	① 事前のやり取りに関する文章		り取りに関する文章 ② 追加要求された試験項目及び		有無、再評価結果	④ 再評価評価書	<ol> <li>5 規制内容/その他</li> </ol>		
機関			その内容に関する文章						
EPA	2017年8月 [MEMORANDUM] Drinking Water Assessment for Registration Review https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0368-0028 (PYU11)	◇EFED は、食品経由リスク 評価において、健康影響部 (HED)が使用している地下 水の最高濃度 404 ℓ/L 及 びその後の平均値を 367 μ g/L とすることを推奨する。		2017 年 12 月 [MEMORANDUM] Draft Human Health Risk Assessment for Registration Review https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0368-0017 (PYU9) 2017 年 12 月 [MEMORANDUM] Acute, Chronic, and Cancer Dietary Exposure and Risk Assessments in Support of the Registration Review Risk Assessment https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0386-0018 (PYU10)	<ul> <li>◇FQPA に基づきけの健康 影響リスク評価に係る毒性デ -9ヘ、-スは+分。</li> <li>◇免疫毒性試験と亜急性 吸入毒性試験と主急性</li> <li>吸入毒性試験と主急性</li> <li>吸入毒性試験と正急性</li> <li>吸入毒性試験にえれらの試 験は必要ないと判断。</li> <li>◇哺乳類では、標的臓器は</li> <li>肝臓。さらに急性、亜慢性</li> <li>及び発達神経毒性試験で</li> <li>神経影響がみられた。</li> <li>◇ラット及びウサギにおける発</li> <li>生毒性試験こおいて、出生</li> <li>前の感受性の増加が認められた。うかりの生殖毒性試験</li> <li>ぐれまいすいた、出生</li> <li>前の感受性の増加が認められた。</li> <li>◇毒性及び暴露データを評 価した結果、FQPA 安全係</li> <li>数(SF)を X10 と設定した。</li> <li>◇ 毎性及び暴露データを評 価した結果、FQPA 安全係</li> <li>数(SF)を X10 と設定した。</li> <li>◇HED は、本有効成分を「ヒ ト発がん性物質の可能性が 高い」と分類し、マウス(オス,メ ス)及びラッド(メス)における複</li> <li>合肝腫瘍(良性肝腫及び/ またはがん腫)について、発 がん性リスクの定量化を求める。</li> <li>◇食品経由暴露評価に基 づき、急性、慢性及び食品</li> <li>経由の発がん性リスクは、- 般的に懸念レハルを超過して いる。その原因は食品経由</li> <li>ではなく飲料水に含まれる</li> <li>残留物である。</li> </ul>		2017 年 7 月 [MEMORANDUM] Tier I Update Review of Human Incidents and Epidemiology for Draft Risk Assessment https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0368-0019 (PYU12)	◇疫学データ 2012 年 1 月から 2017 年 6 月の IDS 及び SENSOR- Pesticide のデータヘ´ースにお いて、本有効成分による事 故は報告されていない。	

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	<ol> <li>② 追加要求され</li> </ol>	た試験項目及び	③ ②の提出の4	<b>「無、再評価結果</b>	④ 再評価評価書		⑤ 規制	内容/その他
機関			その内容	に関する文章						
EPA	2017 年 7 月 【Open Literature Review Summary】 <u>https://beta.regulations.gov</u> <u>/document/EPA-HQ-OPP-</u> <u>2013-0368-0048</u> ( <b>PYU13</b> )	◇公表論文(ミッパチ影響野 外試験)のレビュー: Assessment of Side Effects of Pymetrozine 50 WG (A- 9364 A) on the Honey Bee (Apis mellifera L.) in Plum Orchard Following Application during Bee- Flight, Study code: 20011079/SI-BFEU	2014年5月 【GENERIC DATA CALL-IN NOTICE】 https://beta.regulations.gov /document/EPA-HQ-OPP- 2013-0368-0014 (PYU14)	◇公開された PWP で概説された データギャップ についての 追加要求であり、FWP で満 たされていると指摘された デ ータキ * ャップ は不要。 、>追加要求 データ ・好気的水中代謝試験 ・発芽影響 ・ 植物体影響 ・ 鳥類経口急性毒性 ・ >分析 バリデーション ・ シンハ 7 半野外影響						
	2013 年 12 月 【MEMORANDUM】 EFED Response to Public Comments on Registration Review <u>https://beta.regulations.gov</u> /document/EPA-HQ-OPP- 2013-0368-0013 (PYU16) 2013 年 6 月 【PWP】 Preliminary Work Plan	<ul> <li>◇ハブコメへの回答</li> <li>シンジェンタ社からのコメントに対するEFEDの回答。主に環境動態、飲料水経由の暴露、生態影響関連。</li> <li>◇予想されるデータキ*ャップ・加水分解性</li> <li>・好気的水中代謝</li> </ul>	2013 年 12 月 【FWP】 Final Work Plan Regulations.gov - Supporting & Related Material Document (PYU15)	(ハハ・エヨハ・ション ◇ エビデンスの重み付け (WOE)に基づき、免疫毒性 試験は不要と結論付けた。 ハブコメ結果から、加水分解 試験及び土壌分解試験は 不要となった。 ◇ データギャップ更新 ・好気的水中代謝 ・土壌、水中の分析パリデーション ・鳥類経口急性毒性 ・陸生植物影響 ・ミツパチ影響半野外試験				[M BE Re <u>Re</u> Su Ma	013年5月 MEMORANDUM】 BEAD Chemical Profile for Regulations.gov — Regulations.gov — Re	◇登録審査が開始された農 薬の使用情報と、害虫管理 の役割について概説。
	Regulations.gov – Supporting & Related Material Document (PYU17)	<ul> <li>・土壌、水中の分析パリデーション</li> <li>・</li> <li>・</li> <li>に</li> <li>・</li> <li>に</li> <li>・</li> <li>に</li> <li>・</li> <li>に</li> <li>・</li> <li>に</li> <li>・</li> <li>ホーン</li> <li>・</li> <li>ホーン</li> <li>・</li> <li>ホーン</li> <li>・</li> <li>ホーン</li> <li>ホーン</li></ul>								

	のやり取りに関する文章	れた試験項目及び	③ ②の提出の	有無、再評価結果	④ 再評価評価書	⑤ 規制	内容/その他
評価 ① 事前( 機関 EPA	のやり取りに関する文章	<ul> <li>れた試験項目及び</li> <li>客に関する文章</li> <li>EFEDによる環境影響及び 生態影響に関するレビューと 推定デ<sup>-</sup>クキ 'ャップ 今環境動態         <ul> <li>・加水分解性</li> <li>・好気的水中代謝</li> <li>・甘壤残留試験</li> <li>◇生態影響</li> <li>・鳥類急性経口</li> <li>・植物影響</li> <li>・ミッハ・チ影響半野外試験</li> </ul> </li> </ul>	③ ②の提出の 2013年5月 [MEMORANDUM] Human Health Assessment Scoping Document in Support of Registration Review Regulations.gov - Supporting & Related Material Document (PYU19)	「無、再評価結果 ◆亜急性吸入毒性及び免疫毒性を除き、毒性データハ、 -スは十分。 ◇HEDのHASPOCは、亜 急性吸入試験は不要と判 断した。 ◇フット及びマウスの経口投与 試験により、肝臓が主な標 的臓器で、肝臓腫で陽性であると考えられる。 ◇予がんぜ物質リスが評価が イトラインに基づき、CARC は、「比発がん性物質の可 能性が高い」と分類。提出された試験からは、変異原性 及び遺伝毒性は認められない。 ◇ラット及びウサギの発生毒性 試験、ラットの2世代生殖毒 性試験では、胎内、出生前/ 後の暴露による感受性の増 加は認められなかった。 ◇フットの発達神経毒性試験 (DNT)では、母体毒性を生 じない投与量で仔の脳の形態変化が認められたが、懸 念の程度は低い。 ◇ラット発達神経毒性試験の レントボインを用いて、仔で認められた脳形態学的変化、	<ul> <li>④ 再評価評価書</li> </ul>	⑤ 規制 2013年2月 [MEMORANDUM] Review of Human Incidents <u>Regulations.gov</u> — <u>Supporting &amp; Related</u> <u>Material Document</u> (PYU21)	内容/その他 IDS 及び NIOSH SENSOF Pesticides の両方のデー ースを用いた事故例の頻度 と重症度が低いため、現 点では、懸念はないと推 定。事故情報を継続的に 視し 懸念が生じた場合に は、リスク評価を含めた追 グ析を実施する。 農業衛生調査(AHS)も特 の農薬のLY健康影響に見 する潜在的な情報源であ が、本有効成分は対象と っていない。
				<ul> <li>X10、不確実係数を×1000</li> <li>とした。</li> <li>◇唯一のデ<sup>-</sup>-9<sup>+</sup>*ップ<sup>-</sup>は免疫 毒性試験</li> </ul>		2013年1月 【PRD Label Report】	◇食用及び非食用に使用 れる農薬ラベルリスト
						<u>Regulations.gov –</u> <u>Supporting &amp; Related</u> <u>Material Document</u> (PYU22)	
						2012年6月 [SLUA] Screening Level Usage Analysis (SLUA) <u>Regulations.gov</u> - <u>Supporting &amp; Related</u> <u>Material Document</u> ( <b>PYU23</b> )	◇スクリーニンヴレベルでの使 実態調査

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	<ol> <li>②の提出の有無、再評価結果</li> </ol>	④ 再評価評価書	⑤ 規制内容/その他
機関		その内容に関する文章			
EPA	2010 年 8 月 【federal Register】 https://www.govinfo.gov/co ntent/phg/FR-2010-08- 06/pdf/2010- 19423.pdf#page=1 (PYU24) 2010 年 8 月 ◇ The Natural Resource Council (NRDC) から提出さ れた反対意見請願への対 応として以前のリスク評価を 更新。 ◇ リスク評価に完全な安全係 数 (X1.000)を適用したが、 許容値が安全であるという 結論を変更しなかった。従っ て、NRDCの請願は却下さ れた。				
		1999 年 9 月       条件付登録認可         [NOTICE OF PESTICIDE]       ·分析法(6 ヶ月)         U.S. EPA, Pesticide Product       ·保存安定性、腐食性(15 ヵ         Label, ENDEAVOR,       月)         09/30/1999       ·発達神経毒性(2 年)         (PYU26)       ·創類繁殖性(2 年)         ·飲料水モニタリングデータ(3 年)			2000 年 8 月 【FACT SHEET】 https://www3.epa.gov/pesti cides/chem_search/reg.acti ons/registration/fs PC- 101103.01-Aug-00.pdf (PYU25) (PYU25)

#### 略号(米国 EPA)

BEAD	Biological and Economic Analysis Division	EFED	Environmental Fate and Effects Division	IDS	Incident Data System	PWP	Preliminary Work Plan
CDC	Centers for Disease Control	FQPA	Food Quality Protection Act	LOAEL	Lowest-Observed-Adverse-Effect Level	SF	Safety Factor
СТА	Comparative Thyroid Assay	FWP	Final Work Plan	NIOSH	National Institute for Occupational Safety and Health	WOE	Weight of Evidence
DCI	Data Call-In	HSPOC	Hazard and Science Policy Committee	NOAEL	No-Observed-Adverse-Effect Level		
DER	Data Evaluation Record	HASPOC	Hazard and Science Policy Council	OCSPP	Office of Chemical Safety and Pollution Prevention		
DNT	Developmental NeuroToxicity	HED	Health Effects Division	OPP	Office of Pesticide program		
DWA	Drinking Water exposure Assessment	ID	Interim Registration Review Decision	PID	Proposed Interim Registration Decision		

#### 引用 URL とその PDF ファイル (又はワードファイル、エクセルファイル)

PYU-1	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0060	EPA-HQ-OPP-201 3-0368-0060_cont
PYU-2	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0036	EPA-HQ-OPP-201 3-0368-0036_cont
PYU-3	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0032	EPA-HQ-OPP-201 3-0368-0032_cont
PYU-4	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0030	EPA-HQ-OPP-201 3-0368-0030_cont
PYU-5	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0035	EPA-HQ-OPP-201 3-0368-0035_cont
PYU-6	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0031	EPA-HQ-OPP-201 3-0368-0031_cont
PYU-7	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0034	EPA-HQ-OPP-201 3-0368-0034_cont
PYU-8	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0016	EPA-HQ-OPP-201 3-0368-0016_cont
PYU-9	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0017	EPA-HQ-OPP-201 3-0368-0017_cont

PYU-10	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0018	EPA-HQ-OPP-201
		3-0368-0018_cont
PYU-11	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0028	
		EPA-HQ-OPP-201 3-0368-0028_cont
PYU-12	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0019	
		EPA-HQ-OPP-201 3-0368-0019_cont
PYU-13	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0048	
		EPA-HQ-OPP-201 3-0368-0048_cont
PYU-14	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0014	
		EPA-HQ-OPP-201 3-0368-0014_cont
PYU-15	<u>Regulations.gov – Supporting &amp; Related Material Document</u>	
		EPA-HQ-OPP-201 3-0368-0012.pdf
PYU-16	https://beta.regulations.gov/document/EPA-HQ-OPP-2013-0368-0013	
		EPA-HQ-OPP-201 3-0368-0013_cont
PYU-17	Regulations.gov – Supporting & Related Material Document	
		EPA-HQ-OPP-201 3-0368-0008.pdf
PYU-18	Regulations.gov - Supporting & Related Material Document	
		EPA-HQ-OPP-201 3-0368-0005.pdf
PYU-19	Regulations.gov – Supporting & Related Material Document	
		EPA-HQ-OPP-201 3-0368-0003.pdf
PYU-20	Regulations.gov - Supporting & Related Material Document	
		EPA-HQ-OPP-201 3-0368-0002.pdf
PYU-21	<u>Regulations.gov – Supporting &amp; Related Material Document</u>	
		EPA-HQ-OPP-201         EPA-HQ-OPP-201           3-0368-0007.pdf         3-0368-0007.xls

PYU-22	Regulations.gov - Supporting & Related Material Document	EPA-HQ-OPP-201         EPA-HQ-OPP-201           3-0368-0006.pdf         3-0368-0006.doc
PYU-23	Regulations.gov - Supporting & Related Material Document	EPA-HQ-OPP-201 3-0368-0006.pdf
PYU-24	https://www.govinfo.gov/content/pkg/FR-2010-08-06/pdf/2010-19423.pdf#page=1	2010-19423 (1).pdf
PYU-25	https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-101103_01-Aug-00.pdf	fs_PC-101103_01 -Aug-00.pdf
PYU-26	U.S. EPA, Pesticide Product Label, ENDEAVOR, 09/30/1999	000100-00913-19 990930.pdf

### 9.1.4. 欧州 EFSA におけるピメトロジンの再評価概要と関係資料

評価	① 事前のやり	取りに関する文章	<ol> <li>追加要求され</li> </ol>	れた試験項目及び	③ ②の提出の	有無、再評価結果	④ 再評	価評価書	<ol> <li>5 規制内容/その他</li> </ol>	
機関			その内容	に関する文章						
EFSA									2021年3月	EU データベース
									【EU database】	・農薬有効成分として認可
										はない。
									https://ec.europa.eu/food/	<ul> <li>·認可失効時期::2015 年</li> </ul>
									plant/pesticides/eu-	12月31日.
									pesticides-	·最大猶予期間:2020年1
									database/active-	月 30 日
									substances/?event=as.detai	
									Is&as_id=1405	
									(URL のみ)	
									2021年3月	ECHA データベース
									【ECHA database】	
									https://echa.europa.eu/de/	
									information-on-	
									chemicals/cl-inventory-	
									database/-	
									/discli/details/73456	
									(URL のみ)	
									2021年2月	◇MRL 設定削除
									[Office Journal]	
									https://eur-	
									lex.europa.eu/legal-	
									content/EN/TXT/PDF/?uri	
									=CELEX:32021R0155&from=	
									EN	
	Ĺ	Ĺ							(PYE1)	

評価	① 事前のやり取りに関する文章	<ol> <li>追加要求された試験項目及び</li> </ol>	③ ②の提出の有無、再評価結果	④ 再評価評価書	⑤ 規制内容/その他		
機関		その内容に関する文章					
·機関 EFSA					2018 年 10 月 [Office Journal]         ◇ビホロジへの認可は、2019 年 6 月 30 日に失効する。 ◇経緯概要           https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32018R1501&fromE         ・申請者は認可更新申請、 補足書類を提出した。RMS は、この申請は完全である と判断し、RARを作成し、2013 年 6 月 28 日にEFSA 及び欧州委員会に提出。 ・EFSA は該当するすべて の地下水シリオにおいて、 関連する代謝物 CGA371075 あるいは他の 代謝物が基準値 0.1 µ/Lを 超える地下水はζ露が生じ る可能性が高いと結論付けた。 ・リスク評価のための作物残 留の代謝物の毒性プロ77/ル は確認できず、代謝物 MMF への曝露による水生 生物へのリスク評価は、最終 的に決定することはできな いと結論付けた。 ・EFSA は、異なる種及び年 齢で内分泌器官に悪影響を 及ぼすと結論づけた。しか し、潜在的な内分泌かく乱 特性に関する科学的評価 は EFSAが最終的に決定す ることができなかった。 ・欧州委員会は意見を提出 するよう申請者に求め、申 請者はコシルを提出し、その 内容は慎重に検討された。 しかし、懸念取り除くこと はできず、有効成分の認可 を更新しないこととた。		

評価	① 事前のやり取りに関する文章		<ol> <li>② 追加要求された試験項目及び</li> </ol>	<ol> <li>③ ②の提出の有無、再評価結果</li> </ol>		<ol> <li>④ 再評価評価書</li> <li>⑤ 規制</li> </ol>		内容/その他	
機関			その内容に関する文章						
EFSA	2017年12月	◇本有効成分の使用が難		2018年6月	最終再評価報告書			2018年6月	認可期間延長
LION	[Scientific Report]	防除害虫防除に必須である		[Final Renewal Report]	◇エンドポイント			[Office Journal]	·失効:2018 年 6 月 30 日
		との申請者シンジェンタの主張			・ADI:0.03mg/kg/日				·有効期限 2019 年 6 月 30
	https://efsa.onlinelibrary.wil	の評価。			•ARfD:0.1mg/kg			https://eur-	日
	ey.com/doi/epdf/10.2903/j.	◇10 加盟国の 100 以上の		(PYE4)	•AOEL:0.03mg/kg/日			lex.europa.eu/legal-	
	efsa.2018.5129	作物/害虫を評価。			•AAOEL:0.1mg/kg			content/EN/TXT/PDF/?uri	
	(PYE5)				◇該当するすべての地下水			=CELEX:32018R0917&from=	
					シナリオで、毒性関連代謝物			EN	
					CGA371075 及び他の代謝			(PYE3)	
	2017年10月	♦Draft EFSA Report(PYE5			物は基準値 0.1 με/Lを超え				
	[Member States'	のドラフト)に対する MS か			る曝露の可能性があり、人				
	comments on the draft EFSA Report】	らのコメント及び EFSA のコメント			健康影響評価ができない ◇RAR 記載の GAP では				
	EFSA Report				ORAR 記載の GAP Cla FOCUS シナリオで				
	(PYE6)				FOCUS 975月で CGA371075が0.1度/L以				
	(F120)				下になる可能性があると示				
					されているが、この GAP は				
	2017年9月	♦ application report(PYE8)			代表的な使用方法ではな				
	[Member States'	に対する MS からのコメント及			く、嫌気性の土壌条件が発				
	comments on the	び EFSA のコメント			生しうる地域におけるナタネ				
	application Report]				のリスク評価を最終化できな				
					いと結論づけた。				
	(PYE7)				·嫌気性土壤代謝物				
					CGA180777 及び GS23199				
	不明	◇申請者(Syngenta)は、本			の土壌生物、水生生物、地				
	[Request for derogation in	有効成分がナタネ、ジャガイモ、			下水質への影響の可能性				
	compliance with Art. 4.7 of	野菜、果物、その他マイナー用			·嫌気性土壤代謝物				
	Regulation (EC) No	途における作物栽培のため			CGA249257 の土壌生物、				
	1107/2009】	に必要であることを示す資			地下水水質への影響の可				
	(7)(7)	料を提出。			能性				
	(PYE8)	<ol> <li>1. 抵抗性の管理:抵抗性の 発生を抑制することは、重</li> </ol>			◇内分泌かく乱作用性: 異なる生物種と生育ステージ				
		光王を抑制することは、重 要事項である。ユニークな作用			英なる主物種と主角 「一」で内分泌器官に有害な影響				
		要事項 (803)。ユニール41F用 機序を持ち、殺虫剤の抵抗			をもたらしたが、潜在的な内				
		性対策には欠かせない本			分泌かく乱特性に関する科				
		有効成分が EU から排除さ			学的評価は最終的に決定				
		れると、ナダネの Pollen			できない。				
		Beatle、多くの作物のアブラム			◇提出された情報に基づい				
		シ、コナジラミなどの主要害虫			て行われた評価の結論とし				
		を防除するための薬剤の選			て、本有効成分の認可は				
		択肢が、減少する。			抹消されるべき。				
		2.IPM:本有効成分は、他の							
		代替作用機序とは異なり、							
		IPM に適合している。選択							
		性があり、吸汁性害虫を統							
		合的に防除するための非化							
		学的手法との併用に適して いる。独自の作用機序によ							
		いる。独自の作用機序により、交差耐性のJスクが低い。							
		9、交差 の1人のか低い。 3.副次的用途:本有効成分							
		3.副次的用途:本有効成分 は、代替品がない、あるい							
		は少数の代替品しかない多							
		くの作物に使用されている。							
I	1			1	1	1		l	L

評価機関	① 事前のやり取りに関する文章	② 追加要求された試験項目及び その内容に関する文章	③ ②の提出(	の有無、再評価結果	④ 再	評価評価書	⑤ 規制	内容/その他
評価 機関 EFSA	① 事前のやり取りに関する文章         ① 事前のやり取りに関する文章	② 追加要求された試験項目及び その内容に関する文章	③ ②の提出( 2016年12月 【STATEMENT OF EFSA】 <u>https://efsa.onlinelibrary.wil</u> <u>ey.com/doi/epdf/10.2903/j.</u> <u>efsa.2017.4678</u> ( <b>PYE10</b> )	D 有無、再評価結果 ○ 有無、再評価結果 ◇EFSA は、「the draft technical guidance on assessment of negligible exposure of an active substance in a plant protection product under realistic conditions of use.] IC従って、申請者シンジェンタが提案したジャカイモ、ナタネに対する代表的な使用方法について、無視できる暴露を考慮した評価を実施。 ◇EFSA は、本有効成分の再評価のためのビアレビューの	④ 再	評価評価書	<ul> <li>⑤ 規制</li> <li>2017年5月 [Office Journal]</li> <li>https://eur- lex.europa.eu/legal- content/EN/TXT/PDE/?uri =OELEX:32017R0841&amp;from= EN (PYE9)</li> </ul>	内容/その他   認可期間延長 ・有効期限:2018 年 6 月 30 日
				考慮した評価を実施。 ◇EFSA は、本有効成分の				
				重要な懸念事項として特定。 ◇従って無視できるレヘ・ルとして判断することはできない。				

評価	<ol> <li>事前のや</li> </ol>	り取りに関する文章	② 追加要求された試験項目及び	③ ②の提出	の有無、再評価結果	④ 再評	· 価評価書	⑤ 規制内容/その他		
機関			その内容に関する文章							
	2016 年 8 月 【LIST OF INFORMATION】 (PYE12)	有効成分の認可更新のた めの評価用に RMS が 2016 年 8 月 11 日付の RAR 確認 した情報、試験等のリスト		2016 年 12 月 【PEER REVIEW REPORT】 (PYE11)	<ul> <li>◇無視できる暴露に関する</li> <li>申請者の提出書類に対するコメント</li> <li>◇無視できる暴露に関する</li> <li>Draft EFSA STATEMENT</li> </ul>					
	(1) (1) (2)				に対するコメント					
	2016 年 5 月 【Request for revised assessment of negligible	◇2015 年 6 月: Syngenta は無視できる暴露量に関す る資料を EU 委員会に提						2016 年 4 月 【OFFICE Journal】	認可期間延長 ・有効期限:2017 年 6 月 30 日	
	exposure]	出。 ◇2016 年 5 月 : EU 委員会						https://eur- lex.europa.eu/legal-		
	(PYE13)	は Syngenta に対し、以下の がイダンス文書を考慮して改 訂するよう要求。 ・Technical guidance on the						<u>content/EN/TXT/PDF/?uri</u> =CELEX:32016R0549&from= <u>EN</u> (PYE14)		
		interpretation of points 3.6.3. to 3.6.5, and 3.8.2 of Annex II to Regulation (EC)								
		No 1107/2009, in particular regarding the assessment of negligible exposure to an								
		active substance in a plant protection product under realistic conditions of use";								
		SANCO-2014-12096. •EFSA Guidance on the assessment of exposure of								
		operators, workers, residents and bystanders in risk assessment for plant protection products (EFSA								
		Journal 2014; 12(10):3874). ◇主な変更点								
		<ul> <li>・技術的に可能な限りばく露 を低減するための緩和策の 検討</li> <li>・急性 AOEL の設定と、操</li> </ul>								
		作者、作業者、傍観者、居 住者の関連暴露量の算出								
								2015 年 10 月 【Office Journal <u>】</u>	認可期間延長 ・有効期限:2016 年 6 月 30 日	
								https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX:32015R1885&from=		
								EN (PYE15)		

評価         ① 事前のやり取りに関する文章         ② 追加要求された試験項目及び         ③ ②の提出の有無、再評価組	結果         ④ 再評価評価書         ⑤ 規制内容/その他
機関 その内容に関する文章	
	告書        ifa31xyh

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	③ ②の提出(	の有無、再評価結果	④ 再評価評価書	<ol> <li>5 規制内容/その他</li> </ol>
機関		その内容に関する文章				
	① 事前のやり取りに関する文章		③ ②の提出。 2014年8月 [CONCLUSION ON PESTICIDE PEER REVIEW] https://www.efsa.europa.eu /en/efsajournal/pub/3817 (PYE18)	ビアルビュー結論 ◇データギャップ ・哺乳類毒性:不純物の毒 性影響で1つのデータギャップ ◇発がん性カテュリー2 及び 生殖毒性カテュリー2 みび 生殖毒性カテュリー2 への分 類で、内分泌かく乱性に関 す重要な懸念事項が特定さ れた。 ◇本有効成分の内分泌系 への影響についての情報が 限定的であるため、OECD Conceptual Framework で現 在示されているレイル2 試験 について、データギャップとして 認識される。 ◇地下水中の代謝物は詳 価・規制の対象となる。 ◇作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、トマト及びナス の作物残留試験、レマン についての一下水経由のの指 すったにし、嫌気性土 壊代謝物 CGA19077、 GS23199、CGA249257 につ いての地下水経由の影響は 不明。 ◇評価した地下水シナリオに おいて、関連代謝物 CGA31075 による地下水 基準値 0.1 μg/L を超える ばく靄の可能性があること が重大な懸念事項。 ◇生態影響 ・水生生物への影響について データギャップあり。 ・地下水中代謝物 M3MF、 地表水中及び上壊代謝物 CGA180777、GS23199、 CGA249257 についてデータ ギャンプあり ・散布から開花前の期間ま	④ 再評価評価書	<ul> <li>⑤ 規制内容/その他</li> </ul>
				が重大な懸念事項。 ◆生態影響 ・水生生物への影響につい て データギャッフあり。 ・地下水中代謝物 M3MF, 地表水中及び土壌代謝物 CGA180777, GS23199, CGA249257 についてデータ ギャッフあり		

		[OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters]	上記(PYE18)で引用されて いる OECD Conceptual Framework		
		https://www.bing.com/sear ch?q=OECD+Conceptual+Fr amework&form=ANNTH1&re fig=85f4d2394d184198b786			
		<u>cdbb523b1007&amp;sp=-</u> <u>1&amp;pq=oecd+conceptual+fra</u> <u>mework≻=2-</u>			
		25&qs=n&sk=&cvid=85f4d23 94d184198b786cdbb523b10 07 (PYE19)			

評価	① 事前のやり取りに関する文章	<ol> <li>追加要求された試験項目及び</li> </ol>	③ ②の提出	の有無、再評価結果	(d) Ī	<b>再評価評価書</b>	⑤ 規制	间内容/その他
機関		その内容に関する文章						
EFSA			2013 年 8 月 【REASONED OPINION】 <u>Scientific Opinion on the</u> <u>safety evaluation of the</u> <u>process "INTERSEROH</u> <u>Step 2" used to recycle</u> <u>polypropylene crates for</u> <u>use as food contact</u> <u>material (wiley.com)</u> (PYE20)	アザロール、セロリ、フェンネルの MRL 改訂 ◇エンドボイント・ ADI:0.03mg/kg/日・ ADI:0.03mg/kg/日・ ADI:0.1mg/kg ◇植物代謝 葉面散布後の果実、果菜 類. 根茎類、塊根類、豆類、 菜種類、穀類の代謝試験に 基づき、残留物定義は親化 合物と定義。 ◇作物残留 アザロール(0.7mg/kg)、セロリ (0.03mg/kg)のMRL提案 は、残留物の保存安定性に 関するデータキャワンがあるた め暫定的。 ◇提案されているビメトロジン のアザロール、セロリ、フェンネルへ の使用は、消費者の暴露が 毒性学的参照値を超えるこ とはなく、消費者の健康リスク を引き起こす可能性は低い と結論づけた。	2013 年 5 月 【RAR】 <u>https://www.efsa.europa.eu</u> <u>/sites/default/files/consult</u> <u>ation/consultation/519.zip</u> (PYE21)			
EFSA			2012年10月 【REASOND OPINION】 <u>https://efsa.onlinelibrary.wil</u> <u>ey.com/doi/epdf/10.2903/j.</u> <u>efsa.2012.2939</u> ( <b>PYE22</b> )	Iamb's lettuce と豆類(さや 付き)のMRL改訂 ◇Iンドボイント ・ADL0.03mg/kg/日 ・ARfD:0.1mg/kg ◇果実、根茎類、葉種類、 教類の代謝試験に基づき、 残留物定義は親化合物と 定義。 ◇作物残留試験データの試 料の保存安定性において、 分解を考慮した補正係数を 適用することでの提案は受 け入れられない。従って、 MRL提案は十分でないと結 論付けた。				

評価	① 事前の	のやり取りに関する文章	② 追加要求された	に試験項目及び	③ ②の提出の	D有無、再評価結果	④ 再評価評	価書	⑤ 規制	内容/その他
機関			その内容に関する文章							
機関	2012 年 2 月 【DOSSIER】 (PYE24)	◇申請者 Syngenta が 2010 年 12 月 7 日に再評価用とし てドシェ提出 ◇本有 対成分の登録期限 を 2015 年 12 月 31 日まで 延長。	その内容に関	1する文章	2012年10月 【REASOND OPINION】 https://efsa.onlinelibrary.wil ey.com/doi/epdf/10.2903/j. efsa.2012.2919 (PYE23)	MRL 改訂 ◇エントボイント ・ADI:0.03mg/kg/日 ・ARFD:0.1mg/kg ◇植物代謝 ドマト、ジャガイモ、綿、米の薬 面散布した植物代謝試験で は代謝経路がよく特定さ れ、代謝パターンが類似して いることから残留定義を確 立。一次作物と二次作物の 代謝パターンは類似しており、 輪作作物での重大な影響 はないと結論付けた。 ◇家畜代謝 乳用反芻動物、肉用反芻動 物、豚の代謝試験の結果か ら豚にも外挿可能、組織中 の残留物定義、本リスク評価 時にはビメトロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキン ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンと6-ビトロキシ ルチルビメロジンの合計と定 義。 ◇ 〇 四日 値について一部の MRL 値について一部の MRL 値について一部の MRL 値について一部の MRL 値と見なる検討が必要なため、AnnexII への掲 載を推奨しない。特にこいて てのの雪定的な MRL およ び既存の EU MRL について は、追加の作物残留試験等				
EFSA					2010年11月 【REASOND OPINION】 https://www.efsa.europa.eu /en/efsajournal/pub/1881 (PYE27)	<ul> <li>が必要。</li> <li>lamb`s lettuce と豆類(さや 付き)のMRL改訂</li> <li>◇エンドボイント</li> <li>ADI:0.03mg/kg/日</li> <li>・AR行D:0.1mg/kg</li> <li>◇植物代謝</li> <li>教類気、果実、果菜類、菜種 類気、根茎類、塊茎類の代謝</li> <li>試験において類似した代謝</li> <li>経路が確認されたため、残 留物定義は親化合物と定 義。菜菜類の追加代謝試験</li> <li>は必要ない。</li> </ul>			2011 年 5 月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX.32011R0540&from= EN [CPYE25] 2010 年 11 月 [Office Journal] https://eur- lex.europa.eu/legal- content/EN/TXT/PDF/?uri =CELEX.32010L0077&from= EN (PYE26)	有効成分の認可リ入ト ・純度 950g/kg ・発行日:2001年11月1日 ・有効期限:2015年12月31 日 ・殺虫剤用途 Annex1掲載期限 ・純度:950g/kg ・発効日:2001年11月1日 ・有効期限:2015年12月31 日 ・殺虫剤用途

評価	① 事前のやり取りに関する文章	② 追加要求された試験項目及び	③ ②の提出	の有無、再評価結果	④ 再評価評価書	⑤ 規制	内容/その他
機関		その内容に関する文章					
			2002年7月 【Final Renewal Report】 (PYE29)	最終再評価報告書 ◇エン/ボイン/ ・ADI:0.03mg/kg/日 ・ARED:0.1mg/kg ◇AOEL:0.03mg/kg/日 ・AAOEL:0.1mg/kg ◇該当する地下水シナリオ で、毒性関連代謝物 CGA371075 及び他の代謝 物は基準値0.1 μc/Lを超え る可能性があり、人健康影 響評価に影響がないことは 証明できない。 ◇RAR 記載の GAP では FOCUS シナリオで CGA371075 が0.1 μc/L 以 下になる可能性があると示 されているが、この GAP は 代表的な使用方法ではな く、嫌気性の土壌条件が発 生しうる地域におけるナタネ のリスク評価を最終化できない。 ◇内分泌かく乱作用性: 異なる生物種と生育ステージ で内分泌器官に有害な影響 をもたらしたが、潜在的な内 分泌かく乱作相性: 異なるたが、古在的な内 分泌かく乱作相に基づい て行われた情報に基づい て行われた情報に基づい て、本有効成分の認可は 抹消されるべき。		2007年12月 [Office Journal] https://eur- lex.europa.eu/LexUriServ/L exUriServ.do?uri=OJ:L:2007: 329:0040:0051:EN:PDF (PYE28)	MRL 改訂
EFSA						2001年10月 【Office Journal】 <u>https://eur-</u> <u>lex.europa.eu/legal-</u> <u>content/EN/TXT/PDF/?uri</u> <u>=CELEX:32001L0087&amp;from=</u> <u>EN</u> (PYE30)	Annex I 収載 • 原体純度: 950g/kg • 発行日: 2001 年 11 月 1 日 • 有効期限: 2011 年 10 月 31 日

略号(欧州 EFSA)

AIR	Annex I Renewal	EFSA	European Food Safety Authority	JRC	Joint Research Centre	RASFF	Rapid Alert System for Food and Feed
BfR	Bundesinstitut fur Risikobewertung	EMS	Evaluating Member State	PAFF	Plants, Animals, Food and Feed	RMS	Rapporteur Member State
DAR	Draft Assessment Report	GAP	Good Agricultural Practice	PHI	Pre Harvest Interval	TTC	Threshold of Toxicological Concern
ECHA	European Chemicals Agency	JMPR	Joint FAO/WHO Meeting on Pesticide Residues	RAR	Renewal Assessment Report		

#### 引用 URL とその PDF ファイル(又はワードファイル、エクセルファイル)

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		EFSA Peer Review Report on						
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PYE-19		OECD Conceptual Framework for Te						
PYE-20	Scientific Opinion on the safety evaluation of the process "INTERSEROH Step 2" used to recycle polypropylene crates for use as food contact material (wiley.com)	j.efsa.2013.3348. pdf						
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Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides

December 28, 2016

Office of Pesticide Programs US Environmental Protection Agency



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#### I. PURPOSE & SCOPE

The Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) is a licensing program regulating pesticides in the U.S under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). As part of this program, OPP evaluates a substantial body of toxicology and exposure data to assess the effects of pesticides on human health and the environment. In evaluating human health, EPA looks first for information directly evaluating the potential for effects to people, including epidemiological data. Historically, however, few epidemiology studies have been available to inform the potential toxicity of pesticide chemicals. As such, OPP has in the past primarily relied on toxicology studies in laboratory animals to assess the hazard potential and to estimate human health risk. With the publication of numerous papers from the Agricultural Health Study<sup>1</sup> and from the National Institute of Environmental Health Sciences (NIEHS)/EPA Children's Centers<sup>2</sup>, among others, the availability of epidemiology studies conducted on U.S.-relevant exposures to pesticides is increasing. Nevertheless, since the number of pesticides for which quality epidemiology data either exist or are being developed remains relatively low in the near term, experimental laboratory data will likely continue to be the primary source of data for use in quantitative risk assessment for most pesticides.

OPP's goal is to use such information -- when available -- in a scientifically robust and transparent way. To accomplish this, OPP has developed a general epidemiologic framework, as described in this document, that outlines the scientific considerations that OPP will weigh in evaluating how such studies and scientific information can be more fully integrated into risk assessments of pesticide chemicals. The current document is neither a binding regulation nor is it intended to be or serve as a reviewer's guide or manual or as a Standard Operating Procedure for assessing or using epidemiology data. Nor is it intended to be a full treatise on more modern or advanced epidemiological methods or to adequately convey the nuances and complexity that is important for interpreting these types of studies. As such, it does not discuss (or does not discuss in any detail) such important epidemiological topics as causal inference and causal diagrams (Rothman et al., 2012a; Glymor and Greenland, 2012); more recent approaches to confounder identification, assessment, and control; meta-analysis and heterogeneity and its assessment/evaluation (Borenstein et al., 2009; Greenland and O'Rourke, 2012); or sensitivity/quantitative bias analysis for epidemiologic data (Lash et al., 2009; Lash et al, 2014; Ioannidis, 2008; Greenland and Lash, 2012; Jurek et al., 2007). All these topics, concepts, and issues can and do apply to epidemiology studies concerning pesticides, but are not covered in this OPP framework document. Instead, this document provides overall conceptual considerations concerning the evaluation and use of epidemiology studies on pesticides in

<sup>&</sup>lt;sup>1</sup> https://aghealth.nih.gov/

 $<sup>^{2}\</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers$ 

the context of human health risk assessments to support OPP's FIFRA and FFDCA activities. An earlier version of this document was reviewed favorably by the FIFRA Scientific Advisory Panel (SAP) in February, 2010 (USEPA, 2010; FIFRA SAP, 2010). This document incorporates improvements recommended by the SAP, public comments, and the experience gained since 2010 conducting assessments on several pesticides for which epidemiological data were available, and should be considered a document that will be updated from time-to-time as we progress and on as-needed basis

#### II. INTRODUCTION

Two reports by the National Research Council (NRC) of the National Academy of Science (NAS), "Toxicity Testing in the 21st Century: A Vision and A Strategy (2007)" and "Science and Decisions (2009)," together provide new directions in toxicology and risk assessment. These two NRC reports advocate far reaching changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made. Specifically, the 2007 report on 21<sup>st</sup> century toxicity testing advocates a shift away from the current focus of using apical toxicity endpoints to using toxicity pathways<sup>3</sup> to inform toxicity testing, risk assessment, and ultimately decision making. This approach is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function in human cells. The goal for the new toxicity testing paradigm is to determine how exposure to environmental agents can perturb these pathways, thereby causing a cascade of subsequent key events leading to adverse health effects. Human information like that found in epidemiology studies, human incident databases, and biomonitoring studies, along with experimental toxicological information are expected to play a significant role in this new approach. Specifically, these types of human information provide insight into the effects caused by actual chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. In addition, epidemiologic and human incident data can guide additional analyses or data generations (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies), identify potentially susceptible populations, identify new health effects, or confirm the existing toxicological observations.

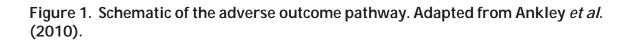
This new vision of toxicity testing and risk assessment will involve data from multiple levels of biological organization ranging from the molecular level up to population-based surveillance with a goal of considering chemical effects from their source to the ultimate health outcome and effects on populations. Such data will come from *in vitro* and *in vivo* experimental studies along with *in silico* and modeled data. OPP's framework for incorporating epidemiology and incident data is conceptually consistent with the 2007 NRC report on 21<sup>st</sup> century toxicity testing in that both emphasize the use of the best available information from multiple data sources are compiled in a weight of the evidence (WOE) analysis.

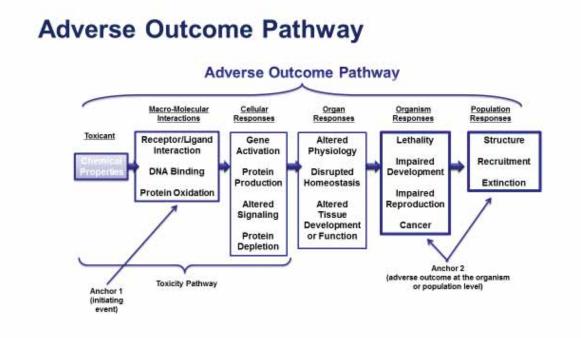
<sup>&</sup>lt;sup>3</sup> Toxicity pathways are cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects.

As a general principle, occupational and environmental epidemiology studies are conducted only on widely used pesticides; these pesticides also tend to have to be wellstudied in the scientific literature. Thus, OPP expects in many cases where epidemiologic data are available, a significant body of literature data on toxicology, exposure, pharmacokinetics (PK), and mode of action/adverse outcome pathway information (MOA/AOP) may also be available. Human incident data are available on a broader range of chemicals, some of which have robust databases and others which do not. In those situations, where there are significant human incident cases and little is known about the MOA/AOP or PK of a particular pesticide, the WOE analysis can be used to identify areas of new research.

OPP's approach in this framework for incorporating epidemiology and human incident data is not a new or novel approach. Instead, this approach is a reasonable, logical extension of existing tools and methods. This document relies on existing guidance documents and frameworks (Table 1) as the starting point for reviewing and evaluating epidemiology and human incident data for use in pesticide risk assessment. This framework on using epidemiology and incident data in human health risk assessment is consistent with the recommendations of the NRC in its 2009 report on *Science and Decisions*, and with the agency's recent Human Health Risk Assessment Framework (USEPA, 2014a) with respect to emphasizing the use of problem formulation as a tool for scoping, planning, and reviewing available, particularly in the context of risk management needs.

Similarly, OPP's framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety MOA/human relevance framework, which highlights the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al., 2014). The MOA/HR framework begins with identifying the series of key events that are along the causal path, that are established on weight of evidence, using principles like those described by Bradford Hill, taking into account factors such as dose-response and temporal concordance, biological plausibility, coherence and consistency (Hill, 1965). Using this analytic approach, epidemiologic findings can be evaluated in the context of other human information (including human incident findings) and experimental studies and for identifying areas of uncertainty and future research. However, it is noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. As the agency continues to move forward in implementing the transformative approach in the 2007 and 2009 NRC reports and as OPP gains experience in integration of epidemiology and human incident information, OPP will re-evaluate and update this framework as appropriate.





### Table 1. Key guidance documents and frameworks used by OPP

	1983: Risk Assessment in the Federal Government: Managing the Process	
	1994: Science and Judgment	
NAS	2007: Toxicity Testing in the 21st Century	
	2009: Science and Decisions: Advancing Risk Assessment	
	2011: NAS report on Formaldehyde	
	2014: Review of EPA's Integrated Risk Information System (IRIS) Process	
	2001-2007: Mode of Action/Human Relevance Framework	
WHO/IPCS	2005: Chemical Specific Adjustment Factors (CSAF)	
	2014: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis.	

	1991-2005: Risk Assessment Forum Guidances for Risk Assessment (e.g., guidelines for carcinogen, reproductive, developmental, neurotoxicity, ecological, and exposure assessment, guidance for benchmark dose modeling, review of reference dose and reference concentration processes) <sup>4</sup>	
EPA	2000: Science Policy Handbook on Risk Characterization	
	2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment	
	2014a. Framework for Human Health Risk Assessment to Inform Decision Making.	
	2014b. Guidance for Applying Quantitative Data to Develop Data-Derived	
	Extrapolation Factors for Interspecies and Intraspecies Extrapolation	
	2001: Aggregate risk assessment	
OPP	2001 and 2002: Cumulative risk assessment	
OECD	2013: Organisation for Economic Co-operation and Development Guidance Document On Developing And Assessing Adverse Outcome Pathways	

Although there are other sources of human information, the focus of this framework is on interpreting and using *epidemiology* and *human incident data* in human risk assessment; other sources of human information are not addressed in this document in any depth. Specifically, this document does not extensively discuss research with pesticides involving intentional exposure of human subjects<sup>5</sup> or on studies done to measure dermal or inhalation exposures in agricultural workers as they perform their activities<sup>6,7</sup>.

<sup>&</sup>lt;sup>4</sup> https://www.epa.gov/osa/products-and-publications-relating-risk-assessment-produced-office-science-advisor

<sup>&</sup>lt;sup>5</sup> Both the conduct of such research and OPP's reliance on data from such research are governed by EPA's Rule for the Protection of Human Subjects of Research (40 CFR Part 26.) Among other things, these rules forbid research involving intentional exposure of pregnant or nursing women or of children, require prior review of proposals for new research by EPA-OPP and by the Human Studies Review Board (HSRB), and require further review by EPA-OPP and the HSRB of reports of completed research.

<sup>&</sup>lt;sup>6</sup> In the last several years, OPP has extensively evaluated existing observational studies with agricultural workers in efforts to improve the data and approaches used in worker exposure assessment; those evaluations can be found elsewhere (http://www.epa.gov/scipoly/sap/meetings/2007/010907\_mtg.htm)

<sup>&</sup>lt;sup>7</sup> For additional information on how such worker exposure studies are conducted and used by OPP, see PPP-48 "Pesticides and human Health Risk Assessment: Policies, Processes, and Procedures "available at <u>https://www.extension.purdue.edu/extmedia/PPP/PPP-48.pdf</u>.

#### III. SYSTEMATIC REVIEW IN PESTICIDE RISK ASSESSMENT: EPIDEMIOLOGY

In recent years, the NRC has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making (NRC 2011, 2014). The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies" (NRC, 2014). Consistent with NRC's recommendations, the Office of Chemical Safety and Pollution Prevention (OCSPP) employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting our decisions.

According to the NRC, systematic reviews "have several common elements: transparent and explicitly documented methods, consistent and critical evaluation of all relevant literature, application of a standardized approach for grading the strength of evidence, and clear and consistent summative language (NRC, 2014)." In recent years, several groups (Rooney et al., 2014; Woodruff and Sutton, 2014; Hartung, 2010) have published systematic review approaches for use in environmental health sciences. The OCSPP approach to systematic review is consistent with the principles articulated in the Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine and with the principles of the Grading of Recommendations Assessment, Development and Evaluation (GRADE). GRADE guidelines used by systematic review approaches for environmental health sciences developed by the National Institute of Environmental Health Sciences (NIEHS) Office of Health Assessment and Translation (OHAT) (Rooney et al., 2014) and University of California, San Diego (Woodruff and Sutton, 2014). According to the *Cochrane Handbook*, the key characteristics of a systematic review are:

- a clearly stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings from the identified studies;
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

Each approach mentioned above share common themes and workflow starting with a statement of scientific context (e.g., problem formulation or protocol) followed by literature review with explicit search strategy methods, analysis of study quality (often called risk of bias), evaluation of the quality of the totality of the evidence (e.g., integration) and ultimately leading to a conclusion(s). Each approach recommends transparent and pre-determined criteria for inclusion/exclusion of scientific literature, evaluation of study quality, and reporting of study quality (e.g., high, medium, low). Each approach recommends a pre-stated tool for data integration that provides the foundation for the conclusion(s).

So far, no single nomenclature has been agreed upon by the risk assessment community for systematic review and OCSPP expects terminology to evolve over time as more broad experience is gained. OCSPP considers its systematic review process and workflow as starting with problem formulation followed by data collection, data evaluation, data integration, and summary findings with critical data gaps identified. Scientific analysis is often iterative in nature as new knowledge is obtained.

### A. Problem Formulation

In the NRC report *Science and Decisions-Advancing Risk Assessment*, the National Academy of Sciences (NAS) recommended to EPA that risk assessments and associated scientific analyses be developed to be useful to policy makers; in order to attain this goal, the NRC recommended that the agency more broadly use problem formulation in developing its risk assessments. In response to the NRC, the agency published the Human Health Risk Assessment Framework (USEPA, 2014) which highlights the importance of problem formulation. Problem formulation entails an initial dialogue between scientists and risk managers and provides the regulatory context for the scientific analysis and helps define the scope of an analysis. Problem formulation draws from regulatory, decision-making and policy context of the assessment, informs the technical approach to the assessment and systematically identifies the major factors to be considered. As such, the complexity and scope of each systematic review will vary among the different risk assessment contexts. In other words, an OCSPP systematic review is conducted as "fit-for-purpose" (NRC, 2009) based on the pre-determined scope and purpose determined from problem formulation.

The problem formulation involves consideration of the available information along with key gaps in data or scientific information. OPP uses problem formulation as a tool to identify exposure pathways and potential health outcomes along with the appropriate methods, data sources, and approaches for the scientific analysis. If missing data are critical to the assessment, options are discussed as to how best to obtain that information (e.g., required testing, research). The peer review process is identified and the timeline for completing the assessment is defined.

Systematic review provides a transparent tool for organizing available information and identifying gaps in information for the regulatory purpose for the analysis. As such, in problem formulation, the regulatory context of a scientific analysis is described which in turn defines the scope of and purpose for collection and evaluation of scientific literature. Some considerations in problem formulation may be related to population or life-stage, exposure pathways (e.g., route, duration, frequency), and/or health outcomes of interest identified from *in vitro* or *in vivo* laboratory studies along with epidemiology or human incident studies along with resources available and regulatory timeframe. In the context of considering epidemiology and human incident information, an initial evaluation of the study quality, study design, and uncertainties are considered. Key scientific issues related to hazard assessment considered in problem formulation include: What are the effects associated with exposure? What are the MOA/AOPs associated with these effects? What are the temporal aspects of the effects? Are there susceptible populations and if so, who are they and what factors contribute to susceptibility? Are there differences in PK or pharmacodynamics (PD) between laboratory animals and humans? Exposure information is also evaluated in problem formulation. Key scientific issues related to exposure assessment considered in problem formulation include: How is the pesticide used? What are all of the relevant use sites of exposure? To what chemical substances will people be exposed? What are the routes, durations, and frequencies of exposures? Who may be exposed? Does the exposure pose different risks to different groups (e.g., due age or activity patterns?) In the specific case of epidemiology data, this review considers a variety of factors including, but not limited to, research hypothesis, study design (i.e., sample size, sufficient controls, quality of measurements, etc.), exposure dose/concentration, statistical analysis, and conclusions.

# B. Data Collection

The data collection phase of systematic review is the collection of available information from various published and unpublished sources, such as the open scientific literature and submitted studies for pesticide registration. OPP reviews data collected under the Organisation for Economic Cooperation and Development (OECD) test guidelines, OCSPP harmonized test guidelines, and other pesticide (OPP guidelines). These guideline studies are collected primarily from in-house databases of submitted studies and are found through searches of such internal databases.

In the case of epidemiology, most studies are expected to be found in the open scientific literature. Although in some cases supplemental analyses or information may be available, dialogue with the researchers may provide additional, important information not published in the original paper in understanding and interpreting epidemiology studies. The sources of human incident information are summarized in Section IV.

Open literature search strategies use specified criteria to retrieve health effects information from the open scientific literature and unpublished sources. After identifying and selecting the most appropriate sources/databases and determining the most resource effective strategy utilizing classification codes, medical subject headings, and/or keywords, a search is conducted of the literature. Depending on the complexity of the scientific evaluation, support from a reference librarian may or may not be needed. The goal of a human health literature search is to perform a reliable and reproducible literature search by providing proper documentation of the literature search process. The following steps are conducted to retrieve relevant studies:

- The purpose of the scientific analysis and inclusion criteria are established.
- Combinations of terms/key words and/or MeSH (Medical Subject Heading) terms and their Boolean combinations (AND; OR; NOT) are used and documented.

Advanced Search and Field Search by author, title, keywords or subject heading may also be performed as needed. Knowledge of database structure, and using a separate search strategy for a specific database is helpful in retrieving relevant studies. In addition to an initial comprehensive search, periodic searches may be conducted to update the literature list.

- The search strategy is documented, including the date(s) of the search(es) to ensure that all the searches of all the databases are reproducible.
- Reference lists of retrieved articles are examined<sup>2</sup> for additional background and to look for articles that were not discovered in the initial search.
- After combining the retrieved articles from different databases and removing duplicates, the available titles and abstracts are screened. For some of the articles where relevance could not be determined from the title and the abstract, the article is retrieved for further review.
- Following the initial screening, articles that were not relevant (exclusion criteria) such as opinion articles, studies not in English, and those consisting only of abstracts are excluded. Additional exclusion criteria can be identified on a case by case basis. All exclusion criteria are documented. The rest of the articles, even those that found no adverse health effects, are included for review and evaluation.

# C. Data Evaluation

In the data evaluation phase, data quality is reviewed and conclusions are made about the utility of such data. Study quality reflects the overall confidence that reports findings are correct (Balshem et al., 2011). As such, study quality can include:

- reporting quality (how well or completely a study is reported);
- how credible the findings are based on the design and conduct of the study;
- and how well the study addresses the topic under review (Rooney et al., 2014).

Study quality is first considered on an individual study basis, and the quality is judged. For example, one may have stronger confidence in a well conducted case control study than a poorly conducted cohort study. Credibility of the scientific findings, often called risk of bias, is evaluated using pre-determined criteria for specific domains related to study design and conduct (See Table 2).

OPP initially developed a guidance on using the open scientific literature considerations called the "Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment" (USEPA, 2012) and generally continues to follow this guidance. However, with the acceleration of systematic review in risk assessment, some aspects of the literature guidance may need updating in the future. Conclusions about the quality of the data are made and can be described in conclusion statements or categories (e.g., acceptable/not acceptable; low, medium, high).

Specific considerations used in evaluating epidemiology studies on pesticide chemicals are provided in Section III.C below. As part of the data review, a concise written review of the study is developed. This written review describes the study design, results, conclusions, and the strengths and weaknesses of the study. The quality of the epidemiologic exposure assessment is an important factor in determining what role epidemiologic data will play in the risk assessment. As such, it is important to fully characterize the assumptions used in the epidemiologic exposure assessment and the degree to which these assumptions affect the interpretation and generalizability of the epidemiologic findings. The evaluation of the epidemiologic exposure assessment may include a consideration of past and present exposure patterns (e.g., exposed populations, pathways, routes, and levels of exposure) and may include significant changes in use patterns (e.g., risk mitigation actions or new use patterns). With regard to evaluating meta-analyses, reporting guidelines for Meta-analysis Of Observational Studies in Epidemiology (MOOSE) have been developed by Stroup et al., (2000) that are useful in evaluating the quality and interpreting meta-analysis.

# D. Data Integration: Weight of Evidence (WOE)

OPP's human health characterizations involve the consideration of all available and relevant data, including but not limited to human studies/epidemiology, biomonitoring data, *in vitro* and *in vivo* experimental laboratory toxicological studies, MOA/AOP information, pharmacokinetic studies, and structure-activity relationships (SAR). Once the different types of hazard data are collected and a full evaluation of each relevant study is conducted and documented, the next step is to integrate multiple lines of evidence.

Data integration is based on the principle of reaching a judgment of the totality of the available negative and positive data for relevant hazards. OPP uses a WOE analysis for evaluating epidemiology and human incident data, such conclusions are made on the preponderance of the information rather than relying on any one study. OPP uses the modified Bradford Hill criteria like those in the MOA/human relevance framework as a tool for organizing and integrating information from different sources (Hill, 1965; U.S. EPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003; Seed et al., 2005; OECD AOP Wiki Users Handbook<sup>8</sup>). It is important to note that the Hill Criteria are not intended as a check box approach but instead are points to consider when evaluating the totality of evidence. In addition, the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. However, even in the absence of a fully developed MOA/AOP, collection and evaluation of mechanistic data may provide support for biological plausibility and help explain differences in tissue sensitivity, species, gender, life-stage, or other factor. The MOA/human relevance framework is a flexible tool which provides a foundation for organizing information without rigidity. It is this

<sup>&</sup>lt;sup>8</sup> https://aopwiki.org/wiki/index.php/Main\_Page#OECD\_User\_Handbook

flexibility that makes it a useful tool for a variety of purposes such as evaluating causality, integrating information across multiple lines of scientific evidence, and identifying data gaps and areas of future research. In this analysis, epidemiologic findings and human incident data can be evaluated in the context of other human information and experimental studies to evaluate biological plausibility, to identify areas of uncertainty and areas of further research. To describe how Bradford Hill aspects are considered in the WOE evaluations, OPP has used some definitions of terms as outlined in EPA's Preamble to the Integrated Science Assessments (ISAs) which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS). (USEPA, 2015).

- **Key events.** In cases where the MOA/AOP are established for a particular health outcome, a clear description of each of the key events (i.e., measurable parameters) that underlie the MOA/AOP are given. Data to inform the key events may come from a combination of *in vitro* or *in vivo* data sources (human or animal). These key events can be a combination of PK and PD events. However, it noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment.
- Biological Gradient/Exposure-Response/Dose-Response Concordance & Relationships. The Preamble to the ISAs notes that "In the context of epidemiology, a well-characterized exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times) (USEPA, 2015)." When the MOA/AOP is known, dose-response relationships are identified for each key event. Dose-response relationships are compared among key events. In some cases, the earlier key events may be more sensitive than later key events. In other cases, key events may share similar dose-response curves.
- **Temporal association**. Evidence of a temporal sequence between the introduction of an agent and appearance of the effect constitutes another argument in favor of causality (USEPA, 2015). The Preamble to the ISAs notes that "Strong evidence for causality can be provided through 'natural experiments' when a change in exposure is found to result in a change in occurrence or frequency of health."

This analysis considers key events which occur rapidly (e.g., metabolism to an active metabolite which could occur within minutes of exposure) and those which occur after longer durations (e.g., development of a tumor) to ensure coherence of the effects. Specific to considering epidemiology data, the temporal relationship between the exposure and health outcome may be considered.

# • Strength, consistency, and specificity.

*Consistency:* An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. Statistical significance is not the sole criterion by which the presence or absence of an effect is determined. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered (USEPA, 2015).

Consistency of findings across studies is informed by the repeated observation of effects or associations across multiple independent studies. Further support is provided by reproducibility of findings in different populations under different circumstances. However, discordant results among independent investigations may be explained by differences in study methods, random errors, exposure, confounding factors, or study power, and thus may not be used to rule out a causal connection (USEPA, 2015).

*Strength of the observed association:* The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may or may not represent a substantial effect in a population (USEPA, 2015).

*Specificity of the observed association:* Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, do environmental exposures invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes (USEPA, 2015).

# • Biological plausibility and coherence.

*Coherence:* An inference of causality from one line of evidence (e.g., epidemiologiccontrolled human exposure, animal, or ecological studies) may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. There may be coherence in demonstrating effects from evidence across various fields and/or across multiple study designs or related health endpoints within one scientific line of evidence (USEPA, 2015).

When animal and human data show a similar toxic profile, both quantitatively and qualitatively, there is high confidence in the human health risk assessment. Whereas in other cases, animal and human data may show a qualitatively similar toxic profile but quantitative differences are observed. For example, a particular chemical exhibits the same MOA/AOP in animals and humans but there may be species differences in dose-response characteristics. These dose-response differences could be due to tissue dosimetry (i.e., PK) or from different response characteristics (i.e., PD). In contrast, animal and human data can, in some instances, show qualitatively dissimilar outcomes. This situation highlights the need to fully and objectively evaluate all available information in a

transparent and comprehensive manner to consider factors such as species, gender, and life-stage differences and potential susceptibilities along with study design considers and exposure potential.

*Biological plausibility:* An inference of causality is strengthened by results from experimental studies or other sources demonstrating biologically plausible mechanisms. A proposed mechanism, which is based on experimental evidence and which links exposure to an agent to a given effect, is an important source of support for causality (USEPA, 2015).

Similarly, information on MOA/AOP for a chemical, as one of many structural analogs, can inform decisions regarding likely causality. Structure activity relationships and information on the agent's structural analogs can provide insight into whether an association is causal (USEPA, 2015).

EPA's Cancer Guidelines (2005) indicate:

"evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies. [p. 39]."

However, The Cancer Guidelines further state that *"lack of mechanistic data, however, is not a reason to reject causality* [p. 41]." As such, lack of established MOA/AOP is not necessary knowledge when using epidemiology data and epidemiology associations may still be valid even in the absence of an established MOA/AOP and may also provide insight into potential MOA/AOP.

• **Uncertainties**. Uncertainties are discussed in the WOE transparently and objectively.

# E. Overall conclusions, recommendations for risk assessment, statement of areas of confidence and uncertainty

It is important to document a summary of the evidence, the procedures or methods used to weigh the evidence, the basis for the WOE conclusion or recommendation, any uncertainties and areas for further research. Recommendations are made on the role of the epidemiologic or human incident data in the risk assessment. Generally, OPP does not use human incident information for quantitative risk assessment but instead to inform risk assessment/risk management activities such as indicating a potential need for a new risk assessment or new risk management measures, evaluating the success of risk mitigation actions after they are implemented, and targeting possible enforcement activities. In contrast to more limited role of human incident data, epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to estimate a risk metric quantitatively. Alternatively, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and animal studies to evaluate the human relevance of animal findings (Hertz-Picciotto, 1995) and may be useful in assessing the biological plausibility of epidemiologic outcomes. In the final portion of the proposed WOE analysis, the overall conclusions along with statement of areas of confidence and uncertainty. This section also identifies areas of additional research. This section recommends the source of data for regulatory values and the appropriate approach for extrapolating between species (if necessary) and among humans.

### IV. REVIEWING EPIDEMIOLOGY STUDIES FOR USE IN PESTICIDE RISK ASSESSMENT

### A. Introduction

Epidemiology is a science that seeks to identify and evaluate relationships between exposure to chemical, physical or biological agents, and the health status of populations (Boyes et al., 2007). It has been defined as the "study of how disease is distributed in populations and the factors that influence or determine this distribution" (Gordis, 2009). More broadly, it is considered as "the study of the occurrence and distribution of healthrelated events, states, and processes in specified populations, including the study of the determinants influencing such processes and the application of this knowledge to control of relevant health problems" (Porta, 2014). The objective of much epidemiologic research is to obtain a valid and precise estimate of the effect of a potential cause on the occurrence of disease. A key objective of epidemiology, like other sciences, is determining cause and effect or - said differently - of identifying the etiology of a disease or health outcome and the risk factors with which it might be associated. Calderon (2000) described four major uses of such studies: 1) describe the health status of a population and discover important time trends in disease and exposure frequency; 2) explain the occurrence of diseases by identifying factors that are associated with specific diseases or trends; 3) predict the number of disease occurrences and the distribution of health states in specific populations; and 4) improving the health status of the population by identifying factors that affect environmental or human health. In the case of pesticides, epidemiology focuses on the relation between exposure and adverse health effects in the general population and in specific sub-populations, such as occupationally exposed workers or applicators.

Epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to quantitatively estimate risk or an appropriate risk surrogate such as an odds ratio or risk ratio. However, many epidemiology studies that deal with pesticides and pesticide exposure suffer some limitations in size, scope, exposure assessment, or data analysis which prevent or otherwise impede their full use in quantitative risk assessment

(Ntzani et al., 2013). Pesticide use in the US has changed significantly over the last few decades. As the use changes, so does the exposure to workers. Changes in pesticide use have occurred due to risk mitigation actions by EPA, resistance management activities, introduction of new chemistries, and increased use of genetically modified crops. These significant changes in exposure have to be taken into account when interpreting epidemiology studies and, ultimately, the decision to use such studies in quantitative risk assessment. Even so, epidemiology studies may be used to compare with evidence from experimental animal studies to characterize assumptions used in deriving such values. In other cases, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and laboratory animal studies to evaluate biological plausibility or human relevance of animal findings (Hertz-Picciotto, 1995). Human information like that found in epidemiology studies are expected to potentially play a significant role in the new vision of toxicity testing recommended by the NRC (2007). Specifically, epidemiology studies can provide insight on health outcomes that may arise from real-world chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. Human information may guide additional studies (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies); and identify novel health effects or host susceptibilities which can be investigated with future research.

When laboratory data from animal studies provide the primary source of information for hazard characterization, one potential source of uncertainty is the relevance of animal models to humans. In the absence of data to support the contrary, animal findings are assumed to be relevant to humans. Furthermore, EPA assumes that humans are more sensitive than laboratory animals in the absence of data to support the contrary. In actuality, humans may be more or less sensitive to pesticides than other animal species. Epidemiology and human incident data can provide scientific information and support to inform uncertainties associated with species extrapolation. With respect to population variability, epidemiology studies better characterize potential variability than do animal studies. Specifically, epidemiologic data include the genetic diversity, and variability inherent in human populations and thus can better account for and represent actual population response to environmental chemicals than laboratory animals (Calderon, 2000).

With respect to dose-response characterization, animal toxicology studies have the benefit that studies can be designed to cover a broad range of exposure levels. However, animal toxicology studies generally use exposures which are much larger (sometimes orders of magnitude) than those that occur in the environment. These high exposure levels in animal studies dictate the need for extrapolation from high to low doses. This extrapolation introduces added uncertainty into the risk assessment. Epidemiology studies and human incident data involve actual real-world exposures and thus high dose extrapolation may in many cases not be needed. Epidemiology studies conducted over a range of exposures (from low to high) are most useful.

Animal studies do not replicate the length, magnitude, duration, routes of exposure and variability in exposure experienced by humans (Calderon, 2000). Human exposure often occurs through multimedia exposure pathways, including food, water, air, and indoor and outdoor environments. In contrast, controlled laboratory studies typically use a single

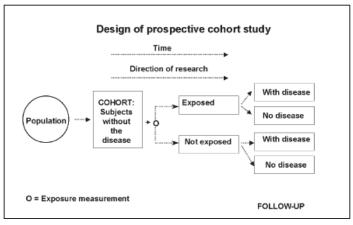
route of exposure. In addition, humans may experience exposure to multiple chemicals and/or non-chemical stressors simultaneously, whereas most animal studies involve a single chemical stressor. On one hand, this multi-chemical exposure in epidemiology studies can provide a challenge when attempting to attribute epidemiologic outcomes to a single pesticide chemical. On the other hand, epidemiologic research considers real-world exposures and may help, when considered along with experimental approaches, address questions associated with multiple chemical exposures which can be difficult to evaluate in an experimental setting.

# B. Types of Epidemiology Studies

The major types of observational epidemiologic studies are described briefly below with consideration of their strengths and weaknesses (Lilienfeld and Lilienfeld, 1979; Mausner and Kramer, 1985; Kelsey et al., 1996; Rothman and Greenland, 2012; Paddle and Harrington, 2000; USEPA, 2005; Purdue Pesticide Programs, PPP-43).

**Cohort studies** begin with a group of people that share common characteristics—the cohort—and evaluate their health over an extended follow-up time period during which the occurrence of disease is recorded (see figure box from van den Brandt et al. (2002)). The common characteristic is often the presence vs. absence of "risk factors" (such as

exposures)<sup>9</sup>. In such studies, differences in disease occurrence between the "exposed" and "nonexposed" individuals are identified and studied over time to determine differences in the rate of disease<sup>10</sup>. This difference in the rate of disease occurrence is then investigated to determine if the rate of disease differs between the exposed and non-exposed groups. Cohort studies have the ability to simultaneously evaluate multiple disease outcomes



under study (which is not true for case-control studies, which are generally limited to evaluating only a single (pre-specified) disease outcome, discussed below). Cohort studies can also be performed either prospectively, like the Agricultural Health Study (AHS, <u>http://aghealth.nci.nih.gov/</u>), or retrospectively from historical records. A prospective cohort design focuses on a group of people from a current point in time through a future point in time. A retrospective cohort design focuses on a group exposed at some point in the past, and compares disease rates after exposure occurred (generally through existing

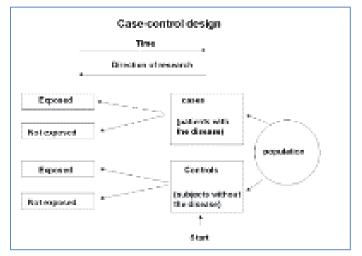
<sup>&</sup>lt;sup>9</sup> While exposure is often dichotomized on an exposed vs. non-exposed basis in cohort studies, exposure can also be measured on a quantitative scale (e.g., by a continuous measure or by quantiles)

<sup>&</sup>lt;sup>10</sup> Cohort studies commonly study differences in rates of disease, but these can also include other focal outcomes of interest such as birth weight, mental abilities, blood pressure, etc.

available exposure databases (or records) available on a person-by-person (individual) basis). Prospective cohort studies can be relatively lengthy and expensive to conduct, particularly for rare diseases, and require a large number of subjects to be under study. Importantly, significant resources and professional staff are required for a long period of time to collect high quality data.

**Case-control studies** are studies in which groups of individuals with (cases) and generally without (controls) a given disease are identified and compared with respect to (generally past<sup>11</sup>) exposure to determine whether those with the disease of interest are

more likely or no more likely to have been exposed to the agent(s) or factor(s) of interest. That is, the analysis of case-control studies contrasts the frequency of exposure of the agent or factor in the cases with those in the controls to determine if these differ and, thus, whether there is a differential association. In case-control studies, determination of the disease status (i.e., cases with the disease; controls without) generally precedes determination of the exposure status (see figure box from van den Brandt et



al. (2002)) Because disease has already occurred at the time of selection into the casecontrol study, this study design is particularly useful in studying uncommon diseases or diseases with long latency and can be utilized to evaluate the relation between many different exposures and a specific (pre-specified) disease outcome of interest. And because case-control studies begin with individuals who have the disease, the studies can involve fewer subjects than cohort studies and can be completed in a comparatively shorter time frame. Challenges in case-control investigations include the selection of an appropriate control group and the assessment of exposures which may have occurred long before the disease was diagnosed (Rothman, 2012; Wacholder et al. 1992a; Wacholder et al. 1992b; Wacholder et al. 1992c; Shultz and Grimes, 2002; Grimes and Schultz, 2005). Case-control studies can be particularly susceptible to "recall bias" in which diseased individuals may remember exposures or events differently (generally better) than those who serve as the controls and are healthy.

**Nested case-control studies** are an example of a hybrid design and contain the elements of a cohort and a case-control study. These designs can be useful when the analytical costs for determining pesticide exposure are too high for the entire cohort to be studies. For example, a cases that that have developed the disease or health outcome in an

<sup>&</sup>lt;sup>11</sup> It is possible for case-control studies to be done prospectively in which the cases have not yet developed the disease until after the study begins under which circumstance the cases are enrolled in the study over time.

ongoing cohort study can be matched with appropriate controls from the study that have not yet developed the disease or outcome of interest at the time of the analysis. One recognized advantage of the nested case-control study (as opposed to a more standard case-control study) is that the issues of selection bias and recall bias are minimized.

Cross-sectional studies focus on the prevalence of disease (e.g., birth defects, smallfor-gestational age or SGA), symptoms, biological/physical and physiologic response measurements (e.g., pulmonary function tests, blood pressure, chest X-ray, clinical examinations, liver and kidney biomarkers). A key feature of such studies is that they are observational studies which focuses on the *prevalence* as a frequency measure, with the presence or absence of disease determined at the time of sampling or over a sampling period. Prevalence is the proportion of individuals in a population that has the disease and can either be determined as a "point prevalence" or as a "period prevalence".<sup>12</sup> A prevalence is a proportion not a rate and thus the cross sectional studies do not involve a follow up period. Typically, the exposure status (e.g., exposed or unexposed), disease status/outcome, and demographic characteristics are determined at a point in (or over) time. The major comparison in this study design is a comparison of the prevalence of the outcome in the exposed population vs. the prevalence of that outcome in the non-exposed population, with the risk measure being the prevalence risk ratio or odds ratio. Crosssectional studies are generally used to identify patterns or trends in disease occurrence over time or in different geographical locations, and can be conducted guickly and relatively inexpensively. However, they measure the prevalence of a disease outcome which is affected by both incidence – the rate of occurrence of new cases – and duration of the disease, and it can be difficult in any analysis to sufficiently separate these factors. Thus, they involve "survivor populations" and do not measure, evaluate, or consider those that have left the population of interest because they became ill. Another important limitation of cross-sectional studies is they do not allow one to determine whether exposure precedes the disease. As such, cross-sectional studies are unable to establish temporal relationships between disease and exposure and typically require additional studies to confirm a hypothesized causal association suggested by a cross-sectional study.

**Ecologic studies** examine exposure and disease patterns using information reflecting group or population-level data. In an ecologic study, the unit of analysis is a group and not an individual<sup>13</sup>. Here, groups of subjects are sampled, with the exposure, disease, and potential confounding factors measured at this group (or cluster) level. Groups are generally defined on a geographic, administrative, or organizations unit basis (e.g., districts, towns, counties, schools, workplaces, etc.) with all exposure, disease, or confounder measurements made or summarized at the group level rather than at the level of the individual. An ecological (group-based) study contrasts with an individual-level study in that in the former there is no information on whether the cases are the actual individuals

<sup>&</sup>lt;sup>12</sup> The former involve measurements at a particular place and/or a particular time while the latter involves determinations of the proportion of cases over a given time period.

<sup>&</sup>lt;sup>13</sup> Some studies can be "partially ecologic" in design in which either the exposure or the disease outcome is measured on a group level but the other variable is measures at an individual level with the researcher making inferences to the individual level.

with the exposure whereas in the latter exposure information is tied to the individual. As an example, a study of disease rates by contaminant levels in water can be ecologic with respect to evaluation of the exposure, but the health outcome or disease status may have determined on an individual basis. In these instances, the term "semi-ecological" can sometimes be used when exposure is determined at the group level but outcome is determined at the level of the individual.

Using this design, it is not possible to know whether all members of the exposed group are individually exposed (or the individual exposure levels) nor is it possible to infer individual-level effects from the group level effects that result. If the intent of the study is to direct inferences to the *group* (rather than the individual), then this is <u>not</u> a concern and these studies can be appropriate, particularly if measurements are constrained or difficult to perform at the individual level and exposures within the group are generally homogenous. If the intent of the study is instead to direct inferences to the individual, then this study design suffers from what is termed the ecological fallacy: the assumption that an observed relationship in an aggregated or grouped data set will reflect what would have been observed had the sampling occurred at the individual level. In addition to this ecological fallacy issue, an additional bias arises a result of the inability to appropriately control for confounding variables at the level of the individual as opposed to the group when information on confounding factors is only available at the group level.

In most cases, ecologic studies are considered as hypothesis-generating studies and best used for suggesting research hypotheses for future studies and may contribute to problem formulation. Nevertheless, it is important to assess ecological studies on the basis of the quality of their design, and useful information can be gleaned from an ecologic study if it is well-designed (FIFRA SAP, 2010). Ecologic studies alone generally do not have the ability to establish a causal association. When taken with other these studies can be useful under certain circumstances and should be noted in the hazard characterization. In particular, stable populations, clear exposure contrasts, and large differences in risk can be important factors that might increase the utility of these studies.

### C. Evaluating epidemiology studies for use in pesticide risk assessment

OPP searches the peer reviewed literature for observational epidemiology studies of potential adverse acute and chronic health effects linked to chemical use. Details regarding literature search protocols and strategies are provided elsewhere. Epidemiologic research utilizing cohort, case-control, or cross-sectional study designs may provide information to OPP to strengthen OPP's understanding of the potential hazards, exposure-response characterization, exposure scenarios. or assessment methods, and – ultimately -- risk characterization (van den Brandt, 2002). In addition, compelling case reports or case series analysis may illumine a health effect or mechanism of action previously unidentified.

Generally speaking, the quality of epidemiologic research, sufficiency of documentation of the study (study design and results), and relevance to risk assessment is considered when evaluating epidemiology studies from the open literature for use in OPP's

risk assessments. It is important that these criteria are endpoint-specific as various methodological details become more or less important given the endpoint of concern. For example, it is important to understand relevant factors that influence outcome ascertainment (*e.g.*, is there a test or a biomarker available to indicate presence of an effect, or are symptoms gradual and non-specific initially leading to physician diagnosis upon advanced disease state). In addition, for environmental and occupational epidemiology studies, the quality of the exposure assessment is vitally important. Prior consideration must be given to aspects of exposure and confounder measurement to the question under consideration.

When considering individual study quality, various aspects of the design, conduct, analysis and interpretation of the epidemiology studies are important. These include:

- 1. Clear articulation of the hypothesis, even if the study is hypothesis-generating in nature;
- 2. Adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment,
- 3. Reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population),
- 4. Appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias,
- 5. Adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed,
- 6. Overall characterization of potential systematic biases in the study including errors in the selection of participation and in the collection of information; this can include performing sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented (*e.g.*, Greenland's formula)
- 7. Evaluation of the statistical power of the study to observe health effects with appropriate discussion and/or presentation of power estimates,
- 8. Use of appropriate statistical modeling techniques, given the study design and the nature of the outcomes under study

Other Federal and non-Federal entities have offered such guides (*e.g.*, OHAT, Navigation Guide, National Toxicology Program [NTP] Report on Carcinogens [ROC<sup>14</sup>], IRIS, Cochrane ACROBAT-Non-Randomized Studies of Interventions) (Sterne et al., 2015 as well as the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for observational epidemiological studies (see <u>www.strobe-statement.org</u> and Vandenbroucke et al., 2007; Von Elm, 2014) As OPP gains experience with integrating epidemiology studies into human health risk assessment, relevant adjustments to its evaluation approach will be made.

<sup>&</sup>lt;sup>14</sup> http://ntp.niehs.nih.gov/pubhealth/roc/index.html

Independent study evaluation is performed and documented prior to the development of evidence- tables of detailed summary tables which are informative to hazard identification and exposure response assessment. Table 2 provides a structure to the major considerations evaluated and the associated weight (low, medium, high) for each consideration. Table 2 provides a generic set of considerations and should not be considered a checklist. The specific scientific considerations appropriate for particular science analysis are adjusted on a case by case basis.

The culmination of the study evaluation process would be to provide professional/expert opinion as to the nature of the potential bias that may result from systematic errors in each specific study identified through study specific evaluations, and an assessment of overall confidence in the epidemiological database. In this way, data integration (animal, human, mechanistic, other) would be informed by level of confidence in the human epidemiological studies that inform human health effects of environmental and occupational exposures.

	aKind et al., 2014)					
Parameter	High	Moderate	Low			
Exposure assessment	Accurate and precise quantitative relationship with external exposure, internal dose, or target dose, possibly associated with an MOA/AOP. If questionnaire utilized, questionnaire and/or interview answered by subjects for chemical-specific exposure	Evidence exists for a relationship between biomarker in a specified matrix and external exposure, internal dose, or target dose. Questionnaire and/or interview for chemical- specific exposure answered by subjects or proxy individuals	Poor surrogate Low-quality questionnaire and/or interview; information collected for groups of chemicals rather than chemical-specific; no chemical-specific exposure information collected; ever/never use of pesticides in general evaluated			
Outcome Assessment	Standardized tool, validated in study population; medical record review/diagnosis confirmation by trained staff; appropriate consideration of prevalence/incidence of cases	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated	Selected sections of test, or maternal report, other; or, maternal/paternal self-report; unclear/no consideration for whether prevalent or incident cases are appropriate			
Confounder control	Good control for important confounders relevant to scientific question, and standard confounders	Moderately good control confounders, standard variables, not all variables relevant for scientific question	Multi-variable analysis not performed no adjustments; no stratification, restriction, or matching			
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly			
Risk of (other) bias (selection, differential misclassification, effect size magnification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding			

# Table 2. Study Quality Considerations <sup>a</sup> (Adapted from Munoz-Quezada et al., 2013; LaKind et al., 2014)

<sup>a</sup> Overall study quality ranking based on comprehensive assessment across the parameters.

# 1. Exposure Assessment

Exposure assessment can be defined as the "process of estimating or measuring the magnitude, frequency and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, pathways, routes, and the uncertainties in the assessment. (Zartarian et al., 2005)." In environmental epidemiology, exposure assessment poses a unique challenge, particularly for toxicants that are found in low concentrations in environmental media (NRC, 1991; NRC, 1997). Given the complexity of exposure pathways, researchers have developed a number of different approaches to assess exposure, which vary in accuracy, precision, and resource requirements (Niewenhuijsen, 2003). Some of these approaches are not specific to epidemiologic research but may be used to inform exposure assessment in a variety of scientific analyses. These approaches include indirect methods, based on historical records, questionnaires, and environmental monitoring, and direct methods, based on personal monitoring and biomonitoring. A brief description of each method and its strengths and limitations is summarized below.

Table 5. Summary of multi-ect and un ect exposure assessment methods.			
Approach	Method/Tools	Example	Exposure Estimation
Indirect	Historical Records	Estimating proximity to agricultural crops using address information	Dichotomous or ordinal exposure
	Questionnaires	Determine potential for exposure based on pesticide-use responses	Dichotomous or ordinal exposure
	Environmental Monitoring	Measuring pesticide levels in community water drinking system	Dichotomous or ordinal exposure, although exposure can be estimated using modeling
Direct	Personal Monitoring	Measuring pesticide inhalation and dermal contact	Quantified exposure
	Biomonitoring	Measuring pesticide levels in blood and urine	Quantified internal dose

Table 3. Summary	of indirect and direct ex	posure assessment methods.
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Historical records and questionnaires are used to characterize key characteristics which may be associated with chemical exposure. When used in epidemiologic studies, historical records and questionnaires are not typically used to predict quantitative levels of exposure. Rather, historical record information or questionnaire responses are used to assign categorical levels of exposure. Examples of historical record information that can be used to assign exposure levels includes address in proximity to an agricultural crop and employment history information on job title and history. Similarly, questionnaires can be used to determine if individuals recall using pesticides or identify individuals that perform specific job functions that increase their potential for exposure. While historical records and questionnaires can be cost-effective sources of data on potential exposure, they do have limitations. Data collected from historical records and questionnaires is only a surrogate of exposure. As a result, these data sources may be an oversimplification of exposure and not accurately rank individual's exposure potential.

**Environmental monitoring** is used to characterize the levels of contaminants in environmental media, including air, water, soil, food, and home and work environments. Many state and Federal programs collect environmental monitoring data that may be useful in epidemiologic studies. Environmental monitoring is particularly useful for exposure that can be defined by geographic boundaries, such as air pollution and drinking water. As such, many epidemiologic studies have utilized ambient air monitoring data and community drinking water system data to characterize exposure to air pollution and drinking water contamination, respectively. While environmental monitoring data is useful for estimating exposures defined by geographic boundaries, it can be less reliable for the purposes of assigning individual-levels exposures, particularly when individuals live, work, and spend time in many different locations.

**Personal monitoring** is used to characterize exposure at the point of contact of a body boundary. Examples of personal monitoring include the use of dosimeters to assess dermal contact with pesticides, personal air sampling devices to assess inhalation exposure, and collection of duplicate diet samples to determine pesticide levels in food. The advantage of personal monitoring is that it is likely to provide more accurate estimates of individual-level exposure than indirect methods. Personal monitoring also makes it possible to guantify exposure levels that can be useful for prioritizing the relevance of different routes of exposure. Additionally, personal monitoring can also be used to assess longitudinal exposure when repeated measurements are taken over time. While personal monitoring offers many advantages over indirect approaches, it also tends to be labor and resource intensive (Niewenhuijsen, 2003). As a result, it is not typically feasible to conduct large-scale epidemiologic studies that assess exposure using personal monitoring. Furthermore, personal monitoring is highly dependent on the measurement techniques and analytic tools used to obtain samples and it is less likely that information that characterizes exposures during the relevant time period (usually in the past) will be available. In addition, it is unlikely that the full range of exposures over the time period of interest will be captured, and sampling may not be over a sufficient time period to capture peaks and fluctuations As such, it is extremely important to consider the scientific rigor and reliability of personal monitoring methodologies that are used in epidemiologic studies, and such monitoring may need to be supplemented by other monitoring (e.g., environmental, biological, and/or interview/questionnaire data).

**Biomonitoring** is used to characterize exposure by measuring a chemical, its metabolite(s), or reactive product(s) in biological samples, such as blood, urine, saliva, milk, adipose, and other body tissues (Needham et al., 2007). Zartarian et al. (2005) state that "a biomarker/biological marker has been defined as an "indicator of changes or events in biological systems. Biological markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicators of health effects (LaKind et al., 2014). Table 4 provides scientific considerations for evaluating the quality and relevance of biomonitoring data

collected from epidemiology studies. Assessing exposure using biomonitoring has expanded rapidly as analytical tools have become more cost-effective and more biomarkers are identified. Compared with self-reported questionnaire or interview data, biomonitoring may reduce exposure misclassification and enhance the precision of the risk estimates. Similarly, biomonitoring integrates exposures from different routes and can be used to determine the amount of exposure that is absorbed into the body (Checkoway et al., 2004). Furthermore, knowledge as to the role of the biomarker in the natural history of disease is known in certain instances, such that biomarkers may help resolve temporality of exposure issues.

While biomonitoring has many advantages over others exposure assessment methods, it also has its own limitations. In many studies, biological sample are only taken from a single point in time and may not reflect accurately reflect longitudinal patterns, particularly if exposures are highly variable. Furthermore, evaluation of biomarkers also requires an understanding of degradation and metabolism of chemicals in both the environment and human body. As such, biomarkers of exposure may differ between individuals for reasons other than exposure level. Differences in metabolism, comorbidities such as kidney disease in relation to urinary measurements, uncertainty as to whether the biomarker measures exposure to the active ingredient or the environmental degradates may all account for apparent differences in biomarkers of exposure among individuals, and possibly between comparison groups. Table 4. Considerations of biomonitoring data from environmental epidemiology research (Adapted from LaKind et al. (2014).

Biomarker Consideration	Tier 1	Tier 2	Tier 3
Exposure biomarker	Biomarker has accurate and precise quantitative relationship with external exposure, internal dose, or target dose.	Biomarker has an unknown quantitative relationship with external exposure, internal dose, or target dose or is poor surrogate (low accuracy and precision) for exposure/dose.	NA
Effect biomarker	Bioindicator of a key event in a MOA/AOP.	Biomarkers of effect for which the relationship to health outcome is understood	Biomarker has undetermined consequences (e.g., biomarker is not specific to a health outcome).
Specificity	Biomarker is derived from exposure to one parent chemical.	Biomarker is derived from multiple parent chemicals with similar toxicities.	Biomarker is derived from multiple parent chemicals with varying types of adverse endpoints.
Method sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question.	Frequency of detection too low to address the research hypothesis.	NA
Biomarker stability	Samples with a known history and documented stability data.	Samples have known losses during storage but the difference between low and high exposures can be qualitatively assessed.	Samples with either unknown history and/or no stability data for analytes of interest.
Sample contamination	Samples are contamination-free from time of collection to time of measurement (e.g., by use of	Study not using/documenting these procedures.	There are known contamination issues and no documentation that the issues were addressed

Biomarker Consideration	Tier 1	Tier 2	Tier 3
	certified analyte-free collection supplies and reference materials, and appropriate use of blanks both in the field and lab). Research includes documentation of the steps taken to provide the necessary assurance that the study data are reliable.		
Method requirements	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (e.g., GC- HRMS, GC-MS/MS, LC-MS/MS)	Instrumentation that allows for identification of the biomarker with a high degree of confidence and the required sensitivity (e.g., GC-MS, GC- ECD).	Instrumentation that only allows for possible quantification of the biomarker but the method has known interferants (e.g., GC-FID, spectroscopy)
Matrix adjustment	Study includes results for adjusted and non-adjusted concentrations	Study only provides results using one method (matrix-adjusted or not).	NA

FP = false positive; FN = false negative; GC-HRMS = gas chromatography/high-resolution mass spectrometry; GC-MS = gas chromatography/mass spectrometry; GC-ECD = gas chromatography-electron capture detector; GC-FID = gas chromatography-flame ionization detector], ICC = intra-class correlation coefficient; NA = not applicable; PFP = probability of false positive

Indirect exposure assessment methods are common in retrospective studies and based on factors that are surrogates of chemical exposure. As described above, indirect exposure data cannot generally be used to estimate quantitative exposure levels without additional modeling. For example, a questionnaire can be used to determine if an individual has ever used a pesticide, but can less reliably collect data on all the environmental and behavioral factors that are needed to calculate that individual's exposure. As such, indirect exposure data are often used to classify exposure using a dichotomous exposure variable (i.e. exposed/unexposed) or ordinal exposure scale. In contrast, direct exposure assessment methods are based on data on actual individual-level exposure through personal monitoring and biomonitoring. Thus, direct methods are more common in prospective studies, but are also used in retrospective studies when existing biological samples are available from well-defined population groups.

<u>**Ouantified personal measurements**</u>, such as personal monitoring and biomonitoring, are generally considered the best source of data for estimating actual exposure levels (NRC, 1991; NRC, 1997). While this is the case, accurate qualitative measures of exposure (e.g. dichotomous and ordinal exposure metrics) from indirect methods can be just as accurate for the purpose of epidemiology. Moreover, indirect methods are often easier to interpret and may require less additional research and development to demonstrate their utility in exposure assessment.

Regardless of the approach, exposure assessment methods should be able to provide exposure estimates that are reliable and valid. In the context of epidemiology, *reliability* general refers to the ability to reproduce results and *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). When evaluating a particular exposure assessment's reliability and validity, it is important to consider the exposure assessment's strengths and weaknesses in the context of the study's research objectives. Less refined exposure assessment may be suitable for exploratory studies. This is because exploratory studies help raise awareness about potential hazards that can encourage investment in more focused research. Conversely, studies with more focused hypotheses can be greatly strengthened through the use of more refined exposure assessment methods. Therefore, indirect and direct exposure assessment methods represent a spectrum of tools that are complimentary and can be used at different stages of research when exploring exposure-disease relationships.

# 2. Confounding Factors

Confounding occurs when the relationship between the exposure and disease is to some extent attributable to the effect of a second (confounding) risk factor. This can happen when this second (i.e., confounding) risk factor is an independent, causally-associated risk factor for the disease but is also associated -- causally or non-causally -- with the exposure under analysis and does not also serve as an intermediate variable in the causal pathway between the exposure and the outcome of interest. If not properly measured and accounted

for, confounders have the ability to change the magnitude (and potentially the direction) of the estimated association between an exposure and health outcome. This can result in an over- or under-estimation of the relationship between exposure and disease because the effects of the two risk factors have not been appropriately separated, or "disentangled". As an example: a given pesticide may be associated with lung cancer in a given study, but this may be due to a confounding effect of farm tractor diesel fumes: here, this second factor farm tractor diesel fumes - would be a confounder if it was causally associated with the disease outcome (here, lung cancer) but also associated with pesticide exposure. Confounding factors may include less intuitive lifestyle exposures such as cigarette smoking, dietary factors (e.g., high energy/calorie laden diet), and physical activity (e.g., lack of physical activity) genetics, comorbidity, medication use, alcohol consumption, etc., all of which may adversely affect health and may be statistically associated with pesticide use. In epidemiological analyses, confounding factors are measured in the study sample and typically "adjusted for" in the final risk estimate in either the design phase of the study or the analysis phase. With respect to the former, the epidemiological researcher can "restrict" the study population to individuals that share a characteristic which the researcher wishes to control; this has the result of removing the potential effect of confounding caused by that (now controlled) characteristic. A second available method – also applicable to the design phase of the study -- is for the researcher to control confounding by "matching" individuals based on the confounding variable. This ensures that the confounding variable is evenly distributed between the two comparison groups and effectively controls for this. It is important to note that the relationship between the confounder and the exposure or outcome does not need to be found to be statistically significant in order for it to have an impact on the risk estimate for the main effect<sup>15</sup>.

At the analysis stage, one method by which confounding can be controlled is by stratification. Under this means of control, the association is measured separately under each of the (potentially) confounding variables; the separate estimates are "brought together" statistically -- if determined to be appropriate -- to produce a common odds ratio or other effect size measure by using Mantel-Haenszel approaches which weight the estimates measured in each stratum. Stratification can be difficult if there are multiple potential confounders that need to be controlled simultaneously. In such cases, confounding is typically dealt with by means of statistical modelling. (e.g., logistic regression).

It is important that careful consideration be given to confounders prior to any epidemiological studies being initiated in the field and it is important that any study adequately describe how this was done: epidemiological studies are frequently critiqued for ignoring or paying insufficient attention to potential confounders. For this reason, a sensitivity analysis can be helpful to demonstrate the potential effects that a missing or unaccounted for confounder may have on the observed effect sizes (see Gustafson and

<sup>&</sup>lt;sup>15</sup> This is why it is generally considered inappropriate to "statistically test" for a confounder to determine whether the confounder needs to be adjusted for. Instead, some consider a change in the effect size of 10% or more after adjustment for (inclusion of) a potential confounder to be sufficient evidence for the confounder to be incorporated into the analysis.

McCandless, 2010). If unmeasured confounders are thought to affect the results, researchers should conduct sensitivity analyses to estimate the range of impacts and the resulting range of adjusted effect measures. Such sensitivity analyses -- generally not uniformly conducted in most published epidemiological studies – can be used when available to estimate the impact of biases and potential confounding by known but unmeasured risk factors.

Depending upon the specific exposure-disease association under study, a factor may or may not be a confounding factor that is necessary to control: in order for a substantial distortion in the effect size estimate to occur due to confounding, the confounder must be not only a relatively strong risk factor for the disease of interest<sup>16</sup>, but also be strongly associated with the exposure of interest. Assessment of potential confounding is made on a study specific basis and - if unmeasured confounders are thought to affect the results -researchers should conduct a sensitivity analysis to estimate the range of impacts and resulting range of adjusted effect measures. When evaluating the guality of observational epidemiology studies, OPP will consider whether relevant confounding factors are properly identified, described, measured and analyzed such that an unbiased estimate of the specific association under study can be made, and, when possible, may consider sensitivity analysis as a potential tool to assist in determining the degree to which such confounding might potentially affect the estimate of the effect size. It should be emphasized that a confounder must be a relatively strong risk factor for the disease to be strongly associated with the exposure of interest to create a substantial distortion in the risk estimate. In such cases, it is not sufficient to simply raise the possibility of confounding; one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be, and how important that impact might be to the interpretation of findings. (p. 23-25, FIFRA SAP Report, 22 April 2010)

Finally, it is important to distinguish between confounding, effect modification, synergy, and other mediating effects of covariates. Confounding is a bias that results from not controlling for a variable that is associated causally with the disease and associated – causally or non-causally -- with the exposure of interest. Epidemiologic researchers seek to minimize this bias. Effect modifiers -- on the other hand -- are variables that differentially affect the magnitude of the effect size, by strata (e.g., age, race/ethnicity, SES status, genetic polymorphisms). Effect modifiers may or may not also be confounders. Typically, they are modelled by either introducing interaction terms in multivariable models or by evaluating effect sizes by strata after stratifying the data by levels of the effect modifier. A study frequently needs to be specifically designed to evaluate effect modifiers in order to have a sufficient sample size in each population strata of interest. Epidemiologic researchers seek to understand effect modifiers (not minimize them, as they do with confounders) because they can be important in evaluating risk differences across population strata, in evaluating the association between exposure and the effect of interest, and in identifying susceptible

<sup>&</sup>lt;sup>16</sup> Consideration needs to be given not only to ensuring that the confounding factor is indeed a risk factor on its own but also to ensuring not only related to the exposure of interest. Adjusting for a factor that has an association with the disease of interest wholly or partly because of its association with the exposure of interest will lead to attenuation of the exposure-disease relationship if it truly exists.

subpopulations. Effect modifiers may or may not also be confounders. For example, smoking may be a confounder in a study associating lung cancer with a pesticide often used on tobacco, but it may also be an effect modifier if the risk of exposure to this pesticide is higher among smokers than non-smokers. Synergy is often introduced as a biological or pharmacological/toxicological concept rather than an epidemiological one and relates to the ability of two chemicals, together and acting jointly, to magnify or exaggerate the effect beyond that which would be seen considering the (mathematical) sum of each chemical's effects alone. In epidemiological and statistical terms, this is often expressed as effect modification or interaction.

### 3. Statistical Analysis

Epidemiologic studies are designed to measure an association between a specific exposure and a disease. When evaluating the quality of pesticide epidemiology studies, OPP will also consider the statistical methods used. Specifically, OPP will consider the extent to which the analytic methods described in the study are appropriate to the research question; the completeness of the description of the statistical methods utilized; the appropriateness of the methods for identification, assessment and adjustment of potentially confounding variables in the exposure-disease relation; and, the description, extent of, and presentation of any sub-group analyses which may have been performed (including whether statistical corrections for multiple comparisons have been made).

Epidemiologic investigations typically utilize statistical modeling to estimate risk (e.g. generalized linear models such as logistic (for odds ratios) or Poisson (for count data) regression. To do so, researchers must consider not only the relevant main exposure and outcome variables, but also consider relevant confounding factors, and whether the association under investigation may differ by level of these factors, i.e., effect modification or interaction (Szklo et al., 2004). Upon identification of a potentially confounding variable -- one that substantively changes the magnitude and/or direction of the association under study -- adjustment through regression modeling can help to isolate the risk estimate of interest, i.e., the association under study. In addition, OPP will evaluate the stratification of statistical interaction. If the magnitude and direction of the association of interest differs greatly by level of a third variable, then the stratified results should be considered primary.

When performing statistical modeling when the outcome is rare or the sample size is relatively small, it is important to be cautious about including too many covariates in the model. Any resulting effect size estimate may be too high or too low and is unlikely to reflect the true estimate of effect. Such issues due to rare events or low sample sizes are also possible when conditional methods are used (e.g., conditional logistic regression when the design includes matching of the comparison group under study): if too few discordant pairs (or discordant sets) are observed, the estimated effect size may also be unreliable. Thus: while controlling for confounders and other covariates is important, the assessor must take care not to over-control or end up with too few degrees of freedom to produce a

reliable test. In these cases, it may be more important to seek parsimonious models that adjust for only a smaller number of the most influential confounders and other covariates so that the effective sample size remains adequate.

Finally, it is important in any statistical modeling exercise to consider statistical significance in the context of clinical/biological/scientific significance of the result. It may be that some results are statistically significant but unimportant in a clinical/biological/ scientific context. The reverse can be true: it may be that results are not statistically significant but may be important in a clinical/biological/scientific context. The former may suggest a sample size that is larger than necessary while the latter may suggest one than is smaller than needed. The latter case may be important from a public health perspective and warrant further exploration, especially when the association is strong (despite it being imprecise)

### 4. Potential Bias in Observational Research

Bias is a systematic error in the design or conduct of a study that gives rise to study results that are systematically different from the (unobserved) true situation. This contrasts with random errors which relate to sampling variability and precision (or, equivalently, confidence bounds) around the effect size measure, but which do not "drive" or "push" the result in one particular direction (e.g., either toward or away from the null).

Bias is a reflection of methodological imperfections in the design or conduct of the study and should be addressed or discussed by researchers as part of their analysis. There are a number of ways that bias can be introduced into a study: studies may be biased in the way in which participants are selected into the study (selection bias), or the way in which information about exposure and disease status is collected (information bias, including recall bias discussed earlier for case-control studies). One example of a common occupational selection bias is the "healthy worker effect" which can create an important bias in occupational epidemiology studies, leading to bias toward the null, and even below (creating the interpretation that the exposure is "protective") No study is totally devoid of bias and one should consider the extent to which authors of published studies described potential bias in the study, and how (if at all) they attempted to address it and characterize it in the study. Bias can result from differential or non-differential misclassification (Greenland, 1998). Differential misclassification (bias) means that misclassification has occurred in a way that depends on the values of other variables, while non-differential misclassification (bias) refers to misclassifications that do not depend on the value of other variables. Misclassification biases – either differential or non-differential – depend on the sensitivity and specificity of the study's methods used to categorize such exposures and can have a predictable effect on the direction of bias under certain (limited) conditions: this ability to characterize the direction of the bias based on knowledge of the study methods and analyses can be useful to the regulatory decision-maker since it may allow the decision maker to determine the extent to which, if any, the epidemiological effect sizes being considered (e.g., OR, RR) are likely underestimates or overestimates of the true effect

size<sup>17</sup>. It is not atypical to find degrees of misclassification in the range of 10 to 20 percent and it can be helpful in reviewing epidemiological studies to consider a form of sensitivity (or "what if") analysis which evaluates such a degree of misclassification -- and whether it is differential or non-differential – and the degree to which such misclassification might impact the odds ratio or relative risk with respect to both magnitude and direction<sup>18</sup>. (p.25, FIFRA EPA SAP report, 22 April, 2010). As mentioned earlier with respect to confounding, such quantitative sensitivity analysis is only rarely performed or practiced in published epidemiology studies, with bias instead more typically evaluated in a narrative manner without any quantitative assessment of its potential magnitude and the effect it may have on the epidemiological effect size estimates (Jurek at al., 2006). This may be due – in part -- to a general lack of availability of computational tools for such analysis by epidemiologists or their unfamiliarity with them. Such tools are becoming increasingly available and may be valuable in developing more rigorous quantitative methods for evaluation of potential biases.

# 5. Interpretation of Null studies

"Null" studies -- or well-conducted studies which report no association between exposure to the pesticide and an adverse health outcome -- will be evaluated carefully for their potential usefulness in human health risk assessment. The study may report a null result either because the investigated association indeed does not in reality exist, or because the study was conducted failed to detect an association at a given predetermined level of significance. This latter result – the failure to detect an association -- should not necessarily be interpreted to mean that no association exists, but rather as simply one was not found in the particular study<sup>19,20</sup>. To evaluate which of these two conditions may be correct when reviewing "null" studies, one should consider other research reported concerning the same or similar research question, the manner in which exposure and outcome were assessed, the extent to which exposure misclassification may have biased the study to the null, the statistical methods used including the identification and analysis of confounding variables in the association, the extent to which the exposure is below a threshold at which an effect would occur or be detected, as well as the power of the study and its ability to detect an effect size of substantive interest. Statistical power refers to the probability that researchers may correctly identify that there is a difference between the two comparison groups, i.e., there is an association between exposure and disease, when in

<sup>&</sup>lt;sup>17</sup> The direction of bias that results from the degree of non-differential misclassification will also depend on the categorization of exposure (either dichotomous or polytomous).

<sup>&</sup>lt;sup>18</sup> Such sensitivity analyses might be especially recommended for exposure misclassification biases which in many cases are expected to result in more substantive effects on the effect size estimate than those from confounding.

<sup>&</sup>lt;sup>19</sup> The old adage that "the absence of evidence does should not be interpreted as the evidence of absence" is true here.

<sup>&</sup>lt;sup>20</sup> See also the American Statistical Association's Statement on Statistical Significance and P-values at <u>https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf</u>

fact there is in fact a true difference (or association). Studies that are "low powered" may falsely conclude there is no association, when an association actually exists<sup>21</sup>.

Finally, it is important to consider the effects of publication bias in any systematic review of the literature with respect to interpretation of null studies. The term publication bias refers to the tendency for the available published literature to disproportionately exclude such null studies. Studies that demonstrate such a "null" association between a disease or health outcome can be as equally informative as those that do provided that the study in question meets the quality criteria established as part of the epidemiological review process. These may include such factors as study design; the existence of an *a priori* hypothesis vs. an exploratory analysis; sample size and statistical power to detect an effect size of interest; proper ascertainment of outcome *vis-à-vis* sensitivity and specificity; the quality of the exposure assessment and the potential for differential and non-differential misclassification; adequacy of the measurement of key potential confounders and other forms of bias (information, selection, etc.); and evaluation of effect modifiers; appropriate statistical analyses, including consideration of and possible correction for multiple comparisons that a unsupported by a priori hypotheses, biological plausibility, or other supporting information.

# 6. External Validity (Generalizability)

As noted above, *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). *External validity*, or *generalizability*, refers to the ability to extend the epidemiologic study results derived from a sample of the population (e.g., pesticide applicators) to other populations (e.g., all agricultural workers). To assess external validity, comparison of characteristics in the sample to the larger population (if known) can be made. Such evaluation should include not only demographic factors, but also whether exposures (e.g., dose, timing, duration) are similar and whether important effect modifiers (e.g., sensitivity of vulnerable populations) were considered. Generalizability is of particular importance because it is important to understand whether and how individual study results may be applied to the larger group or targeted sub-groups in regulatory risk assessment. For example, the AHS has reported statistical associations between some cancer and non-cancer health outcomes for some pesticide chemicals. OPP has an interest in evaluating the extent to which the reported findings may apply to pesticide applicators in states other than North Carolina and lowa or to farm workers who primarily do post-application activities.

<sup>&</sup>lt;sup>21</sup> Studies that are low-powered but find statistically significant effects may also be subject to the phenomenon of effect size magnification and this can be important to investigate as well. (loannidis, 2008).

## V. HUMAN INCIDENT SURVEILLANCE DATA

Generally speaking, epidemiology studies on pesticides such as those described above focus on lower exposures (over a longer time period) that are less likely to result in acute clinical symptoms. OPP is also interested in exposures that are higher and occur over shorter-intervals (often on an acute "one-time" basis). This "human incident," or poisoning data can be useful for evaluating short term, high exposure scenarios that can be readily attributed to the pesticide in question.

OPP uses such "human incident information" for several purposes. Most broadly, the program uses incident data to inform risk assessment/risk management activities; this forms an integral part of our registration review activities under our Pesticide Registration Improvement Act (PRIA) responsibilities. To this end, OPP evaluates human incident data for trends over time and examines patterns in the severity and frequency of different pesticide exposures. In some cases, incident information can indicate need for additional information or additional risk management measures. Incident information can also help assess the success of risk mitigation actions after they are implemented, and incident information is an important part of OPP's performance accountability system to ensure the effectiveness of risk management actions that OPP has taken to protect human health and the environment. Lastly, incident information can be useful in providing real world use information with respect to usage practices and also in potentially targeting enforcement or educational activities, where appropriate.

OPP obtains this information from a variety of sources. Sources of human incident data include both (human) **medical case reports** appearing in the medical and toxicological literature as well as information from a variety of national **toxico-surveillance activities** for acute pesticide poisonings which are considered jointly to aid acute and chronic hazard identification and as an integral part of the risk assessment process.<sup>22</sup>

**Medical case reports** (first-hand accounts written by physicians) or medical case series (a compendium of medical case reports across individuals that share common source or symptomology) are valuable tools for analyzing all available evidence of health effects, and to complement the findings of animal studies and epidemiological studies. In addition, they can identify unusual or novel occurrences of an adverse health effects plausibly associated with use of a specific pesticide providing "advance notice" to the agency for toxico-vigilance purposes. Published case reports for pesticides typically describe the effects from an atypical (high exposure/dose, illegal, off-label) acute or short-term exposure. The reports are often anecdotal and can be highly selective in nature. They can, however, can be particularly valuable in identifying previously unidentified toxic effects in humans and in learning about the effects, health outcomes, and medical sequelae following high exposures. They frequently have more detailed medical information (including sequelae), detailed follow-up, and generally higher quality and/or quantitative

<sup>&</sup>lt;sup>22</sup> OPP is aware of efforts by IPSC to consider human incident data in risk assessment. <u>http://www.who.int/ipcs/publications/methods/human\_data/en/index.html</u>

information about dose. If similarities are seen across multiple medical case studies or patterns emerge – in symptoms, exposure scenarios or usage practices -- these can provide valuable information for the risk assessment process and strengthen any findings. Medical case studies and series that include quantitative exposure information can be compared to exposure estimates in the risk assessment (which are based on labeled application rates and surrogate exposure information) to characterize margins of exposure expected from typical use, when appropriate.

The following considerations are evaluated in assessing medical case reports and medical case series:

- A detailed history of exposure (when, how, how much); time of onset of adverse effects; and signs and symptoms of the patient, are reported.
- Information on the product/chemical/pesticide, such as name, pesticide label, registration number, etc.
- Patient information (e.g. age, race, sex); underlying health conditions and use of any medications that can produce similar signs and symptoms; relevant medical history; and the presence of any risk factors.
- Description of events and how the diagnosis was made.
- Management and treatment of the patient, and laboratory data (before, during and after the therapy), including blood levels of pesticides and chemicals.
- Whether the medical report is reliable, reasonable and whether it is consistent with current knowledge, including other research, reviews and guidelines.
- Clinical course of the event and patient outcome (e.g. patient recovered and discharged from hospital; condition of patient after the discharge, any chronic health effects or premature death related to the pesticide or chemical exposure).

In addition to using medical case reports/series as a source of real-world exposure and toxicological information, OPP also engages in toxico-surveillance activities using a variety of pesticide poisoning incident databases are also available. Specifically, OPP has access to the following five human incident data sources: the *OPP Incident Data System* (IDS); the American Association of Poison Control Centers (PCC) summary reports from their *National Poison Data System* (NPDS); data from the EPA-funded *National Pesticide Information Center* (NPIC), currently at Oregon State University; the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health *Sentinel Event Notification System for Occupational Risk-Pesticides* (NIOSH SENSOR-Pesticides) and the *California Pesticide Illness Surveillance Program* (PISP). Each of these are described, in turn below:

□ <u>OPP Incident Data System (IDS)</u> is maintained by OPP and incorporates data submitted by registrants under FIFRA section 6(a)(2)<sup>23</sup>, as well as other incidents reported directly to EPA. OPP has compiled the pesticide related

<sup>&</sup>lt;sup>23</sup> Under FIFRA 6(a) (2), pesticide registrants are required to notify EPA if and when they become aware of "factual information regarding unreasonable adverse effects on the environment of the pesticide."

incident reports in the IDS since 1992. The IDS includes reports of alleged human health incidents from various sources, including mandatory FIFRA Section 6 (a) (2) reports from registrants, other federal and state health and environmental agencies and individual consumers. IDS include information on incidents involving humans, plants, wild and domestic animals where there is a claim of an adverse effect. The vast majority of IDS reports are received by the agency in paper format. IDS entries act as a "pointers" to copies of original reports retained on microfilm and scanned images in OPP's Information Service Center.

While IDS includes both occupational and non-occupational incidents, the majority of incidents reported relate to non-occupational/residential scenarios The reports are obtained from across the U.S. and most incidents have all relevant product information (such as the EPA Registration Number) recorded. As IDS is populated mostly by information provided by pesticide registrants under their FIFRA 6(a)(2) reporting requirements, the agency has relatively high confidence in the identification of the specific product which is involved. Severity rankings are included for each incident (as specified by CFR §159.184). Symptom information is sometimes included in the narrative portion of the incident, but this information is usually not validated/confirmed by a healthcare professional. IDS also includes narrative information on exposure scenario and hazard information. Many companies use standardized, industry-developed Voluntary Incident Reporting Forms.

OPP collects and evaluates the data from the IDS and identifies potential patterns with respect to the extent and severity of the health effects due to pesticides exposure. While IDS reports are broad in scope and can in some cases contain detailed information, the system does not necessarily consistently capture detailed information about incident events, such as occupational exposure circumstances or medical outcome.

In addition, most cases data going into IDS is not validated or verified, though some reports are collected from calls to contract poison control centers. Nevertheless, incident information can provide an important post-marketing feedback loop to the agency following initial registration of the product: IDS incidents of a severe nature, or a suggested pattern or trend among less severe incidents can signal the agency to further investigate a particular chemical or product. Because IDS has such extensive coverage, it can assist in providing temporal trend information and determining whether risk mitigation has helped reduce potential pesticide exposure and decreased the number of potential incidents reported to IDS. Overall, IDS provides good information about national trends and frequency of incidents for pesticides and can provide valuable insights into the hazard and/or exposure potential of a pesticide. □ The National Poison Data System (NPDS) -- formerly called the Toxic Effects Surveillance System (TESS) -- is maintained by the American Association of Poison Control Centers (AAPCC) and is supported with funding from several federal agencies. NPDS is a computerized information system with geographically specific and near real-time reporting. Although the main mission of Poison Control Centers is in helping callers respond to emergencies, NPDS data can help identify emerging problems in chemical product safety. Hotlines at 61 PCC's nationwide are open 24/7, 365 days a year and are staffed by specially trained nurses, pharmacists, and other clinical health care specialists to provide poisoning information. Using computer assisted data entry, standardized protocols, and strict data entry criteria, local callers report incidents. These reported incidents are retained locally and are updated in summary form to the national database maintained by AAPCC. Information calls are tallied separately and not counted as incidents. The PCC system covers nearly all the US and its territories and has undergone major computer enhancements since 2001.

NPDS includes mainly non-occupational incidents. NPDS does not include narrative information and the product information may not be complete. NPDS provides severity rankings and symptom information that are designated/recorded by trained specialists, and the agency has relatively high confidence in this information. NPDS also provides some information on the likelihood of the adverse effect being a result of the reported exposure. Overall, NPDS provides good information about national trends, frequency of incidents for pesticides, as well as the hazard potential for particular pesticides. However, resource limitations permit the agency to only access AAPCC summary reports published each year (e.g., see <u>http://www.aapcc.org/annual-reports/</u>) and these serve as a supplement to other data sources for which the agency has more complete access.

### The National Pesticide Information Center (NPIC)

(http://npic.orst.edu/index.html) is funded by EPA to serve as a source of objective, science-based pesticide information in response to inquiries and to respond to incidents. NPIC functions nationally during weekday business hours and is a cooperative effort between Oregon State University (currently) and EPA; it is intended to serve as a source of objective, science-based pesticide information and to respond to inquiries from the public and to incidents. Similar to Poison Control Centers, NPIC's primary purpose is not to collect incident data (about 10% of NPIC's annual calls are considered "incident" related), but rather to provide information to inquirers on a wide range of pesticide topics, and direct them to other sources for pesticide incident investigation and emergency treatment. Nevertheless, NPIC does collect information about incidents (approximately 4000 incidents per year) from inquirers and records that information in a database. NPIC is a source of national incident information, but generally receives fewer reports than IDS. Regardless, if a high frequency is observed in IDS for a given pesticide or

product, NPIC provides a source of information that can prove valuable in determining consistency across national data sets.

As with IDS and PCC, the incidents in NPIC are mainly non-occupational. NPIC incidents include narratives and product information when the caller provides the information. Although the scope is national, there are significantly fewer incidents reported to NPIC than to NPDS or IDS but considerably more information is provided and the agency can request custom reports on an as-needed basis. Hazard information includes severity rankings, route of exposure and symptoms – which are recorded by trained personnel. NPIC also provides information on how likely the link between exposure and adverse effect is (which they call a certainty index). NPIC also publishes annual reports and analyses in the open literature which are valuable resources.

The Center for Disease Control and Prevention National Institute for Occupational Health (CDC/NIOSH) manages a pesticide surveillance program and database entitled the Sentinel Event Notification System for Occupational Risk (SENSOR)-Pesticides.<sup>24</sup> This database includes pesticide illness case reports in 12 states from 1998-2013. Participating states are: California, Florida, Iowa, Louisiana, Michigan, Nebraska, New Mexico, New York, North Carolina, Oregon, Texas and Washington. The participating states for a given year vary depending on state and federal funding for pesticide surveillance.

Cases of pesticide-related illnesses in the SENSOR-Pesticides database are ascertained from a variety of sources, including: reports from local Poison Control Centers, state Department of Labor workers' compensation claims when reported by physicians, reports from state Departments of Agriculture, and physician reports to state Departments of Health. Although both occupational and non-occupational incidents are included in the database, the SENSOR coordinators primarily focus their follow-up case investigation efforts on the occupational pesticide incidents. The SENSOR coordinator at the state Department of Health will follow-up with cases and work to obtain medical records in order to verify exposure scenario, symptoms, severity, and health outcome. Using standardized protocol and case definitions, SENSOR coordinators at state Departments of Health enter the incident interview description provided by the case, medical report, physician and patient into the SENSOR data system.

All SENSOR-Pesticides cases must report a minimum of two health effects in order to be included in the aggregate database that EPA uses for incident

<sup>&</sup>lt;sup>24</sup> SENSOR-Pesticides webpage: <u>http://www.cdc.gov/niosh/topics/pesticides/overview.html</u>

analyses. Evidence for each case is evaluated, based on the NIOSH case classification matrix, for its causal relationship between exposure and illness. 98% of SENSOR-Pesticides cases are classified as definite, probable, or possible, and 2% of the cases are classified as suspicious. Unlikely, asymptomatic, and unrelated cases, as well as those with insufficient information, are not included in the SENSOR-Pesticides database.

Overall, SENSOR-Pesticides provides very useful information on both occupational and non-occupational incidents, and sometimes valuable insights into the hazard and/or exposure potential of a pesticide. SENSOR-Pesticides also conducts analyses of its own data and publishes these in the Morbidity and Mortality Weekly. Unlike the aforementioned databases and although it contains both non-occupational/residential and occupational incidents, SENSOR's has traditionally focused on occupational pesticide incidents, and is of particular value in providing that information. SENSOR-Pesticides data from 1998-2011 is available online at: <u>http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides</u>.

The California Pesticide Illness Surveillance Program (PISP) is maintained by the State of California. This database documents pesticide-related illnesses and injuries. Case reports are received from physicians and via workers' compensation records. The local County Agricultural Commissioner investigates the circumstances of the exposure. Medical records and investigative findings are then evaluated by California's Department of Pesticide Regulation (DPR) technical experts and entered into an illness registry. All reported pesticide illnesses in the California PISP program are investigated by the county agricultural commissioners, and the DPR evaluates the reports and compiles them into a database, which is used to improve the state's program to protect workers and others from the adverse effects of pesticide exposure (http://apps.cdpr.ca.gov/calpiq/).

Currently, OPP evaluates human incident data on a chemical-specific basis. Incidents from each database are analyzed for hazard potential (deaths, frequency of more severe incidents, and patterns/trends of reported symptoms) and exposure potential (frequency of incidents/ trends over time, patterns/trends of exposure scenarios, of factors affecting exposure or of products). When evaluating human incident data from the above databases, OPP considers several general criteria. OPP considers the relative severity and frequency of symptoms. Additionally, OPP generally has greater confidence in reports in which temporal association can be verified or are at least plausible. Lastly, other factors that are used to evaluate human incident data include evidence of an exposure response association, consistency in reported health effects, biological plausibility of reported health effects, elimination of alternative causes of health effects. Additionally, narratives of more severe incidents are often evaluated for any temporal association between time-of-exposure and effects reported to determine whether an association is supported by the circumstances. For example, a heart attack in an elderly individual that occurs three

months following an indoor pesticide application may be determined not to be a likely causal association. On the other hand, a severe incident occurring at or shortly after the time of exposure with symptoms consistent with known symptomology for the pesticide class and that occurs without prior medical history may suggest that causal inference is more justified.

In sum, then, incident data -- consisting of both medical case reports/case series appearing the medical and human toxicological literature and toxico-surveillance data derived from the databases that EPA either maintains, funds, or accesses -- can provide useful, complementary information that assists OPP in evaluating the real-world risks of pesticides.

## **VI. SUMMARY & CONCLUSIONS**

This framework describes important factors in reviewing epidemiology and human incident data and describes a proposed WOE analysis for incorporating such data in pesticide human health risk assessment. OPP uses the best available data across multiple lines of evidence and from *in vitro*, *in vivo*, and *in silico* data sources. OPP uses a WOE approach when integrating data from multiple sources to take into account for quality, consistency, relevancy, coherence and biological plausibility using modified Bradford Hill criteria as an organizational tool. Application of WOE analysis is an integrative and interpretive process routinely used by EPA according to in scientific analysis outlined in its risk assessment guidelines. The WOE analysis also evaluates the quality of the combined data set and is consistent with the level of effort and complexity that is appropriate for a particular scientific assessment (U.S. EPA, 2002). OPP acknowledges that toxicology and risk assessment are currently undergoing transformational changes towards implementing the new vision of 21<sup>st</sup> century toxicity testing. As these transformation changes occur, OPP will update this approach as appropriate.

# **VII. REFERENCES**

American Statistical Association. 2016 "AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND P-VALUES" March 7. Available at: <u>https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf</u>

<u>Ankley, GT, Bennett RS, Erickson RJ</u> et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. <u>Environ Toxicol Chem</u> 29(3):730–741.

Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, and Guyatt GH. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401-6. doi: 10.1016/j.jclinepi.2010.07.015. Epub 2011 Jan 5.

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W, Steen W, Dosemeci M, and Alavanja M. 2002. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Ann Epidemiol. Oct 1;10(7):478.

Borenstein M, Hedges LV, Higgins JPT, and Rothstein HR. 2009. Introduction to Metaanalysis. John Wiley and Sons, Chichester, UK.

Boyes WK, Moser VC, Geller AM, Benignus VA, Bushnell PJ, and Kamel F. 2007. Integrating epidemiology and toxicology in neurotoxicity risk assessment. Hum. Exp. Toxicol. 26(4):283-93.

Calderon RL 2000. Measuring risks in humans: the promise and practice of epidemiology. Food and Chemical Toxicology. 38:S59-S63.

Carlile DJ, Zomorodi, K, and Houston, JB. 1997. Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab. Dispos. 25(8):903-911.

Checkoway H, Pearce, N, and Kriebel D. 2004. Research Methods in Occupational Epidemiology, 2<sup>nd</sup> Edition. Oxford University Press, New York.

Clark LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologicallybased pharmacokinetic models for use in safety or risk assessment. Risk Anal. 24(6):1697-1717.

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

Glymor, MM and Greenland, S. 2012. "Causal Diagrams" in Rothman, KJ, Greenland, S, Poole, C, Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 183-212.

Gordis, L. 2009. Epidemiology. 4th Edition. Saunders-Elsevier, New York.

Greenland, S. 1998. "Basic Methods for Sensitivity Analysis and External Adjustment" in Rothman, KJ and Greenland, S. Modern Epidemiology. 2<sup>nd</sup> ed. Lippencott-Raven Publishers, Philadelphia. pp.343-357.

Greenland, S. and Lash T. 2012. "Bias Analysis" in Rothman KJ, Greenland S, Poole C, Lash TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 345-380.

Greenland, S and O'Rourke, K. 2012. "Meta-analysis" in Rothman, KJ, Greenland S, Poole C, and Lash, TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 652-682.

Grimes, DA and Schultz, KF. 2005. Compared to What? Finding controls for case-control studies. Lancet 365: 1429-1433.

<u>Gustafson P</u><sup>1</sup>, and <u>McCandless LC</u>. 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. <u>Int J Environ Res Public Health.</u> 2010 Apr;7(4):1520-39. doi: 10.3390/ijerph7041520..

<u>Hartung T</u>. 2010. Evidence-based toxicology - the toolbox of validation for the 21st century? <u>ALTEX</u>. 2010;27(4):253-63.

Hertz-Picciotto I. 1995. Epidemiology and quantitative risk assessment: a bridge from science to policy. American Journal of Public Health. 85(4): 484-491.

Hill AB. 1965. The Environment and Disease: Association or Causation? President's Address. Proceedings of the Royal Society of Medicine 58:293-300

Hoppin JA, Yucel F, Dosemeci M, and Sandler DP. 2002. Accuracy of self-reported pesticide use duration information for licensed pesticide applicators in the Agricultural Health Study. Journal of Exposure Analysis and Environmental Epidemiology, 12: 313-318.

IPCS (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration response assessment. Harmonization Project Document 2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.

Ioannidis JP. 2008. Why Most Discovered True Associations Are Inflated. Epidemiology. 19(5): 640-8.

Jurek AM, Maldonado G, Greenland G, and Church TR. 2006. Exposure-measurement Error is Frequently Ignored When Interpreting Epidemiological Study Results. Europ. J. Epid. 21: 871-876.

Kelsey JL, Whittemore AS, Evans AS, and Thompson WD. 1996. Methods in Observational Epidemiology. 2<sup>nd</sup> ed. Oxford University Press, New York.

LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, and Weisel CP. 2014. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. <u>Environ Int.</u> Dec;73:195-207. doi: 10.1016/j.envint.2014.07.011.

Lash, TL, Fox, MP, and Fink, AK. 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, and Greenland S. 2014. Good practices for quantitative bias analysis. International Journal of Epidemiology p. 1-17.

Lilienfeld AM and Lilienfeld D. 1979. Foundations of epidemiology, 2<sup>nd</sup> ed. Oxford University Press, New York.

Mausner JS and Kramer S. 1985. Epidemiology, 2<sup>nd</sup> ed. W.B. Saunders, Philadelphia.

Meek, ME, Bucher, JR, Cohen, SM et al. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33:591-653.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

<u>Muñoz-Quezada MT<sup>1</sup>, Lucero BA</u>, <u>Barr DB</u>, <u>Steenland K</u>, <u>Levy K</u>, <u>Ryan PB</u>, <u>Iglesias V</u>, <u>Alvarado S</u>, <u>Concha C</u>, <u>Rojas E</u>, and <u>Vega C</u>. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. <u>Neurotoxicology</u>. Dec;39:158-68. doi: 10.1016/j.neuro.2013.09.003.

Needham LL, Calafat AM, and Barr DB. 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210: 229-238.

Nieuwenhuijsen MJ. 2003. Exposure Assessment in Occupational and Environmental Epidemiology. Oxford University Press, New York.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC.

NRC (National Research Council). 1991. Environmental Epidemiology, Volume 1: Public Health and Hazardous Wastes. National Academy Press, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 1997. Environmental Epidemiology, Volume 2: Use of the Gray Literature and Other Data in Environmental Epidemiology. National Academy Press, Washington, DC.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press, Washington, DC.

NRC (National Research Council). 2009: Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. National Academies Press, Washington, DC. http://www.nap.edu/catalog/13142.html

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) process. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record\_id=18764

Ntzani EE, Chondrogiori MNG, Evangelou E and Tzoulaki I. 2013. Literature review of epidemiological studies linking exposure to pesticides and health effects. External Scientific Report. EFSA supporting publication 2013-EN-497. 159 pp. Available online at <u>www.efsa.europa.eu/publications</u>.

Organisation for Economic Co-operation and Development. 2013. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, ENV/JM/MONO(2013)6, April 17, 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono( 2013)6&doclanguage=en

Paddle GM, and Harrington JM. 2000. Environmental epidemiology--strengths and weaknesses. Int Arch Occup Environ Health. 73:7-14.

Porta MJM. 2014. A Dictionary of Epidemiology. 6<sup>th</sup> ed. Oxford University Press, New York.

Purdue Pesticides Programs. 2003. Pesticides and Epidemiology: Unraveling Disease Patterns. Purdue University Cooperative Extension Service. http://www.btny.purdue.edu/Pubs/PPP/PPP-43.pdf.

Rothman KJ and Greenland S. 2012. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

Rothman, KJ, Greenland S, Poole C, and Lash TL. 2012a. "Causation and Causal Inference" in Rothman, KJ, Greenland S, Poole C, and Lash TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 5-31.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. Jul;122(7):711-8. doi: 10.1289/ehp.1307972.Seed, J., E.W.

Carney, RA, Corley, et al. 2005. Overview: Using mode of action and lifestage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672.

Schultz, KF and Grimes DA. 2002. Case-control studies: research in reverse. Lancet: 359:431-434.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Sterne JAC, Higgins JPT, and Reeves, BC. on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for non-randomized studies of interventions (ACROBAT-NRSI), Version 1.0. 0, 24 September 2014." *www. riskofbias. info* (2015)

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Bennie D, Moher D, Becker BJ, Sipe TA, and Thacker SB for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. 2000. J. American Medical Association 283(15): 2008-2012.

Szklo M and Nieto FJ. 2004. Epidemiology: Beyond the Basics. Jones and Bartlett Publishers, Boston, MA.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Science Policy Council Handbook: Risk Characterization. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/100/B-00/002. Available at <u>http://www.epa.gov/iris/backgr-d.htm</u>.

U.S. EPA (U.S. Environmental Protection Agency). 2001. General Principles For Performing Aggregate Exposure And Risk Assessments Washington, DC. Available at <u>https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf</u>

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. U.S. EPA (U.S. Environmental Protection Agency). 2002a. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity for Information Disseminated by the Environmental Protection Agency. Office of Environmental Information, Washington, DC. EPA/260/R-02/008. Available at

http://www.epa.gov/quality/informationguidelines/documents/EPA InfoQualityGuidelines/documents/EPA Inf

U.S. EPA (U.S. Environmental Protection Agency). 2002b. A Review of the Reference Dose and Reference Concentration Processes. December. Risk Assessment Forum. Washington, DC. EPA/630/P-02/002F.

U.S. Environmental Protection Agency. 2002c. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"; January 14, 2002. U.S. EPA (U.S. Environmental Protection Agency). 2004. An Examination of EPA Risk Assessment Principles & Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science Advisor. U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at <u>http://www.epa.gov/raf</u>.

U.S. EPA. (U.S. Environmental Protection Agency). 2006a. Harmonization in Interspecies Extrapolation: Use of BW<sup>3/4</sup> as Default Method in Derivation of the Oral RfD (External Review Draft). U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-06/001. Available at <u>http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525</u>.

U.S. EPA (U.S. Environmental Protection Agency). 2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Scientific Issues Associated with Field Volatilization of Conventional Pesticides. U.S. Environmental Protection Agency, Washington, DC. OPP Regulatory Public Docket EPA-HQ-OPP-2009-0687.

U.S. EPA (U.S. Environmental Protection Agency). 2010. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. EPA (U.S. Environmental Protection Agency). 2012. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment U.S. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014a. Framework for Human Health Risk Assessment to Inform Decision Making.

https://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. <u>http://www2.epa.gov/osa/guidance-applying-quantitativedata-develop-data-derived-extrapolation-factors-interspecies-and</u>

U.S. EPA (U.S. Environmental Protection Agency). 2015. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA.

https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8C C/\$File/ISA\_PREAMBLE\_FINAL2015.PDF

van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A, and Goldbohm A. 2002. The contribution of epidemiology. Food Chem Toxicol. Feb-Mar;40(2-3): 387-424.

Vandenbroucke JP, Van Elm E, Altman DG, Gotzsche PC, Mulroew CD, Pockock SJ, Pool C, Schlesseman JJ, and Egger, M. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. Ann. Int. Med. Vol 147(8): W163-193

Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, and Vandenbroucke JP. 2014. The Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. Int. J. Surgery 12: 1495-99.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992a. Selection of Controls in Case-Control Studies I. Principles. American J. Epid. 135(9): 1019-1028.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992b. Selection of Controls in Case-Control Studies II. Types of Controls. American J. Epid. 135(9): 1029-1041.

Wacholder, S, McLaughlin, JK, Silverman, DT, and Mandel, JS. 1992c. Selection of Controls in Case-Control Studies III. Design Options. American J. Epid. 135(9): 1042-1050.

<u>Woodruff TJ</u><sup>1</sup> and <u>Sutton P</u>. 2014. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. <u>Environ Health Perspect</u>. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

Zartarian V., Bahadori T, and McKone T. 2005. Adoption of an official ISEA glossary. Journal of Exposure Analysis and Environmental Epidemiology 15:1-5

農薬のリスク評価において ヒトでの疫学データと事例データを導入するための 農薬プログラム局のフレームワーク(基本概念)

2016年12月28日

農薬プログラム局 米国環境保護庁

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#### I. 目的と範囲

米国環境保護庁(EPA)の農薬プログラム(OPP)は、連邦殺虫剤・殺菌剤・殺鼠剤法 (FIFRA)及び連邦食品・医薬品・化粧品法(FFDCA)に基づき、米国内の農薬を規制する認 可プログラムである。このプログラムの一環として、OPPは毒性とばく露の多くの主要データ を評価し、農薬がヒトの健康及び環境に及ぼす影響を評価している。ヒト健康影響を評価する際、 EPAは、まず疫学データを含めたヒトへの影響の可能性を直接評価する情報に注目する。しかし、 これまでは農薬の潜在的な毒性を報告した疫学研究はほとんどない。したがって、OPPはハザ ードの可能性を評価し、ヒトの健康リスクを推定するため、これまでは実験動物を用いた毒性試 験に主に依存してきた。Agricultural Health Study<sup>1</sup>や National Institute of Environmental Health Sciences (NIEHS) / EPA Children's Centers<sup>2</sup>などから数多くの論文が発表されたこ とで、米国で実施された農薬へのばく露に関する疫学研究の有用性が高まっている。とはいえ、 質の高い疫学データが存在する、あるいは充実しつつある農薬の数は、今後短期的には比較的少 ないので、ほとんどの農薬の定量的リスク評価に用いるためのデータの主な情報源は試験データ であることがおそらく継続するだろう。

OPP の目標は、そのような情報が入手可能な場合には、科学的に正確で明白な方法で利用す ることである。これを達成するために OPP は、この文書に記載されているように総合的な疫学 的フレームワークを開発した。このフレームワークでは、そのような研究や科学的情報を農薬の リスク評価にどのようにして一層完全に統合することができるかを評価する際に OPP が検討す る科学的に考慮すべき要点を述べている。現在の文書は、拘束力のある規則ではなく、疫学デー タを評価または使用するためのレビュアー向けのガイドやマニュアル、標準作業手順書であるこ とを意図したものでも、その役割を果たすことを意図したものでもない。また、より最新で進歩 した疫学的方法の完全な専門書となることも、この種の研究を解釈するために重要なニュアンス や複雑さを適切に伝えることも意図していない。したがって、因果推論及び因果関係図 (Rothman ら、2009年; Greenland and O'Rourke、2012年)、交絡因子の特定・評価・制御 に対する最新の研究法、メタ分析と異質性の評価(Borenstein ら、2009 年; Greenland and O'Rourke、2012 年)、または疫学データの感度/定量的バイアス解析(Lash ら、2009 年; Lash ら、2014年; Joannidis、2008年; Greenland and Lash、2012年; Jurek ら、2007年)と いった重要な疫学の話題は(詳細に)論じていない。これらの話題、概念及び問題点のすべては、 農薬に関する疫学研究に応用できるが、この OPP フレームワーク文書では取り上げていない。 代わりに、この文書では、OPP の FIFRA 及び FFDCA の機能支援のためのヒト健康リスク評価 の文脈において農薬の疫学研究の評価と使用に関する全体的な概念を提供している。

<sup>&</sup>lt;sup>1</sup> <u>https://aghealth.nih.gov/</u>

<sup>&</sup>lt;sup>2</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-diseaseprevention-research-centers

本文書の以前のバージョンは、2010年2月にFIFRA科学諮問パネル(SAP)によって好意的に レビューされた(USEPA、2010年、FIFRA SAP、2010年)。この文書は、SAPが推奨する改 善点、パブリックコメント及び2010年以降に疫学データが利用可能であった農薬の評価から得 られた知識が盛り込まれており、進捗及び必要に応じて随時更新される文書と考えるべきである。

#### II. 序章

全米科学アカデミー(NAS)の米国研究評議会(NRC)による2つの報告書、「21世紀の毒 性試験:ビジョンと戦略(2007年)」と「科学と政策調査(2009年)」は、共に毒性学とリス ク評価の新たな方向性を示している。これら 2 つの NRC 報告書は、毒性試験の実施方法、デー タの解釈方法、そして最終的に規制上の政策調査の方法に大きな変更を提唱している。具体的に は、21世紀の毒性試験に関する 2007年の報告書では、毒性試験、リスク評価、そして最終的な 政策調査に情報を提供するために、現在の先端毒性エンドポイントの使用に焦点を当てたものか ら、毒性発現経路3を使用するようにシフトすることを提唱している。このアプローチは、遺伝 子、タンパク質、低分子がどのように相互作用してヒト細胞の機能を維持する分子経路を形成し ているかという急速に進化する科学的理解に基づいている。新しい毒性試験のパラダイムの目標 は、環境因子へのばく露がこれらの経路をどのように乱すかを明らかにし、それによって有害な 健康影響につながる重要な事象を連鎖的に引き起こす原因を明らかにすることである。この新し いアプローチでは、疫学研究、ヒトでの事例データベース、バイオモニタリング研究に見られる ようなヒトの情報と実験毒性学的情報が重要な役割を果たすことが期待されている。具体的には、 このようなヒト情報は、実際の化学物質ばく露による影響についての見識を提供し、問題の定式 化やハザード/リスクの特性評価に貢献することができる。さらに、疫学データやヒトでの事例 データは、追加の解析やデータ生成(例えば、in vitro試験や目標とする in vivo試験で使用する ための用量やエンドポイントの選択)の指針となり、影響を受けやすい集団を特定したり、新た な健康影響を特定したり、既存の毒性学的結果を確認したりすることができる。

この毒性試験とリスク評価の新しいビジョンには、生物学的体系の多種多様なレベル(分子レ ベルから化学物質の影響をその発生源から最終的な健康影響や集団への影響まで考慮することを 目的とした集団を基にした調査レベルまで)のデータが含まれる。このようなデータには、 *in vitro*試験や *in vivo*試験から得られるデータのほかに、*in silico*モデルデータがある。疫学デ ータと事例データを取り入れる OPP のフレームワークは、21 世紀の毒性試験に関する 2007 年 の NRC の報告書と概念的に一致しており、どちらも多種多様なデータソースから得られる最良 の情報の用途はエビデンスの重み付け(WOE)解析にまとめて使用することを強調している。

<sup>&</sup>lt;sup>3</sup>毒性発現経路とは、十分に撹乱された場合に有害な健康影響を及ぼすことが予想される細胞応 答経路のことである。

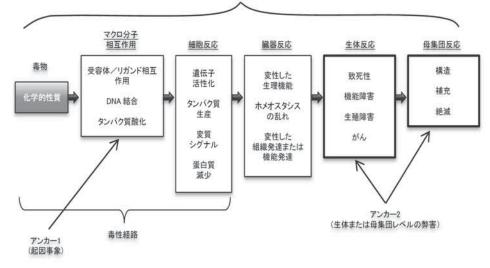
一般的な原則として、産業環境疫学研究は広く使用されている農薬のみを対象に実施されてお り、これらの農薬も科学的文献で十分に研究されている必要がある。したがって、疫学データが 利用可能な多くの場合、毒性、ばく露、薬物動態(PK)、作用機序/有害性発現経路(MOA/ AOP)の情報に関する主要な文献データも利用可能であると OPP は予想している。ヒトでの事 例データは、より広範囲の化学物質について入手可能であるが、中には正確なデータベースを持 つものと持たないものがある。ヒトでの重大な事例があり、特定の農薬の MOA/AOP や PK に ついてほとんど知られていない場合には、WOE 解析を用いて新たな研究分野を特定することが できる。

疫学データとヒトでの事例データを組み込むための当該フレームワークにおける OPP のアプ ローチは、初めてのものもしくは新しいものではない。むしろ、このアプローチは、既存のツー ルや方法を合理的かつ論理的に拡張したものである。当該文書は、農薬リスク評価に使用する疫 学データ及びヒトでの事例データを検討するための出発点として、既存のガイダンス文書及びフ レームワーク(表 1)に依存している。ヒトの健康リスク評価における疫学データ及びヒトでの 事例データの使用に関する当該フレームワークは、2009 年の「*科学と政策調査*」に関する報告 書での NRC の提言や、特にリスク管理の必要性の文脈で、利用可能なものを調査、企画、検討 するためのツールとして問題の定式化の使用を強調する点に関して、政府機関の最新のヒト健康 リスク評価フレームワーク(USEPA、2014 年 a) と一致している。

同様に、OPP のフレームワークは、問題の定式化の重要性と生物学的体系の多種多様なレベルでの情報統合の必要性を強調した「世界保健機関/化学物質の安全性に関する国際プログラムMOA/ヒト関連(MOA/HR)フレームワーク」の最新版と一致している(Meek 6、2014 年)。 MOA/HRフレームワークは、用量反応や時間的一致、ならびに生物学的な妥当性、整合性及び 一致性などの要素を考慮に入れて、Bradford Hill によって記述されたような原則を使用して、 エビデンスの重み付けに基づいて確立された因果経路に沿った一連の重要な事象を特定すること から始まる(Hill、1965年)。この解析アプローチを用いることで、疫学的知見は、他のヒト情 報(ヒトでの事例所見を含む)や実験的研究の文脈で評価することができ、不確実分野や今後の 研究分野を特定することができる。しかし、完全に解明された MOA/AOP があることが、ヒト 健康リスク評価に疫学研究を利用するための要件ではないことは注目に値する。政府機関が 2007 年及び 2009 年の NRC 報告書にある変化するアプローチの実施を引き続き進め、OPP が疫 学情報とヒトでの事例情報の統合の経験を積む中で、OPP は当該フレームワークを適切に再評 価し、更新していく予定である。

図 1. 有害性発現経路の概略図。Ankley ら(2010年)より引用。

有害性発現経路



有害性発現経路

表 1. OPP が使用している主要なガイダンス文書とフレームワーク

NAS	1983: 連邦政府におけるリスクアセスメント:プロセスの管理
	1994: 科学と判断
	2007:21 世紀の毒性試験
	2009:科学と政策調査:リスクアセスメントの推進
	2011: ホルムアルデヒドに関する NAS 報告書
	2014: EPA の統合リスク情報システム(IRIS)プロセスの見直し
	2001~2007: 作用機序/ヒト関連のフレームワーク
WHO/IPCS	2005: 化学物質固有の補正係数(CSAF)
	2014: 作用機序/種のコンコーダンス解析に関する WHO/IPCS フレー ムワークの進化と応用における新たな展開

	1991~2005: リスクアセスメントのためのリスクアセスメントフォーラム 指針(発がん性、生殖毒性、発生毒性、神経毒性、生態毒性及びばく露 評価のための指針、ベンチマーク用量モデリングのための指針、基準用 量と基準濃度プロセスのレビューなど)4
	2000: リスクの判定に関する科学政策ハンドブック
環境保護庁	2006b. 生理的薬物動態(PBPK)モデルのリスク評価への応用のための アプローチとその裏付けとなるデータ
	2014a. 政策調査に情報提供するためのヒト健康リスク評価のフレームワ ーク
	2014b. 異種間・同種間の推定のためのデータ由来の推定係数を開発する ための定量的データの適用ガイダンス
農薬プログラム	2001: 総合的なリスク評価
	2001 と 2002 年: 累積リスク評価
経済協力開発機構	2013: 有害性発現経路の開発と評価に関する経済協力開発機構 (Organisation for Economic Co-operation and Development) ガイダン ス文書

ヒトの情報源は他にもあるが、このフレームワークの意図は、ヒトのリスク評価における *学データととトでの事例データ*の解釈と利用にあり、他にもあるヒトの情報源については当該 文書で深く触れていない。特に、当該文書では、意図的なヒトを対象としたばく露に関する農 薬の研究<sup>5</sup>や、農業従事者の活動に伴う経皮や吸入によるばく露量を測定するために行われた 研究<sup>6,7</sup>については、広く論じていない。

 $<sup>^4\</sup>$  https://www.epa.gov/osa/products-and-publications-relating-risk-assessment-produced-office-science-advisor

<sup>&</sup>lt;sup>5</sup> このような研究の実施及び OPP がこのような研究から得られたデータに依存することは、 EPA の研究対象者保護規則(40 CFR Part 26)によって管理されている。その他に、この規 則は妊婦、授乳婦、子供への意図的なばく露に関する研究を禁じ、EPA-OPP やヒト研究評価 委員会(HSRB)による新規研究計画の事前評価、ならびに研究成果報告の評価を求めてい る。

<sup>&</sup>lt;sup>6</sup> 過去数年間に OPP は、農業従事者のばく露評価に使用されるデータとアプローチを改善する ために、農業従事者を対象とした既存の観察研究を広範囲に評価してきた。それらの評価は (http://www.epa.gov/scipoly/sap/meetings/2007/010907\_mtg.htm) で閲覧できる。

<sup>7</sup> OPP が農業従事者ばく露研究をどのように実施し、成果を利用するかについての追加情報は、 PPP-48「農薬とヒト健康リスク評価: Policy, Processes, and Procedures」 (<u>https://www.extension.purdue.edu/extmedia/PPP/PPP-48.pdf</u>で入手可能)を参照のこと。

#### III. 農薬のリスク評価におけるシステマティックレビュー: 疫学

近年、NRC は、規制上の政策調査に情報を提供するための化学物質固有のリスク評価をサポ ートする科学的文献レビューの明白性を高めるために、システマティックレビュープロセスに移 行するよう政府機関に奨励している(NRC、2011 年、2014 年)。NRC はシステマティックレ ビューを「特定の疑問に焦点を当て、明確で事前に指定された科学的方法を用いて、類似してい るが別個の研究の結果を特定、選択、評価、要約する科学的調査」と定義している(NRC、 2014 年)。NRC の勧告に沿って、化学物質安全・公害防止局(OCSPP)は、政策調査を支え る科学的データの収集、評価、統合のための標準的な方法に依存する、目的にかなったシステマ ティックレビューを採用している。

NRC によると、システマティックレビューには、"明白性があり明確に文書化された方法、関連するすべての文献の一貫した厳しい評価、エビデンスの強さを評価するための標準化されたアプローチの適用、明白で一貫性のある総括的用語(NRC、2014年)"といういくつかの共通要素がある。近年、いくつかの集団(Rooney ら、2014年; Woodruff and Sutton、2014年; Hartung、2010年)が、環境保健科学で使用するためのシステマティックレビューのアプローチを発表している。システマティックレビューでの OCSPP のアプローチは、Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine に明記されている原則と、Grading of Recommendations Assessment, Development and Evaluation (GRADE)の原則と一致している。GRADE ガイドラインは、国立環境保健科学研究所(NIEHS)健康評価・翻訳局(OHAT) (Rooney ら、2014年)とカリフォルニア大学サンディエゴ校(Woodruff and Sutton、2014年)が開発した環境保健科学のためのシステマティックレビューアプローチで使用されているものである。コクラン・ハンドブックによると、システマティックレビューの主な特性は以下の通りである。

- ・目的が明確に示されており、研究の適格性基準があらかじめ定義されていること。
- ・明示的で再現性のある方法論。
- ・適格性基準を満たすすべての研究を特定するための系統的検索。
- ・特定された研究から得られた知見の妥当性の評価。
- ・収録された研究の特性と知見を体系的に提示し、総合的にまとめたもの。

上記の各アプローチは共通のテーマとワークフローを共有しており、科学的な文脈(例えば、 問題の定式化やプロトコル)の記述から始まり、明確な検索戦略の方法を用いた文献レビュー、 研究の質の解析(しばしばバイアスのリスクと呼ばれる)、エビデンス全体の質の評価(例えば、 統合)、そして最終的に結論に至るまでの流れを示している。それぞれのアプローチでは、科学 文献の包含/除外、研究の質の評価、研究の質(例:高、中、低)の報告のための透明性のある 事前に設定された基準を推奨している。各アプローチでは、結論の基礎となるデータ統合のため の事前に定められたツールを推奨している。

これまでのところ、リスクアセスメントに関係する人々の間では、システマティックレビュー のための単一の命名法は合意されておらず、OCSPP は、より広範な経験が積まれるにつれて用 語が進化していくことを期待している。OCSPP は、システマティックレビューのプロセスとワ ークフローを、問題の定式化から始まり、データ収集、データ評価、 データ統合、そして重要 なデータギャップを特定した結果の要約と考えている。

科学的な解析は、新しい知識が得られるたびに、繰り返し行われることが多い。

#### A. 問題の定式化

NRCの報告書「Science and Decisions-Advancing Risk Assessment (科学と意思決定・リス ク評価の促進)」において、全米科学アカデミー(NAS)は EPA に対して、政策立案者に有用 なリスク評価と関連する科学的解析を開発するよう勧告した。この目標達成のために NRC は、 政府機関に対してリスク評価の開発において一層幅広く問題の定式化を用いるよう勧告した。 NRCの勧告を受けて、USEPA は「ヒト健康リスク評価フレームワーク」(USEPA、2014年) を公表し、問題の定式化の重要性を強調している。問題の定式化は、科学者とリスク管理者の間 の初期対話を伴うものであり、科学的解析のための規制上の背景を提供し、解析の範囲を定義す るのに役立つ。問題の定式化は、評価の規制、政策調査及び政策の背景から導き出され、評価の 技術的なアプローチの情報を提供し、考慮すべき主要な要因を体系的に特定するものである。こ のように、各システマティックレビューの複雑さと範囲は、異なるリスク評価の文脈によってさ まざまである。言い換えれば、OCSPP のシステマティックレビューは、問題設定から事前に設 定された範囲と目的に基づいた「目的にかなったもの」(NRC、2009年)として実施される。

問題の定式化には、利用可能な情報とともにデータや科学的情報の主要なギャップを考慮する ことが含まれる。OPP は、ばく露経路と潜在的な健康影響を特定するためのツールとして問題 の定式化を用いており、適切な方法、情報源及び科学的解析のアプローチとともに使用している。 欠損データが評価において重要な場合、情報(例えば、必要とされる試験)を得るための最良の 選択肢が議論される。ピアレビュープロセスが特定され、評価を完了するためのタイムラインが 定義される。

システマティックレビューは、利用可能な情報を整理し、解析に対する規制目的に関する情報 のギャップを特定するための明白なツールを提供する。このように、問題の設定では、科学的解 析の規制上の文脈が説明され、それによって科学的文献の収集と評価の範囲と目的が定義される。 問題の設定における考慮すべき事項は、集団またはライフステージ、ばく露経路(例:経路、期 間、頻度)及び/または in vitro または in vivo の実験室での研究、疫学またはヒトの事故によ る研究から特定された目的の健康結果、ならびに利用可能な資源及び規制の時間枠に関すること であろう。疫学情報及びヒトでの事例情報を考慮する文脈では、試験の質、試験デザイン及び不 確実性の初期評価が考慮される。

問題の設定で考慮されるハザード評価に関連する主な科学的課題は以下の通りである。ばく露 に関連する影響とは何か?これらの影響に関連する MOA/AOPs はどのようなものか?影響の 時間的側面は?影響を受けやすい集団があるのか、あるとすれば、その集団は誰であり、どのよ うな要因によって影響を受けやすいのか。実験動物とヒトの間で PK や薬力学 (PD) に違いは あるか?また、ばく露情報は問題の設定においても評価される。問題の設定で考慮されるばく露 評価に関連する主な科学的課題は以下の通りである。農薬はどのように使用されるのか?ばく露 の関連する使用場所のすべてはどのようなものか?ヒトはどのような化学物質にばく露されるの か?ばく露の方法、期間、頻度はどのようなものか?きがばく露される可能性があるか?ばく露 は異なる集団 (例えば、年齢や行動パターンのため)に異なるリスクをもたらすのか?疫学デー タの具体的なケースでは、このレビューでは、研究仮説、研究デザイン(すなわち、サンプルサ イズ、十分な対照、測定方法の質など)、ばく露量/ばく露濃度、統計解析及び結論を含むが、 これらに限定されない様々な要因を考慮して検討する。

#### B. データ収集

システマティックレビューのデータ収集段階では、公開科学文献や農薬登録のために提出され た研究論文など、様々な公開・未公開の情報源から利用可能な情報を収集する。OPP では、経 済協力開発機構(OECD)試験ガイドライン、OCSPP 調和試験ガイドライン及び農薬ガイドラ イン(OPP ガイドライン)に基づいて収集されたデータをレビューしている。これらのガイド ライン研究論文は、主に提出された研究の社内データベースから収集され、そのような社内デー タベースの検索によって発見される。

疫学の場合、ほとんどの研究は公開されている科学文献に掲載されていることが予想される。 場合によっては補足的な解析や情報が得られることもあるが、研究者との対話によって、疫学研 究を理解し解釈する上で、原著論文には掲載されていない追加の重要な情報が得られることもあ る。ヒトでの事例の情報源はセクション IV にまとめられている。

公開文献検索戦略では、特定の基準を用いて、公開されている科学文献や未発表の情報源から 健康影響情報を検索する。最も適切な情報源/データベースを特定して選択し、分類コード、医 学的主題の見出し及び/またはキーワードを利用した最もリソース効率の良い戦略を調査した後、 文献の検索が行われる。科学的評価の複雑さに応じて、文献管理責任者のサポートが必要な場合 もあれば、必要でない場合もある。ヒトの健康に関する文献検索の目標は、文献検索プロセスの 適切な文書を提供することにより、信頼性が高く再現性のある文献検索を行うことである。有用 な研究を検索するには、以下の手順で行う。

- 科学的解析の目的と包括基準が確立されている。
- 用語/キーワードや MeSH (Medical Subject Heading) 用語の組み合わせと、それらの ブール考案の組み合わせ(AND; OR; NOT)を使用し、文書化する。

必要に応じて、著者名、タイトル、キーワード、主題見出しによる高度な検索やフィー ルド検索を行うこともできる。データベースの構造を知り、特定のデータベースに対し て別の検索戦略を使用することは、有用な研究論文を検索する際に役立つ。最初の包括 的な検索に加えて、文献リストを更新するために定期的な検索を行うこともある。

- すべてのデータベースのすべての検索が再現可能であることを保証するために、検索戦略は検索の日付を含めて文書化されている。
- 検索された論文のリファレンスリスト<sup>2</sup>は、追加の文脈を調べたり、最初の検索では発見 されなかった論文を探したりするために調査されている。
- 異なるデータベースから検索された論文を組み合わせて重複を除去した後、利用可能な タイトルと要約を選別する。タイトルと要約から関連性が判断できなかった論文につい ては、その論文を検索し、さらなる再検討を行う。
- 最初の選別の後、有用性のない論文(除外基準)一例えば、持論の論文、英語で記述されていない研究論文、要約のみで構成された論文は、除外される。追加の除外基準は個別的に特定される。すべての除外基準は文書化されている。残りの論文は、有害な健康影響が認められなかったものであっても、検討と評価のために含まれる。

## C. データ評価

データ評価段階では、データの質が検討され、そのようなデータの有用性について結論が出される。研究の質は、報告された結果が正しいという全体的な信頼性を反映する(Balshem ら、2011年)。このように、研究の質には以下のようなものがある。

- 報告の質(研究の報告がどの程度に十分であるか、または完全であるか)。
- 試験のデザインと実施に基づいて、試験結果がどの程度に信頼性のあるものか。
- さらに、その研究が評価下の主題にどの程度に十分な対応しているか(Rooney ら、 2014 年)。

研究の質は、まず個々の研究基盤で検討され、その質が判断される。例えば、不完全に実施さ れたコホート研究よりも、適切に実施された症例対照研究の方が信頼性は高い場合がある。科学 的知見の信頼性は、しばしばバイアスのリスクと呼ばれ、試験のデザインと実施に関連する特定 の分野について、あらかじめ定められた基準を用いて評価される(表2参照)。

OPP は当初、「ヒトの健康リスク評価を支援するための公表されている科学文献の毒性研究 の検討と使用に関するガイダンス」(Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment)(USEPA、2012年)と呼ばれ る公表されている科学文献の考えの使用に関するガイダンスを作成し、一般的にはこのガイダン スに従っている。しかし、リスク評価におけるシステマティックレビューの促進に伴い、この文 献ガイダンスの一部の側面は将来的に更新が必要になる可能性がある。 データの質についての結論が出され、結論の記述またはカテゴリー(例えば、許容可/許容不 可;低、中、高)で記述される。

農薬に関する疫学研究の評価に用いられる具体的な考えは、以下のセクション III.C に記載されている。データレビューの一環として、研究の簡潔なレビュー文書を作成する。このレビュー 文書には、研究のデザイン、結果、結論と、研究の長所と短所が記載されている。疫学的ばく露 評価の質は、疫学的データがリスク評価においてどのような役割を果たすかを調査する上で重要 な要素である。そのため、疫学的ばく露評価で使用された仮定と、その仮定が疫学的知見の解釈 と一般化の可能性にどの程度影響を与えるかを十分に特性評価することが重要である。疫学的ば く露評価には、過去及び現在のばく露パターン(例えば、ばく露集団、ばく露経路、ばく露方法 及びばく露レベル)の検討が含まれ、また、使用パターンの大幅な変化(例えば、リスク軽減措 置や新たな使用パターン)が含まれている場合がある。メタ分析の評価に関しては、Stroup ら (2000 年)によって報告された「疫学における観察研究のメタアナリシス」Meta-analysis Of Observational Studies in Epidemiology (MOOSE)のガイドラインが、メタ分析の質の評価や 解釈に有用である。

# D. データ統合:エビデンスの重み付け(WOE)

OPP のヒト健康影響評価では、ヒトでの調査/疫学、バイオモニタリングデータ、in vitro の 毒性試験及び in vivo の毒性試験、MOA/AOP 情報、薬物動態試験、構造活性関係(SAR) な どを含む(但し、これらに限定されない)利用可能なすべての関連データを考慮する必要がある。 さまざまな種類のハザードデータを収集し、関連する各試験の完全な評価を実施して記録した後、 次のステップは複数のエビデンスを統合することである。

データ統合は、関連するハザードについて利用可能なネガティブデータとポジティブデータを 総合的に判断するという原則に基づいている。OPP は、疫学データ及びヒトでの事例データの 評価に WOE 解析を使用しているが、結論は、特定の研究に依拠するのではなく、情報の優越性 に基づいて出される。OPP は、異なる情報源からの情報を整理して統合するためのツールとし て、MOA/HR のような修正された Bradford Hill 基準を使用している(Hill、1965 年; U.S. EPA、1999 年、2005 年; Sonich-Mullin ら、2001 年; Meek ら、2003 年; Seed ら、2005 年; OECD AOP Wiki Users Handbook<sup>8</sup>)。Hill 基準は、チェックボックス方式ではなくて、エビデ ンスの総合的評価の検討を示唆していることに注目することが重要である。また、MOA/AOP が完全に解明されていることは、ヒト健康リスク評価に疫学研究を利用するための必須条件では ない。しかし、完全に解明された MOA/AOP がない場合でも、メカニズムデータの収集と評価 は、生物学的な妥当性の裏付けとなり、生体組織の感受性、生物種、性別、ライフステージ、ま たはその他の要因での違いを説明するのに役立つ可能性がある。MOA/HR は、厳密ではない情 報を整理するための基盤を提供する柔軟なツールである。

<sup>&</sup>lt;sup>8</sup> https://aopwiki.org/wiki/index.php/Main\_Page#OECD\_User\_Handbook

これは因果関係の評価、複数の科学的根拠に基づく情報の統合、データのギャップや今後の研究 分野の特定などの様々な目的に有用なツールとなる柔軟性を備えている。この解析では、疫学的 知見やヒトでの事例データは、生物学的な妥当性を評価するために、他のヒト情報や試験の文脈 で評価され、不確実分野や今後の研究分野を特定することができる。Bradford Hill の側面が WOE 評価でどのように考慮されるかを説明するために、OPP は、EPA の全米環境大気質基準 (NAAQS)のレビューのための科学的基盤となる統合科学評価(ISA)の EPA 前文に記載され ている用語の定義を使用している(USEPA、2015 年)。

- 重要事象。特定の健康影響について MOA/AOP が確立されている場合には、MOA/AOP の基礎となる重要事象(すなわち、測定可能なパラメータ)をそれぞれ明確に記述する。重要事象を示すデータは、*in vitro*または *in vivo*のデータソース(ヒトまたは動物)の組み合わせから得られる可能性がある。これらの重要事象は、PK 事象と PD 事象の組み合わせである。しかし、完全に解明された MOA/AOP を利用できることは、ヒト健康リスク評価に 疫学研究を利用するための必須条件ではないことは注目に値する。
- 生物学的勾配/ばく露-反応/用量-反応の一致と関係。ISAs の前文では、「疫学の文脈では、よく特徴づけられたばく露-反応関係(例:ばく露量が多いほど影響が増加する)は、特にばく露期間(例:ばく露時間が長くなると影響が増加する)についても因果関係を強く示唆する」(USEPA, 2015)と指摘している。MOA/AOPが明らかである場合、各重要事象について用量反応関係が特定される。用量反応関係は、重要事象間で比較される。場合によっては、初期の重要事象の方が後期の重要事象よりも感度が高い場合がある。他の場合では、重要事象は類似した量反応曲線を共有しているかもしれない。
- 時間的関連付け。薬剤の投与と影響の出現との間に時間的な順序があるというエビデンスは、
   因果関係を支持するもう1つの根拠となる(USEPA、2015年)。ISAsの前文では、「ばく露の変化が健康影響の発現または発生頻度の変化をもたらすことが判明した場合には、
   『自然実験』を通じて因果関係を示す強固なエビデンスを提供することができる」と指摘している。

この解析では、影響の整合性を確保するために急速に発現する重要事象(例えば、ばく露後数 分以内に発現する活性代謝物への代謝)と長い継続期間の後に発現する重要事象(例えば、腫瘍 の発生)を考慮している。疫学データを検討する際には、ばく露と健康影響の間の時間的関係が 考慮される。

#### 強固性、一致性、特異度。

一致性。複数の独立した研究でリスクの上昇が観察された場合に、因果関係の推測は強固になる。結果の再現性は因果関係を示す最も強力な根拠の一つである。統計的な有意性は、効果の有無を判断する唯一の基準ではない。研究間で不一致な結果がある場合は、ばく露の違い、交絡因子、研究の検出力などの理由が考えられる(USEPA、2015年)。

複数の研究における結果の一貫性は、複数の独立した研究における効果または関連付けの繰り 返しの観察から得られる。さらに、異なる状況下での異なる母集団における結果の再現性によっ ても支持される。しかし、独立した調査の間での結果の不一致は、調査方法の違い、ランダムエ ラー、ばく露、交絡因子、または研究の検出力の違いによって説明される可能性があり、因果関 係を否定するために使用することはできない(USEPA、2015年)。

**観察された関連付けの強固性。**大規模で正確なリスクの発見は、関連付けが偶然、偏りまたは その他の要因によるものではない可能性が高いという確信を得られる。しかし、影響の推定が小 さい場合は、母集団における実質的な影響を表す場合もあれば、そうでない場合もあることに注 意が必要である(USEPA、2015 年)。

**観察された関連付けの特異度。**特定の結果をばく露に結びつけるエビデンスは、因果関係を示 す強い根拠となる。しかし、環境ばく露が結果の発生を必ず予測できることは稀であり、結果に は複数の原因がある可能性を認識しなければならない(USEPA、2015 年)。

### 生物学的な妥当性と整合性。

**整合性。**1 つのエビデンス(例えば、疫学的に管理されたヒトのばく露研究、毒性試験、または生態毒性試験)からの因果関係の推測は、関連付けの因果関係の解釈を支持する他のエビデンスによって強化されることがある。1 つの科学的エビデンスグループの中で、さまざまな分野にわたるエビデンス、複数の試験デザインにわたるエビデンス、または健康影響に関連するエビデンスから、効果を示す一貫性があるかもしれない(USEPA、2015年)。

動物でのデータとヒトでのデータが、定量的にも定性的にも同様の毒性プロファイルを示してい る場合、ヒト健康リスク評価の信頼性は高い。一方、動物でのデータとヒトでのデータが定性的 には類似した毒性プロファイルを示していても、定量的な相違が観察される場合もある。例えば、 ある化学物質が動物とヒトでは同じMOA/AOPを示していても、用量反応特性に種差がある場 合がある。このような用量反応性の相違は、生体組織薬量測定(すなわちPK)に起因するか、 または異なる反応特性(すなわちPD)に起因する可能性がある。一方、動物でのデータとヒト でのデータは、場合によっては質的に異なる結果を示すことがある。このような状況から、種、 性別及びライフステージの違いや潜在的な感受性などの要因を考慮するために、入手可能なすべ ての情報を透明性のある包括的な方法で十分かつ客観的に評価する必要性が明らかになった。

**生物学的妥当性。**因果関係の推測は、生物学的に妥当なメカニズムを示す試験またはその他の 情報源からの結果によって強化される。提案されたメカニズムは、実験的エビデンスに基づいて おり、ある物質へのばく露を所定の効果に結びつけるもので、因果関係を支持する重要な情報源 である(USEPA、2015 年)。

同様に、多くの構造的類似物の1つとしての化学物質のMOA/AOPに関する情報は、因果関係の可能性が高いかどうかの判断に役立てることができる。構造活性相関及び薬剤の構造的類似物に関する情報は、関連性が因果関係にあるかどうかについての洞察が提供される(USEPA、2015年)。

EPA のがんガイドライン(2005年)は以下の通りに指摘している。

「疫学研究で観察された関連付けの生物学的妥当性の評価は、潜在的作用機序(MOA)の 特定に関連する毒性学的エビデンスとばく露関連因子の両方を考慮したものである。同様に、 疫学的文献で報告された健康影響の関連付けの整合性を考慮することは、毒性試験や疫学研 究で評価された生物学的マーカーの特性に関する情報を幅広く考慮することを示している [p.39]。」

しかし、このガイドラインはさらに、「しかしながら、メカニズムデータの欠如は因果関係を 否定する理由にはならない [p.41]」と述べている。このように、確立された MOA/AOP の欠 如は、疫学データを使用する際に必要な知識ではなく、確立された MOA/AOP がなくても疫学 的関連付けは有効である可能性があり、また、潜在的な MOA/AOP について洞察も得られる可 能性がある。

・不確実性。不確実性は WOE で明白かつ客観的に述べられている。

## E. 全体的な結論、リスク評価の推奨事項、信頼性と不確実性の分野の陳述

エビデンスの要約、エビデンスの重み付けに使用した手順または方法、WOE の結論または勧 告の根拠、不確実性及び更なる研究分野を文書化することが重要である。また、リスク評価にお ける疫学的データまたはヒト由来のデータの役割についても推奨されている。一般的に、OPP はヒトでの事例情報を定量的リスク評価には使用しないが、その代わりに、新たなリスク評価や 新たなリスク管理措置の必要性を示すこと、リスク軽減措置が実施された後の成功度を評価する こと及び可能性のある施行活動を対象とすることなど、リスク評価/リスク管理の活動を対象と する。

ヒトでの事例データの役割がより限定的であるのとは対照的に、疫学研究は様々な方法でリス ク評価の複数の構成要素に情報を提供するのに役立つ可能性を持っている。正確なばく露評価を 行った質の高い研究は、リスク指標を定量的に推定するために使用することができる。

また、疫学的研究で報告された結果は、*in vitro* 試験や動物試験で見られた結果と定性的に比較して動物試験の結果のヒトへの関連性を評価することもでき(Hertz-Picciotto、1995年)、 疫学的結果の生物学的妥当性を評価することにも有用である。提案されている WOE 解析の最後 の部分では、全体的な結論と信頼性と不確実性の分野の記述が行われる。このセクションでは、 追加研究の分野も特定している。本セクションでは、規制値の情報源及び(必要に応じて)種間 及びヒトの間での外挿法での推定のための適切なアプローチを推奨している。

# IV. 農薬のリスク評価に用いるための疫学研究のレビュー A. 緒言

疫学は、化学的、物理的、または生物学的要因へのばく露と集団の健康状態との関係を特定し、 評価することを目指す科学である(Boves ら、2007 年)。疫学とは、「集団における疾病の分 布と、その分布に影響を与えたり、調査したりする要因の研究」と定義されている(Gordis、 2009年)。より広義には、「特定の集団における健康に関連する事象、状態、プロセスの発生 と分布の研究であり、そのようなプロセスに影響を与える調査要因の研究と、この知識を関連す る健康問題の制御に応用することを含む」(Porta、2014 年)と考えられている。多くの疫学研 究の目的は、病気の発生に対する潜在的な原因の影響を有効かつ正確に推定することである。疫 学の主な目的は、他の科学と同様に、原因と結果を明らかにすることであり、別の言い方をすれ ば、病気や健康影響の病因とそれに関連する可能性のあるリスク因子を特定することである。 Calderon(2000年)は、このような研究の4つの主要な用途を説明している。1)集団の健康状 態を記述し、疾病やばく露頻度の重要な時間的傾向を発見すること、2)特定の疾病や傾向に関 連する因子を特定することで疾病の発生を説明すること、3)特定の集団における疾病の発生数 や健康状態の分布を予測すること、4)環境やヒト健康に悪影響を及ぼす因子を特定することで 集団の健康状態を改善すること、である。農薬の場合、疫学は、一般集団と、職業上ばく露され る農業従事者や農薬散布者などの特定の小集団におけるばく露と健康への悪影響との関係に焦点 を当てている。

疫学研究は、様々な方法でリスク評価の複数の要素に情報を提供するのに役立つ可能性がある。 正確なばく露評価を行った質の高い研究は、リスクを定量的に推定するために、あるいはオッズ 比やリスク比のような適切なリスク・サロゲート(代理)を用いることができる。しかし、農薬 や農薬ばく露を扱う疫学研究の多くは、規模、範囲、ばく露評価、またはデータ解析になんらか の制限があり、それが定量的リスク評価への十分な利用を妨げている(Ntzani ら、2013 年)。

米国での農薬使用は、ここ数十年で大きく変化した。使用が変化すると、農業従事者へのばく 露も変化する。農薬使用の変化は、EPA によるリスク軽減措置、抵抗性管理活動、新しい化学 物質の導入、遺伝子組換え作物の使用量の増加によって生じている。疫学研究を解釈し、最終的 には定量的リスク評価にそのような研究を使用するかどうかを調査する際には、ばく露における これらの大きな変化を考慮しなければならない。そうであっても、疫学研究は、実験的動物研究 から得られたエビデンスと比較して、そのような値を導き出す際の仮定を特徴づけるために使用 することが可能である。また、疫学研究で報告された結果を *in vitro* 試験や動物試験で得られた 結果と定性的に比較して、動物実験で得られた結果の生物学的妥当性や人間との関連性を評価す る場合もある(Hertz-Picciotto、1995 年)。疫学研究で得られたようなヒトでの情報は、NRC (2007 年)が推奨する毒性試験の新しいビジョンにおいて重要な役割を果たす可能性があると 期待されている。具体的には、疫学研究は、ヒトの実社会での化学物質ばく露から生じる可能性 のある健康影響についての知見を提供し、その結果、問題の設定やハザード/リスクの特性評価 に貢献することができる。ヒトでの情報は、追加的な研究(例えば、*in vitro* 試験や目標とする *in vivo*試験で使用するための用量やエンドポイントの選択)の指針となり、今後の研究で調査さ れる新たな健康影響や感受性を特定することができる。

動物実験から得られた実験データがハザードの特性評価のための主要な情報源となる場合、潜 在的な不確実性の原因の一つとして、動物モデルのヒトへの関連性が挙げられる。これに反する データがない場合には、動物の結果はヒトとの関連性があると想定される。さらに EPA はヒト は実験動物よりも感受性が高いと仮定しているが、それを裏付けるデータがない。実際には、ヒ トは他の動物種よりも農薬に対して感受性が高い場合も低い場合もある。疫学データ及びヒトで の事例データは、科学的な情報を提供し、外挿法での推定に関連する不確実性を伝えるための裏 付けとなり得る。母集団の変動性に関しては、動物試験よりも疫学研究の方が潜在的な変動性を の特徴をよく表している。具体的には、疫学データは、ヒトの集団に固有の遺伝的多様性と変動 性を含むため、実験動物よりも環境化学物質に対する実際の集団での反応をよりよく説明し、代 表することができる(Calderon、2000 年)。

用量反応の特性評価に関して、動物の毒性試験には、広範囲のばく露レベルをカバーするよう に試験デザインできるという利点がある。しかし、動物の毒性試験では、一般的に環境中でのば く露レベルよりもはるかに高い(時には桁違いの)ばく露レベルが用いられている。動物試験で は、このような高いばく露レベルがあるため、高用量から低用量の外挿法での推定が必要である。 この外挿法での推定はリスク評価に不確実性をもたらす。疫学研究データやヒトでの事例データ は、ヒトの実社会でのばく露を含んでいるため、多くの場合、高用量の外挿法での推定は必要な いと考えられる。疫学研究は低用量から高用量までの広い範囲のばく露が最も有用である。

動物試験では、ヒトが経験するばく露の時間、程度、期間、ばく露経路及びばく露の変動性を 再現するものではない(Calderon、2000年)。ヒトでのばく露は、食品、水、空気、屋内外の 環境を含む多岐のばく露経路を介して行われることが多い。対照的に、制御された実験室での研 究では、通常、単一のばく露経路である。

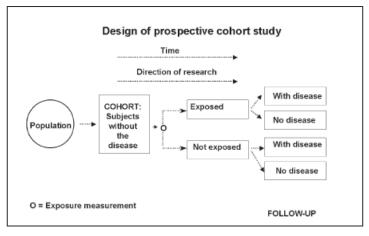
さらに、ほとんどの動物試験では単一の化学的ストレス因子を用いているのに対し、ヒトでは複数の化学物質及び/または非化学的ストレス因子へのばく露を同時に受けている可能性がある。 一方で、疫学研究における複数の化学物質へのばく露は、疫学研究の結果が単一の農薬に帰属させようとする場合に問題となり得る。一方で、疫学研究は実社会のばく露を考慮しており、実験的アプローチと併せて考慮することで、実験環境では評価が困難な複数の化学物質ばく露に関連した問題に対処するのに役立つ可能性がある。

#### B. 疫学研究の種類

観察疫学研究の主なタイプを、その長所と短所を考慮しながら以下に簡単に説明する (Lilienfeld and Lilienfeld、1979年; Mausner and Kramer、1985年; Kelsey ら、1996年; Rothman and Greenland、2012年; Paddle and Harrington、2000年; USEPA、2005年; Purdue Pesticide Programs, PPP-43)。

**コホート研究**では、共通の特性を共有する人々の集団(コホート)から始まり、病気の発生が記録されている長期の追跡期間にわたって健康状態を評価を行う(van den Brandtら(2002年)

から引用の図を参照)。共通の特性 は、しばしば「リスク因子」(ばく露 など)<sup>9</sup>の有無である。このような研 究では疾病発生率の違いを調べるため に、「ばく露者」と「非ばく露者」の 間の疾病発生率の違いを特定し、時間 をかけて調査する<sup>10</sup>。次に、この疾病 発生率の差を調査して、「ばく露群」 と「非ばく露群」の間で疾病発生率が 異なるかどうかを判断する。コホート 研究は、複数の疾病発生を同時に評価 対照研究では当てはまらない。症例対照



研究では、一般的には後述するように単一の(事前に指定された)疾病発生のみを評価すること に限定される)。

コホート研究は、Agricultural Health Study (AHS\_http://aghealth.nci.nih.gov/) のように前 向きで実施することもできるし、過去の記録から後ろ向きに実施することもできる。前向きコホ ート研究は、現在の時点から将来の時点までの人々の集団に焦点を当てている。後ろ向きコホー ト研究は、過去のある時点でばく露された集団に焦点を当て、ばく露が発生した後の疾病率を比 較するものである(一般的には、一人(個人)単位で利用できる既存のばく露データベース(ま たは記録)を利用)。

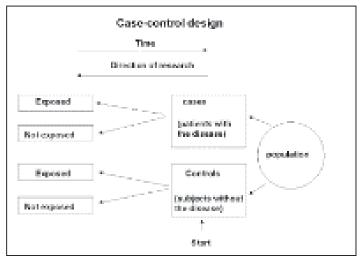
<sup>9</sup> コホート研究では、ばく露はしばしばばく露者と非ばく露者に二分されるが、ばく露は定量的 尺度(例えば、連続的尺度または定量値)で測定することも可能である。

<sup>&</sup>lt;sup>10</sup> コホート研究では、一般的に疾病発生率の違いを研究しているが、これらの研究には、出生時の体重、知能、血圧など、関係のある他の限局的な結果も含まれる。

前向きコホート研究は、特に希少疾病の場合、実施に比較的時間がかかり、費用がかかることが あり、研究対象となる被験者の数が多いことが必要である。重要なことは、質の高いデータを収 集するためには、長期間にわたって多大な資源と専門スタッフが必要であるということである。

**症例対照研究**は、特定の疾病を持つ(症例)個人と、そうでない(対照)個人の集団を特定し、 ばく露(一般的には過去<sup>11</sup>のばく露)に関して比較することで、関係ある疾病を持つ人が、関係 ある薬剤あるいは要因にばく露されていたか否かを判断する研究である。すなわち、症例対照研

究の解析では、症例において関係ある薬剤 または要因のばく露頻度と、対照群におけ るばく露頻度を対比させて、これらが異な るかどうか、要するに関連があるかどうか を判断する。症例対照研究では、一般的 に、疾病状態(すなわち、疾病を持つ症例 と疾病を持たない対照群)を決定すること が、通常、ばく露状態の調査に先立って行 われる(van den Brandt ら、(2002年)か ら引用の図を参照)。この研究デザインは 症例対照研究に選択された時点ですでに疾 病が発生しているので、希な疾病や潜伏期 間の長い疾病の研究で特に有用であり、多 くのばく露と関係ある特定の(事前に指定 された)疾病発生との関連性を評価するた



めに利用することができる。また、症例対照研究は、その疾病に罹患している個人を対象として いるので、コホート研究に比べて被験者数が少なく、比較的短い期間で終了することができる。 症例対照研究の問題は、適切な対照群の選択と、疾病が診断されるずっと前に発生した可能性の あるばく露の評価である(Rothman, 2012 年; Wacholder ら、1992 年 a; Wacholder ら、1992 年 b; Wacholder ら、1992 年 c; Shultz and Grimes、2002 年; Grimes and Schultz、2005 年)。症 例対照研究は、特に"想起バイアス"の影響を受けやすい。これは、罹患した人は、対照あるいは 健康人と比べてばく露や出来事を違った形で(一般的にはよく)覚えている可能性がある。

**コホート内症例対照研究**は、ハイブリッドデザインの一例であり、コホート研究と症例対照研究の要素を含んでいる。この研究デザインは、農薬ばく露を調査するための解析コストが、高すぎて、コホート全体を調査することができない場合に有用である。

<sup>&</sup>lt;sup>11</sup>時間をかけて症例を研究に登録した状況下では研究開始後まで発症していない症例で前向きに 症例対照研究を実施することは可能である。

例えば、現在進行中のコホート研究で疾病または健康影響を生じた症例は、解析時に関係ある疾 病や影響を生じていない研究からの適切な対照群と組み合わせることが可能である。コホート内 症例対照研究(標準的な症例対照研究とは対照的に)の利点として認識されているのは、選択バ イアスと想起バイアスの問題が最小限に抑えられることである。

横断研究では、疾病(例えば、先天性異常、胎児発育遅延(SGA))、症状、生物学的/身体 的及び生理学的反応の測定値(例えば、肺機能検査、血圧、胸部X線、臨床検査、肝臓及び腎臓 のバイオマーカー)の有病率に焦点を当てている。このような研究の主な特徴は、サンプリング 時またはサンプリング期間中に疾病の有無を判定し、有病率を頻度の尺度として重視した観察研 究であるということである。有病率とは、ある集団の中で罹患している人の割合であり、「点有 病率」または「期間有病率」として調査される12。有病率は比率ではなく、割合であるため、横 断研究では追跡調査期間を必要としない。一般的に、ばく露状態(例えば、ばく露または非ばく 露)、疾病の状態/発生、人口統計学的特性は、ある時点(またはその時点以後)で調査される。 この研究デザインにおける主な比較は、ばく露集団における疾病発生の有病率と非ばく露集団に おける疾病発生の有病率の比較であり、リスク尺度は有病率リスク比またはオッズ比である。横 断研究は、一般的に、長年にわたる疾病発生または地理的に異なる場所のパターンや傾向を明ら かにするために使用され、迅速かつ比較的安価に実施することができる。しかし、これらの調査 では、疾病の発生率(新規症例の発生率)と疾病の持続期間の両方に影響される疾病発生の有病 率を測定しているため、これらの要因を十分に分離することは、どのような解析においても困難 な場合がある。したがって、これらの研究は「生存者集団」を対象としており、病気になったた めに関係ある集団から離れてしまった人を測定、評価、考慮することはない。横断研究のもう一 つの重要な限界は、ばく露が疾病に先行しているかどうかを判断できないことである。そのため、 横断研究では疾病とばく露の時間的関係を確立することができず、横断研究で示唆された仮説に 基づく因果関係を確認するために、追加の研究を必要とするのが一般的である。

**生態学的研究**では、グループまたは集団のレベルでのデータを反映した情報を用いてばく露や 疾病のパターンを調べる。生態学的研究では、解析の単位は個人ではなく集団である<sup>13</sup>。ここで は、被験者の集団がサンプリングされ、ばく露、疾病及び潜在的な交絡因子を、この集団(また はクラスター)レベルで測定される。集団は一般的に地理的、行政的、組織的な単位(例えば、 地区、町、郡、学校、職場など)で定義され、すべてのばく露、疾病、交絡因子の測定は、個人 のレベルではなく、集団のレベルで行われたり、まとめられたりする。生態学的(集団ベース) 研究は、個人レベルの研究とは対照的であり、前者では症例がばく露された実際の個人であるか どうかの情報がないのに対し、後者ではばく露情報が個人に結びついている。

<sup>&</sup>lt;sup>12</sup>前者は特定の場所及び/または特定の時間での測定であるのに対し、後者は特定の期間における症例の割合の調査である。

<sup>&</sup>lt;sup>13</sup> 一部の研究では、ばく露または疾病発生のいずれかが集団のレベルで測定されるが、他の変数 は個人のレベルで測定され、研究者は個人のレベルで推測を行うという「部分的に生態学的」 研究もある。

例えば、水中の汚染物質レベルによる疾病率の研究は、ばく露の評価に関しては生態学的である が、健康影響や疾病状態は個人で調査されている場合がある。このような場合、ばく露は集団レ ベルで決定されるが、結果は個人レベルで調査される場合に「半生態学的」という用語が使用さ れることがある。

この研究デザインでは、ばく露集団のすべてのメンバーが個別にばく露されているかどうか (または個人のばく露レベル)を知ることはできないし、グループレベルの影響から個人レベル の影響を推測することもできない。研究の目的が(個人ではなく)*集団*への直接的な推測を目的 としている場合には、これは問題では<u>なく</u>、特に個人レベルでの測定が制約されていたり、困難 であったり、集団内のばく露が一般的に均質であったりする場合には、このような研究が適切で あると考えられる。研究の意図が個人への直接的な推測である場合、この研究デザインは生態学 的誤謬と呼ばれる問題を抱えていることになる。集合またはグループ化されたデータセットで観 察された関係が、個人レベルでサンプリングが行われていたら観察されていたであろうことを反 映するという仮定である。この生態学的誤謬の問題に加えて、交絡因子に関する情報が集団レベ ルでしか得られない場合、集団とは対照的に個人レベルで交絡変数を適切にコントロールできな い結果、追加のバイアスが発生する。

ほとんどの場合、生態学的研究は仮説を生み出す研究と考えられており、今後の研究のための 研究仮説を提案するのに最適であり、問題の提起に貢献する可能性がある。とはいえ、生態学的 研究をデザインの質に基づいて評価することは重要であり、デザインが優れていれば、生態学的 研究から有用な情報を得ることができる(FIFRA SAP、2010 年)。一般的に、生態学的研究だ けでは、因果関係を立証する能力はない。これらの研究を他の研究と併用した場合、特定の状況 下では有用となる可能性があり、ハザード特性評価の際に留意すべきである。特に、安定した母 集団、明確なばく露の対比、リスクの大きな差は、これらの研究の有用性を高める重要な要因と なり得る。

#### C. 農薬のリスク評価に用いるための疫学研究の評価

OPP は、化学物質の使用に関連した潜在的な急性及び慢性の健康影響に関する観察疫学研究 を査読済み文献で検索する。文献検索の手順と戦略に関する詳細は、別の場所で提供されている。 コホート研究、症例対照研究、または横断研究のデザインを利用した疫学研究は、潜在的なハザ ード、ばく露反応の特性、ばく露シナリオ、または評価方法、そして最終的にはリスクの特性評 価に関する OPP の理解を深めるための情報を提供することができる(van den Brandt、2002 年)。さらに、説得力のある症例報告や症例集積解析は、これまで明らかにされていなかった健 康影響や作用機序を明らかにする可能性がある。

一般的には、OPP のリスク評価で用いられる公開文献からの疫学研究を評価する際には、疫 学研究の質、研究の文書化の十分さ(研究デザインと結果)、リスク評価との関連性が考慮され る。

これらの基準は、懸念されるエンドポイントに応じて様々な方法論の詳細が重要になったり、な らなかったりするため、エンドポイントを特定することが重要である。疾病発生の確認に影響を 与える関連因子を理解することが重要である(*例えば、*影響の有無を示す検査やバイオマーカー が利用できるか、あるいは初期段階では非特異的な症状であり、進行した病状では医師の診断に つながるかなど)。さらに、環境及び職域疫学研究では、ばく露評価の質が極めて重要である。 検討中の問題に対して、ばく露と交絡要因の評価の側面を事前に考慮しなければならない。

個々の研究の質を考える際には、疫学研究のデザイン、実施、解析、解釈の様々な側面が重要 である。これらには以下が含まれる。

- 1. 仮説を明確に明示することで、たとえその研究が本質的に仮説生成的なものであったとしても、その仮説を明確に示されていること。
- 健康影響の関連する重大な時期、リスク評価対象集団の関係あるばく露範囲、試験から 得られる用量/ばく露反応の傾向の入手可能性など、ばく露評価の資質の中で、適切な ばく露評価が十分であること。
- 3. 合理的に有効で信頼性の高い結果の確認(研究集団における健康影響の有無を正しく識 別されていること)。
- 4. 対象集団を代表するサンプル集団となり、系統的な偏りがない適切な組み入れ基準と除 外基準。
- 5. 観察されたリスク推定値における複数の農薬ばく露、または混合物ばく露の役割の評価 または考察を含む、潜在的な交絡変数の適切な評価及び解析。
- 6. 参加者の選択や情報収集における誤りを含む、研究における潜在的な系統的な偏りの全体的な特性。これには、提示されたリスク推定値に対する系統的誤差の潜在的な影響を調査するための感度分析の実施を含む(例:Greenland's 公式)。
- 7. 健康影響を観察するための研究の統計的検出力の評価と適切な考察及び/または説明。
- 8. 研究デザインと対象となる結果の性質を考慮した適切な統計的モデル化技術の使用。

他の連邦及び非連邦の機関が次のようなガイドを提供している(*例えば、*OHAT、ナビゲーシ ョンガイド、National Toxicology Program [NTP] Report on Carcinogens [ROC<sup>14</sup>]、IRIS、 Cochrane ACROBAT-Non-Randomized Studies of Interventions)(Sterne ら、2015 年)、及 び観察疫学研究に関する STROBE(Strengthening the Reporting of Observational Studies in Epidemiology)声明(<u>www.strobe-statement.org</u>, Vandenbroucke ら、2007 年; Von Elm、 2014 年)を参照)。OPP が疫学研究をヒトの健康リスク評価に統合する経験を積むにつれ、評 価アプローチに関連する調整が行われることになる。

<sup>&</sup>lt;sup>14</sup> http://ntp.niehs.nih.gov/pubhealth/roc/index.html

独立した研究評価は、ハザードの特定及びばく露反応評価に有益である詳細な要約表を作成す る前に実施され、文書化されている。表2は、評価された主な考慮事項と、各考慮事項に関連す る重み(低、中、高)の構造を示している。表2は考慮事項の一般的な検討事項を示したもので あり、チェックリストと考えるべきではない。特定の科学的解析に適した特定の科学的考慮事項 は、ケース・バイ・ケースで調整される。

研究評価プロセスの最終段階では、研究固有の評価を通じて特定された各研究における系統的 な誤りから生じる可能性のあるバイアスの性質についての専門家/エキスパートの意見を提供し、 疫学データベースに対する全体的な信頼性の評価を行うことである。このようにして、データ統 合(動物、ヒト、メカニズム、その他)は、環境ばく露や職業上ばく露のヒト健康影響を伝える ヒト疫学研究の信頼度に基づいて行われることになる。

パラメータ	高	Quezaua ら、2015 平,Lan 中	低
ばく露評価	MOA/AOP に関連し ている外部ばく露、内 部ばく露量、または電 標本な定量関係。 アンケートを利用した 場合は、能についての するなくなったと のばくなったに ので メタビューの回答。	のバイオマーカーと外部ば く露、内部ばく露量、また は目標ばく露量との間の関 係についてのエビデンス。 調査対象者または代理人が 回答した化学物質固有のば	
発現事象評価		ル;または、方法が明記さ	検査の選択された部 分、または母親の報 告、その他;または母 親/父親の自己報告; 有病症例または事例症 例が適切であるかどう かが不明確/不評価。
交絡因子コント ロール	科学的な疑問に関連す る重要な交絡因子及び 標準的な交絡因子を良 好に制御。	絡因子、標準変数(すべて の変数ではない)を中程度 に良好に制御。	は実施していない;不 層化、不制限、または 不調和。
統計解析	研究の課題とデザイン 適切であること、適 切なサンプル規模に支 えられていること、デ ータを最大限に活用し ていること、適切に報 告されていること(選 択的ではない)。	調査力(特にサブアナリシス)、情報が無く明確に報告されていない解析の選択。	注意、比較の不実施、 または不明記。
アスのリスク(選 択、差のある誤	その他の潜在的なバイ アスの主な原因は、存 在する可能性は低く、 存在するがリスク推定 の規模と方向性に影響 を与える可能性は低く 解析される。		存在するが研究では取 り上げられておらず、

a パラメータ全体の総合的な評価に基づく総合的な研究の質のランキング。

# 1. ばく露評価

ばく露評価は、「ある物質へのばく露の規模、頻度及び期間を、ばく露された集団の数と特性 とともに推定または評価するプロセス」と定義することができる。理想的には、評価において原 因、経路、方法及び不確実性を記述すると記載(Zartarian ら、2005年)されている。環境疫学 において、ばく露評価は、特に環境媒体中に低濃度で存在する毒性物質については、独特の課題 となっている(NRC、1991年; NRC、1997年)。ばく露経路の複雑さを考慮して、研究者はば く露を評価するための多くの異なるアプローチを開発してきたが、その正確さ、精度及び必要な 資源は様々である(Niewenhuijsen、2003年)。これらのアプローチのいくつかは、疫学研究 に特化したものではないが、様々な科学的解析におけるばく露評価の情報を提供するために使用 される可能性がある。これらのアプローチには、過去の記録、アンケート、環境モニタリングに 基づく間接的な方法と、個人モニタリングやバイオモニタリングに基づく直接的な方法がある。 それぞれの方法の簡単な説明とその長所と限界を以下に要約する。

アプローチ	方法/ツール	例	ばく露の推定
間接的	過去の記録	住所情報を利用した農地 への近接推定	二分法または順序のばく露
	アンケート	農薬使用の回答に基づく ばく露の可能性の調査	二分法または順序のばく露
	環境モニタリング	地域の水道における農薬 レベルの測定	モデル化を用いてばく露を 推定することができるが、 二分法または順序のばく露
直接的	個人モニタリング	吸入と経皮でばく露され た農薬の測定	定量化されたばく露
	バイオモニタリング	血中・尿中の農薬の測定	定量化された内部ばく露量

表 3. 間接的ばく露評価方法と直接的ばく露評価方法のまとめ

過去の記録やアンケートは、化学物質へのばく露に関連する可能性のある主要な特性を評価す るために使用される。疫学研究で使用される場合、過去の記録やアンケートは通常、定量的なば く露レベルを予測するためには使用されない。むしろ、過去の記録情報やアンケートの回答は、 ばく露のカテゴリーレベルを調査するために使用される。ばく露レベルを調査するために使用で きる過去の記録情報の例としては、農地に近接した住所や、職位や職歴に関する雇用履歴情報な どが挙げられる。同様に、アンケートは、個人が農薬を使用したことを思い出すかどうかを調査 するために、またはばく露の可能性を高める特定の職務を行っている個人を特定するために使用 される。過去の記録やアンケートは、潜在的なばく露に関するデータの費用対効果の高い情報源 になり得るが、限界がある。過去の記録やアンケートから収集されたデータは、ばく露のサロゲ ート(代理)に過ぎない。

その結果、これらの情報源はばく露の単純化しすぎているため、個人のばく露の可能性を正確 に並べていない可能性がある。

**環境モニタリング**は、大気、水、土壌、食品、家庭環境や職場環境などの環境媒体中の汚染物 質のレベルを特性評価するために使用される。多くの州及び連邦政府のプログラムでは、疫学研 究に有用な環境モニタリングデータを収集している。環境モニタリングは、大気汚染や飲料水な ど、地理的な境界によって定義されるばく露に対して特に有用である。そのため、多くの疫学研 究では、大気汚染と飲料水汚染へのばく露を特性評価するために、大気モニタリングデータと地 域の水道のデータをそれぞれ利用している。環境モニタリングデータは、地理的境界によって定 義されるばく露量を推定するのに有用であるが、個人レベルのばく露量を調査する目的では、特 に個人が多くの異なる場所で生活し、仕事をし、時間を過ごす場合には、信頼性が低くなる可能 性がある。

個人モニタリングは、体の境界の接触点でのばく露を特性評価するために使用される。個人モ ニタリングの例としては、農薬との経皮接触を評価するための線量計の使用、吸入ばく露を評価 するための個人用空気サンプリング装置、食品中の農薬レベルを調査するための対の食品サンプ ルの収集などがある。個人モニタリングの利点は、間接的な方法よりも個人レベルのばく露量を より正確に推定できる可能性が高いことである。また、個人モニタリングは、異なる経路でのば く露の関連性の優先順位付けに有用なばく露レベルの定量化を可能にする。さらに、個人モニタ リングは、時間をかけて測定を繰り返すことで、縦断的なばく露量を評価するためにも使用され る。個人的なモニタリングは間接的なアプローチに比べて多くの利点があるが、労力と資源が必 要とする傾向がある(Niewenhuijsen、2003 年)。その結果、個人モニタリングを用いてばく 露を評価する大規模な疫学研究を実施することは一般的には不可能である。さらに、個人的なモ ニタリングは、サンプルを取得するために使用される測定技術や解析ツールに大きく依存してお り、関連する期間(通常は過去)のばく露を特性評価する情報が利用可能になる可能性は低い。 さらに、対象となる期間のばく露の全容が捕捉される可能性は低く、ピークと変動を捕捉するの に十分な期間でのサンプリングがではないかもしれない。そのため、疫学研究で使用される個人 モニタリング手法の科学的厳密さと信頼性を考慮することが非常に重要であり、そのようなモニ タリングは他のモニタリング(例えば、環境データ、生物学的データ及び/またはインタビュー /アンケートのデータ)で補完する必要があるかもしれない。

バイオモニタリングは、血液、尿、唾液、乳、脂肪、その他の生体組織などの生物学的試料中の化学物質、その代謝物、または反応生成物を測定することであり、ばく露の特性を明らかにするために使用される(Needham ら、2007年)。Zartarian ら(2005年)は、「バイオマーカー/生物学的マーカーは、「生物学的システムにおける変化や事象の指標」として定義されてきた」と述べている。「ばく露の生物学的マーカーとは、組織、細胞、体液などの生物学的媒体から得られる細胞や生化学的、分析的、または分子的な測定値を指し、ある物質へのばく露指標である」と述べている。したがって、バイオマーカーは、ばく露を評価に用いられ、また健康影響の指標として使用される(LaKind ら、2014年)。表4は、疫学研究から収集されたバイオモニタリングデータの質と関連性を評価するための科学的考察を示している。

解析ツールがより費用対効果の高いものになり、より多くのバイオマーカーが同定されるよう になったため、バイオモニタリングを用いたばく露評価は急速に拡大してきた。自己申告のアン ケートやインタビューデータと比較して、バイオモニタリングはばく露の誤分類を減らし、リス ク推定の精度を高めることができるかもしれない。同様に、バイオモニタリングは、異なる経路 からのばく露を統合し、体内に吸収されるばく露量を調査するために使用されている (Checkoway ら、2004 年)。さらに、疾病の履歴におけるバイオマーカーの役割が知られるよ うになってきている場合もあり、バイオマーカーはばく露の時間的な問題を解決するのに役立つ かもしれない。

バイオモニタリングは他のばく露評価方法に比べて多くの利点があるが、それ自体にも限界が ある。多くの研究では、生物学的サンプルはある時点での一点からしか採取されず、特にばく露 が大きく変動する場合には、経時的なパターンを正確に反映していない可能性がある。さらに、 バイオマーカーの評価には、環境とヒトの両方での化学物質の分解と代謝についての理解も必要 である。そのため、ばく露のバイオマーカーはばく露以外の理由で個人間で異なる場合がある。 代謝の違い、尿の測定値に関連した腎臓病などの併発疾患やバイオマーカーが有効成分のばく露 を測定しているのか、環境中分解物のばく露を測定しているのかといった不確実性が、個人間や 想定される比較集団間におけるばく露のバイオマーカーの見かけ上の違いの原因となっているか もしれない。

表 4. 環境疫学研究におけるバイオモニタリングデータの考察(LaKind ら、2014年より引用)

バイオマーカーの考察	ティア1	ティア2	ティア 3
ばく露バイオマーカー	バイオマーカーは、外部ばく露、 内部ばく露量、目標ばく露量と正 確かつ精密な定量的関係にある。	バイオマーカーは、外部ばく露、 内部ばく露量、目標ばく露量との 定量的関係が不明であるか、ばく 露/用量に関してわずかなサロゲ ート[代理](正確さ・精度が低い)である。	
効果バイオマーカー	MOA/AOP では key events のバ イオインジケーター。	健康影響との関係に対する効果の バイオマーカーは明らかである。	バイオマーカーは結論をもたらさ ない(例えば、バイオマーカーは 健康影響を明確にしない)。
特異度	バイオマーカーは、1 つの親化合 物へのばく露に由来する。	バイオマーカーは、類似の毒性を 持つ複数の親化合物に由来する。	バイオマーカーは、様々な有害エ ンドポイントがある複数の親化合 物に由来する。
手法の感度	検出限界は、研究課題に取り組む のに十分な割合のサンプルから化 学物質を検出するのに十分に低 い。	研究仮説に取り組むには、検出頻 度が低すぎる。	NA
バイオマーカーの安定性	既知の履歴と文書化された安定性 データを持つサンプル。		履歴が不明なサンプル及び/また は目的の分析物の安定性データが ないサンプル。

バイオマーカーの考察	ティア1	ティア2	ティア 3
サンプルの汚染	サンプルは、収集時から測定時ま で汚染されていない(例えば、分 析物の無いことが保証された収集 用品及び参照物質の使用及びフィ ールドとラボの両方でのブランク の適切な使用による)。研究に は、研究データの信頼性を保証す るために必要な手順の文書化が含 まれる。		汚染の問題が知られているが、問 題を取り上げた文書はない。
手法の要件		マーカーの同定を可能にする装置	<ul> <li>バイオマーカーの定量のみが可能</li> <li>であるが、その方法には既知の干</li> <li>渉物質がある装置(例:GC-</li> <li>FID、分光法)</li> </ul>
マトリックス調整	研究には、調整濃度と非調整濃度 の結果が含まれている。	研究では、1 つの方法(マトリッ クス調整済みかどうか)を用いた 結果のみを提供している。	

FP=偽陽性、FN=偽陰性、GC-HRMS=ガスクロマトグラフィー/高分解能質量分析、GC-MS=ガスクロマトグラフィー/質量分析、 GC-ECD=ガスクロマトグラフィー-電子補足検出器;GC-FID=ガスクロマトグラフィー-水素炎イオン化型検出器]、 ICC=クラス内相関係数;NA=該当しない;PFP=偽陽性の確率 **間接的ばく露評価**方法は、後ろ向き研究では一般的であり、化学物質のばく露のサロゲート (代理)となる因子に基づいている。上述したように、間接的なばく露データは、追加のモデル 化を行わない限り、一般的には定量的なばく露レベルを推定するためには使用できない。例えば、 ある個人が農薬を使用したことがあるかどうかを調査するためにアンケートを使用することはで きるが、その個人のばく露量を計算するために必要なすべての環境的・行動的な要因に関するデ ータを確実に集めることは困難である。そのため、間接的ばく露データは、二分法のばく露変数 (すなわち、ばく露/非ばく露)または順序ばく露尺度を用いてばく露を分類するために使用さ れることが多い。一方、直接的ばく露評価方法は、個人のモニタリングやバイオモニタリングに よる実際の個人レベルのばく露に関するデータに基づいている。したがって、直接法は、個人の ばく露または内部ばく露量レベルを推定するために使用することができる。直接法は前向き研究 で用いられることが多いが、十分に定義された母集団から既存の生物学的サンプルが入手可能な 場合には、後ろ向き研究でも使用される。

定量化された個人評価には、個人モニタリングやバイオモニタリングがあり、一般的に実際の ばく露レベルを推定するための最良のデータソースと考えられている(NRC、1991年; NRC、 1997年)。その一方で、間接的な方法による正確なばく露の質的測定(例えば、二分式や順序 式のばく露測定)も、疫学の目的では同じように正確であると考えられる。さらに、間接法は解 釈が容易であることが多く、ばく露評価における有用性を示すための追加の研究開発が少なくて 済む可能性がある。

どのようなアプローチであっても、ばく露評価方法は信頼性と妥当性のあるばく露推定値を提 供できなければならない。疫学の文脈では、一般的に*信頼性とは*結果を再現する能力を意味し、 *妥当性とは、一*般的にばく露推定値が真のばく露レベルを反映しているかどうかを意味している (Checkoway ら、2004 年)。特定のばく露評価の信頼性と妥当性を評価する際には、研究目的 の文脈でばく露評価の長所と短所を考慮することが重要である。あまり洗練されていないばく露 評価は、探索的研究に適しているかもしれない。これは、探索的研究は潜在的なハザードについ ての認識を高めるのに役立つため、より焦点を絞った研究への専心を促すことができるからであ る。逆に、より焦点を絞った仮説を持つ研究は、より洗練されたばく露評価法を用いることで大 幅に強固となる。したがって、間接的ばく露評価法と直接ばく露評価法は補完的なツールであり、 ばく露と疾病の関係を調査する際に研究の様々な段階で使用することができる。

## 2. 交絡因子

交絡因子は、ばく露と疾病との関係がある程度で第二のリスク因子(交絡因子)の影響に起因 している場合に発生する。これは、この第二のリスク因子(すなわち、交絡因子)が、疾病の独 立した因果関係のあるリスク因子でありながら、解析対象のばく露と因果関係(因果関係または 非因果関係)があり、ばく露と関係のある疾病との間の因果経路の中間変数としての役割を果た していない場合に起こりうる。交絡因子は、適切に測定され、考慮されなければ、ばく露と健康 影響との間の推定された関連性の大きさ(そして場合によっては方向性)を変化させる可能があ る。

これは、2 つのリスク因子の影響が適切に分離、あるいは「区別」されていないため、ばく露と 疾病の関係が過大または過小に評価される結果となりうる。例えば、ある研究では、ある農薬が 肺がんと関連しているかもしれないが、これは農場のトラクターのディーゼル排気ガスの交絡効 果によるものかもしれない。このように疾病発生(ここでは肺がん)との因果関係が農薬ばく露 とも関連する場合、第二の因子の「農場のトラクターのディーゼル排気ガス」は交絡因子である。 交絡因子には、喫煙、食事因子(例えば、高エネルギー/高カロリーの食事)、身体活動(例え ば、身体活動の欠如)、遺伝子、併存症、薬物使用、アルコール摂取などのあまり直感的でない ライフスタイルでのばく露が含まれることがあり、これらはすべて健康に悪影響を及ぼし、農薬 使用と統計的に関連している可能性がある。疫学的解析では、交絡因子は研究サンプルで測定さ れ、通常、研究のデザイン段階または解析段階のいずれかで最終的なリスク推定値に「調整」さ れる。前者に関しては、疫学研究者は、研究者がコントロールしたい特性を共有する個人に研究 集団を「限定」することができる。これにより、その特性(現在はコントロールされている)に 起因する交絡の潜在的な影響を取り除くことがでる。2番目に利用可能な方法は、研究のデザイ ン段階にも適用可能な方法で、研究者が交絡変数に基づいて個人を「マッチング」することで交 絡をコントロールすることである。これにより、交絡変数が2つの比較群の間で均等に分布し、 交絡変数を効果的にコントロールすることができる。主効果のリスク推定値に影響を与えるため には、交絡因子とばく露または疾病との関係が統計的に有意である必要はないことに注目するこ とが重要である15。

解析の段階で、交絡因子をコントロールできる方法の1つは、層化がある。この方法では、交 絡変数(の可能性がある)ごとに関連性を測定し、適切であると判断された場合には、個別の推 定値を統計的に「まとめる」ことで、各層で評価された推定値に重みをつける Mantel-Haenszel アプローチを使用して共通のオッズ比または他の効果を算出する。層化は同時にコントロールす る必要のある潜在的な交絡因子が複数存在する場合には困難である。このような場合、交絡因子 は通常、統計的モデル化によって対処される。(例:ロジスティック回帰)。

疫学研究が現場で開始される前に交絡因子を慎重に考慮することが重要であり、どのような研 究でも交絡因子をどのようにして考慮したかを適切に記述することが重要である。疫学研究は、 潜在的交絡因子を無視したり、あるいは十分な注意を払っていないと批評されることか多い。こ のため、感度分析は、欠落している交絡因子や考慮されていない交絡因子が観察された効果量に 与える潜在的な影響を示すのに役立つ(Gustafson and McCandless, 2010年を参照)。

<sup>&</sup>lt;sup>15</sup>これが、一般的に、交絡因子を調整する必要があるかどうかを判断するために交絡因子を「統計的に検定」することが不適切であると考えられている理由である。その代わりに、潜在的な交絡因子を調整した(交絡因子を含む)後の効果量の変化が10%以上であれば、交絡因子を分析に組み込むのに十分なエビデンスであると考える人もいる。

測定されていない交絡因子が結果に悪影響を及ぼすと考えられる場合、研究者は感度分析を実施 して、影響の範囲とその結果として得られる調整後の効果測定値の範囲を推定すべきである。こ のような感度分析は、一般的にほとんどの公表されている疫学研究では一様には行われていない が、既知ではあるが評価されていないリスク因子によるバイアスや潜在的な交絡因子の影響を推 定するために、利用可能な場合には使用することができる。

特定のばく露・疾病関連付けに応じて、ある因子はコントロールするために必要な交絡因子で ある場合とそうでない場合がある。交絡因子によって推定された効果量に大きな歪みが生じるた めには、交絡因子は、関係ある疾病が比較的強いリスク因子でなければならない<sup>16</sup>し、関係ある ばく露とも強固に関連付けられていなければならない。潜在的な交絡因子の評価は研究ごとに行 われ、測定されていない交絡因子が結果に悪影響を与えると考えられる場合には、研究者は感度 分析を行い、影響の範囲とその結果として得られる調整された効果評価の範囲を推定すべきであ る。観察疫学研究の質を評価する際、OPP は、関連する交絡因子が適切に同定、記述、評価、 解析されているかどうかを検討し、研究対象となっている特定の関連性を偏りのない推定が可能 であるかどうかを検討し、研究対象となっている特定の関連性を偏りのない推定が可能 であるかどうかを検討する。可能な場合は、交絡因子が推定された効果量の推定値に影響を及ぼ す範囲の判断を助けるツールとして感度分析を考慮する。交絡因子は、疾患の比較的強いリスク 因子でなければならず、リスク推定値に大きな歪みをもたらすためには、対象となるばく露と強 く関連していなければならないということを強調すべきである。このような場合には、交絡因子 の可能性を提起するだけでは十分ではなく、なぜリスク因子が交絡因子となりうるのか、その影 響がどのようなものなのか、そしてその影響が結果の解釈にとってどの程度重要なものなのかに ついて、説得力のある議論が必要である。(p.23・25、FIFRA SAP レポート、2010 年 4 月 22 日)

最後に、共変量の交絡、効果修正、相乗効果、その他の媒介効果を区別することが重要である。 交絡因子とは、疾病と因果関係のある変数及び対象となるばく露と因果関係があるか、否かにか かわらず、関連する変数をコントロールしないことによって生じるバイアスである。疫学研究者 はこのバイアスを最小化しようと努める。一方、効果修飾因子とは、層化(例えば、年齢、人種 /民族、SESの状態、遺伝的多型)によって、効果量に異なる影響を与える変数である。効果修 飾因子は、交絡因子である場合もあればそうでない場合もある。一般的に、効果修飾因子は多変 量モデルに交互作用項を導入するか、効果修飾因子のレベルによってデータを層化した後、層化 に効果量を評価することによってモデル化される。対象となる各集団層において十分なサンプル サイズを確保するためには、効果修飾因子を評価するための特別な研究デザインが必要となるこ とが多い。疫学研究者は、母集団の層化にわたるリスクの違いの評価、ばく露と関係のある効果 の関連付けの評価、影響を受けやすい亜集団の特定が重要であることから、効果修飾因子を理解 することを目指す(交絡因子のように最小化するのではなく、効果修飾因子の理解に努めてい る)。

<sup>&</sup>lt;sup>16</sup>交絡因子がそれ自体が本当にリスク因子であることを確認するだけでなく、関係のあるばく露との関連性だけではないことを確認することも考慮する必要がある。関係のある疾病との関連性を持つ因子の全部または一部を、関係のあるばく露との関連性のために調整することは、ばく露と疾病の関係が本当に存在する場合には、ばく露と疾病の関係を減衰させることにつながる。

効果修飾因子は交絡因子である場合もあればそうでない場合もある。例えば、タバコによく使用 される殺虫剤と肺がんを関連付ける研究では、喫煙は交絡因子になるかもしれないが、この殺虫 剤へのばく露のリスクが非喫煙者よりも喫煙者の方が高い場合には、効果修飾因子になるかもし れない。相乗効果は、疫学的な概念ではなく、生物学的または薬理学的/毒物学的な概念として 紹介されることが多く、2 つの化学物質が一緒になって共同で作用することで、各化学物質の効 果の(数学的な)合計を考慮した場合の効果を超えて、効果を拡大したり、増強したりする能力 に関係している。疫学や統計学の用語では、これは効果の修飾や相互作用として表現されること が多い。

#### 3. 統計解析

疫学研究は、特定のばく露と疾病との関連付けを評価することを目的としている。農薬疫学研究の質を評価する際、OPP は使用された統計的手法も考慮する。具体的には、研究に記載されている分析方法が研究課題に適切であるかどうか、使用された統計的方法の記述の完全性、ばく露と疾病の関係における潜在的な交絡変数の特定、評価、調整のための方法の適切性、実施された可能性のある小集団解析の説明、範囲、提示(多重比較のための統計的補正が行われているかどうかを含む)が考慮される。

疫学研究では通常、リスクを推定するために統計的モデル(例えば、ロジスティック回帰(オ ッズ比)やポアソン回帰(カウントデータ)のような一般化された線形モデル)を利用する。そ のためには、研究者は関連する主要なばく露変数と転帰変数だけでなく、関連する交絡因子を考 慮しなければならず、また、研究における関連付けがこれらの因子、すなわち、効果の修飾や相 互作用のレベルによって異なるかどうかを考慮しなければならない(Szklo ら、2004 年)。交絡 の可能性のある変数(調査対象となる関連性の大きさや方向性を実質的に変化させる変数)が同 定された場合、回帰モデルによる調整を行うことで、目的のリスク推定値、すなわち調査対象と なる関連性を分離することができる。さらに、OPP は、研究における潜在的な効果修飾因子の レベルまたは統計的相互作用の評価による関連付けの層化を評価する。関係のある関連付けの規 模と方向性が第三の変数のレベルによって大きく異なる場合、層化された結果は一次的なものと みなされるべきである。

発現事象がまれな場合やサンプル規模が比較的小さい場合に統計的モデリングを行う場合、モ デルにおける共変量が多すぎないように注意することが重要である。結果として得られる効果量 の推定が高すぎたり、低すぎたりすることがあり、効果の真の推定を反映しているとは考えにく い。稀な事象やサンプル規模が小さいことによるこのような問題は、条件付き手法(例えば、デ ザインに研究における比較群の組み合わせを含む場合の条件付きロジスティック回帰)を使用す る場合にも起こり得る。すなわち不一致ペア(または不一致セット)の観察数が少なすぎると、 推定された効果量もまた信頼性が低くなる可能性がある。このように、交絡因子及びその他の共 変量をコントロールすることは重要であるが、信頼性の高い検定を行うために過度にコントロー ルしたり、自由度が少なくしすぎたりしないように評価者は注意しなければならない。

このような場合には、有効なサンプルサイズを十分に確保するため、最も影響力のある交絡因子 や他の共変量をより少ない数だけ調整して、より解析的なモデルを求めることがより重要である。

最後に、どのような統計モデリング演習においても、結果の臨床的/生物学的/科学的な意義 の文脈で統計的有意性を考慮することが重要である。統計的には有意だが、臨床/生物学的/科 学的な文脈では重要ではないということもある。逆に、統計的には有意ではないが、臨床的/生 物学的/科学的な文脈では重要な結果である場合もある。前者の場合はサンプルサイズが必要以 上に大きいことを示唆し、後者の場合は必要以上に小さなサンプル規模を示唆しているかもしれ ない。後者の場合は、公衆衛生の観点から重要であり、(不正確であるにもかかわらず)特に関 連付けが強固である場合には、さらなる調査が必要である。

#### 4. 観察研究における潜在的なバイアス

バイアスとは、(観察されていない)真の状況とは系統的に異なる研究結果をもたらす研究の デザインまたは実施における系統的な誤差のことである。これは、効果量の評価におけるサンプ リングの変動や精度(または同義で信頼限界)に関係するランダム誤差とは対照的であるが、結 果をある特定の方向(例えば、帰無仮説に向かって、または帰無仮説から離れて)に「追い込む (drive)」または「押し込む(push)」ようなことはない。

バイアスとは、研究のデザインや実施における方法論的な不完全性の反映であり、研究者は解 析の一部として、それに対処したり、議論したりすべきである。研究にバイアスが導入される方 法はいくつかある。研究は、参加者を研究に選択する方法(選択バイアス)や、ばく露や疾病に 関する情報を収集する方法(情報バイアス、先に説明した症例対照研究の想起バイアスを含む) にバイアスがかかっている可能性がある。一般的な職業選択バイアスの例としては、「健康な労 働者効果」があり、職域疫学研究において重要なバイアスを生じさせ、帰無値に向かってバイア スを生じさせ、さらにはそれ以下のバイアス(ばく露が「保護的」であるという解釈を生じさせ る)につながる。バイアスの全くない研究はなく、公表した研究の著者は研究における潜在的バ イアスどの程度説明したか、研究におけるバイアスを(もしあれば)どのように対処し、研究の 特徴を明らかにしたかを検討すべきである。バイアスは、差異的(differential)または非差異 的(non-differential) な誤分類から生じる可能性がある(Greenland、1998 年)。差異的誤 分類(バイアス)とは、他の変数の値に依存する方法で誤分類が発生したことを意味し、非差異 的誤分類(バイアス)とは、他の変数の値に依存しない誤分類を意味する。誤分類バイアス(差 異的または非差異的のいずれか)は、そのようなばく露を分類するために使用される試験方法の 感度と特異度に依存し、特定の(限定された)条件の下でバイアスの方向性を予測可能な影響を 及ぼすことがある。研究の方法と解析の知識に基づいてバイアスの方向を特定できるということ は、規制当局の意思決定者にとって有用である。なぜなら、意思決定者は、検討されている疫学 的効果の大きさ(例:OR、RR)が、真の効果の大きさの過小評価または過大評価である可能性 がどの程度あるかを判断することができるからである 17。

誤分類の程度が 10~20%の範囲にあることは異例ではなく、このような誤分類の程度(それが 差異的か非差異的か)と、そのような誤分類の大きさと方向性の両方に関してオッズ比または相 対リスクに影響を与える可能性の程度を評価する感度(または「もしも」)解析の形態を検討す ることは、疫学研究をレビューする際に有用であると考えられる<sup>17</sup>。(p.25、FIFRA EPA SAP 報告書、2010年4月22日)。交絡因子に関して前述したように、このような定量的感度分析は、 公表されている疫学研究ではほとんど実施されておらず、バイアスの潜在的な規模や疫学的効果 量推定への影響の定量的な評価は行われずに、むしろ一般的には解説文で評価されている (Jurek at al., 2006)。これは次のような理由によるものである。その理由の一部は、疫学者が このような解析のための計算ツールを一般的に利用できないことや、そのようなツールに慣れて いないことにある。このようなツールは次第に利用可能になりつつあり、潜在的なバイアスを評 価するためのより厳密な定量的手法を開発する上で有用であると考えられる。

# 5. 帰無研究の解釈

「帰無」研究、すなわち、農薬へのばく露と有害な健康影響との間に関連付けがないと報告す るよく行われている研究は、ヒト健康リスク評価に有用な可能性があるかどうか慎重に評価され る。研究が無効という結果になるのは、調査された関連付けが実際には存在しないか、あるいは、 実施された研究が所定の有意水準で関連付けを検出することができなかったためである。後者の 結果(関連性を検出できなかった)は、必ずしも関連付けが存在しないことを意味するものでは なく、特定の研究で関連付けが検出されなかったと解釈すべきである<sup>18,19</sup>。「帰無」研究をレビ ューする際に、これら2つの条件のどちらが正しいかを評価するためには、同一または類似の研 究課題について報告された他の研究、ばく露と発現事象の評価方法、ばく露の誤分類が研究を帰 無に偏らせた可能性の程度、関連付けの交絡変数の同定と分析を含む使用された統計的方法、影 響が発生する、あるいは影響が検出される閾値を下回っているばく露の程度、研究の検出力と実 質的な関係のある効果量を検出する能力を考慮する必要がある。統計的検出力とは、実際に真の 違い(または関連付け)がある場な、研究者が2つの比較群間に差があること、すなわち、ばく 露と疾病の間に関連付けがあることを正しく識別できる確率を意味する。

<sup>&</sup>lt;sup>17</sup>このような感度分析は、多くの場合、交絡因子よりも効果量推定に実質的な影響を及ぼすと予想されるばく露の誤分類バイアスに対して特に推奨されるかもしれない。

<sup>&</sup>lt;sup>18</sup>「エビデンスがないからといって、エビデンスがないと解釈すべきではない→エビデンスがないことを、ないことのエビデンスと解釈してはならない」という古い格言は、ここでは真実である。

<sup>&</sup>lt;sup>19</sup>米国統計協会の統計的意義と P 値に関する声明 <u>https://www.amstat.org/asa/files/pdfs/P-</u> <u>ValueStatement.pdf も</u>参照のこと。

「低検出力」の研究は、関連付けが実際に存在しているのに、関連付けがないと誤って結論を下 すことがある<sup>20</sup>。

最後に、帰無研究の解釈に関しては、文献のシステマティックレビューにおける出版バイアス の影響を考慮することが重要である。出版バイアスとは、利用可能な公表文献が、そのような帰 無研究を不釣り合いに除外する傾向のことである。疾病または健康影響の間にこのような「帰無」 関連付けを示す研究は、研究課題が疫学的レビュープロセスの一部として確立された質の基準を 満たしていれば、その研究と同様に有益な情報を得ることができる。これらには、研究デザイン、 *優先*的仮説と探索的解析の比較、関係のある効果量を検出するためのサンプルサイズと統計的検 出力、感度と特異度*に対する*影響の適切な確認、ばく露評価の質と差異的・非差異的な誤分類の 可能性、主要な潜在的交絡因子とその他のバイアス(情報、選択等)の評価の適切性、効果修飾 因子の評価(優先的仮説による裏付けがない仮設、生物学的妥当性、またはその他の裏付け情報 についての多重比較の検討とその補正を含む適切な統計解析)などの要因が含まれる。

# 6. 外的妥当性(一般化可能性)

上述したように、妥当性とは、一般的にはばく露推定が真のばく露レベルを反映している程度 を指す(Checkoway ら)。外的妥当性または一般化可能性とは、母集団のサンプル(例:農薬 散布者)から得られた疫学的研究結果を他の母集団(例:すべての農業従事者)に拡張する能力 のことである。外的妥当性を評価するために、サンプルの特性とより大きな集団(既知の場合) との比較を行うことができる。このような評価には、人口統計学的要因だけでなく、ばく露(例 えば、用量、時期、期間)が類似しているかどうか及び重要な影響修飾因子(例えば、脆弱者集 団の感受性)が考慮されているかどうかも含まれるべきである。一般化可能性は特に重要であり、 個々の研究結果が、規制当局のリスク評価において、より大きな集団または対象となる亜集団に 適用できるかどうか、またどのように適用できるかを理解することが重要である。例えば、AHS は、いくつかの農薬について、いくつかのがんとがん以外の健康影響との間に統計的な関連付け があることを報告している。OPP は、報告された知見が、ノースカロライナ州とアイオワ州以 外の州の農薬散布者や、主に散布後の作業を行う農業従事者にどの程度適用できるかを評価する

<sup>&</sup>lt;sup>20</sup> 検出力は低くても統計的に有意な効果が見られる研究は、効果量の拡大という現象である可能 性があり、これを調査することが重要である(Ioannidis、2008年)。

## V. ヒトでの事例調査データ

一般的に言えば、上記のような農薬に関する疫学研究は、急性の臨床症状をもたらす可能性が 小さい低量ばく露(より長い期間にわたって)に焦点を当てている。OPP はまた、より高く、 より短い間隔(多くの場合、急性の"一回限り"で)のばく露に関心を持っている。この「ヒトで の事例」または中毒データは、問題となっているに起因すると考えられる短期的で高濃度の高量 ばく露シナリオを評価するのに有用である。

OPP はこのような「ヒトでの事例情報」をいくつかの目的で使用している。最も広い意味で は、OPP はリスク評価/リスク管理の活動に情報を提供するためにヒトでの事例データを使用 している。これは、農薬登録改善法(PRIA)の責任のもと、登録審査活動の重要な一部となっ ている。この目的のために、OPP は人の事例データを評価して経時的な傾向を調べ、さまざま な農薬ばく露の重大さの程度と頻度のパターンを調べている。場合によっては、事例情報は、追 加の情報や追加のリスク管理措置の必要性を示すことができる。また、事例情報は、リスク軽減 措置が実施された後の達成の評価にも役立ち、事例情報は、OPP がヒト健康と環境を保護する ために行ってきたリスク管理措置の有効性を保証するための OPP のパフォーマンス・アカウン タビリティ・システムの重要な部分である。最後に、事例情報は、使用方法に関する実際の使用 情報を提供する上で有用であり、また、必要に応じて、施行や教育の活動の対象となる可能性が ある。

**OPP** はこの情報を様々な情報源から入手している。ヒトでの事例データの情報源には、医学的及び毒物学的文献に掲載されている(ヒトの)**医学的症例報告**や急性及び慢性のハザードの特定を促進し、リスク評価プロセスに不可欠な共同で検討されている急性農薬中毒の様々な国の中 **毒サーベイランス活動**からの情報が含まれている<sup>21</sup>。

**医学的症例報告**(医師によって書かれた直接の証言)や医学的症例集積(共通の原因や症状を 持つ個人の症例報告をまとめたもの)は、健康影響に関する利用可能なすべてのエビデンスを解 析するための貴重なツールであり、動物試験や疫学研究の知見を補完するものでもある。さらに、 特定の農薬の使用に関連すると思われる有害な健康影響の稀な発生や新規の発生を特定すること ができ、中毒警戒の行政機関に「事前通知」を提供できる。公表されている農薬の症例報告は、 通常、非定型的な(高量ばく露/大量投与、違法、適用外)急性または短期のばく露による影響 を記述している。報告書はしばしば個々の事例に基づいており、その性質上、選択性が高い。し かし、これらの報告書は、ヒトにおいてこれまでに確認されていなかった毒性影響を特定し、高 量ばく露後の影響、健康影響及び医学的後遺症を知る上で、特に有用である。これらの研究には、 より詳細な医学的情報(後遺症を含む)や詳細な追跡調査が行われていることが多く、一般的に 用量に関する高い質及び/または定量的な情報が得られている。

<sup>21</sup> OPP は、IPSC がリスク評価においてヒトでの事例データを考慮する成果を認識している。 http://www.who.int/ipcs/publications/methods/human data/en/index.html

複数の医療事例研究で類似点が見られたり、症状、ばく露シナリオ、使用方法などにパターン が見られた場合には、リスク評価プロセスと結果の裏付けに有用な情報を提供することができる。 医学的症例研究や定量的なばく露情報集積は、必要に応じて、典型的な使用から予想されるばく 露マージンを特性評価することで(ラベルに明示された適用率や代替ばく露情報に基づく)リス ク評価のばく露推定値と比較できる。

医学的症例報告や医学的症例集積を評価する際には、以下の点を考慮する。

ばく露の詳細な履歴(いつ、どのように、どのくらい)、健康影響の発現時期、患者の 徴候や症状が報告されている。

商品名、農薬表示、登録番号など、商品・化学品・農薬に関する情報。

患者情報(例:年齢、人種、性別);基本的な健康状態及び同様の徴候や症状をもたら す可能性のある医薬品の使用;関連する病歴及びリスク因子の有無。

疾病の説明と診断に至った経緯。

患者の管理・治療、農薬や化学物質の血中濃度などの検査データ(治療前、治療中、治療後)。

医学的報告は信頼性があるか、妥当であるか。他の研究、レビュー、ガイドラインを含む最新の知見と一致しているかどうか。

疾病の臨床経過及び患者の転帰(例:患者の回復及び退院;退院後の患者の状態、農薬 または化学物質へのばく露に関連した慢性的な健康影響または早期死亡)。

実際のばく露情報及び毒物学的情報の情報源として医学的症例の報告/集積を利用することに 加えて、OPP は様々な農薬中毒事例データベースを利用した中毒サーベイランス活動も行って いる。具体的には、OPP は以下の 5 つのヒトでの事例データソースにアクセスできる。OPP Incident Data System (IDS) 、American Association of Poison Control Centers (PCC) の National Poison Data System (NPDS) からの要約報告書、現在オレゴン州立大学にある EPA が資金提供している National Pesticide Information Center (NPIC) からのデータ、疾病管理 予防センター (Centers for Disease Control and Prevention) /National Institute for Occupational Safety and Health for Occupational Risk - Pesticides (NIOSH SENSOR-Pesticides) 及び California Pesticide Illness Surveillance Program (PISP) のデータ。以下、 それぞれについて順番に説明していく。

> □ <u>OPP Incident Data System (IDS)</u>は OPP が管理しており、FIFRA 第 6 条 (a)(2)<sup>22</sup>項に基づき登録者が提出したデータ及び EPA に直接報告されたその他の 事例を含んでいる。

<sup>&</sup>lt;sup>22</sup> FIFRA 6(a)(2)に基づき、農薬登録者は、「農薬の環境への不適切な悪影響に関する事実情報」を知った場合には、EPA に通知するよう求められている。

OPPは1992年以来、IDSに農薬関連の事例報告をまとめている。IDSには、登録者、他の連邦・州の保健・環境機関及び個人消費者からの強制的な FIFRA 第6章(a)(2)報告を含む、様々な情報源から主張されたヒトでの健康影響事例の報告が含まれている。IDS には、ヒト、植物、野生動物、家畜への悪影響を主張している事例に関する情報が含まれている。IDS 報告の大部分は、政府機関が紙形式で受け取っている。IDSの項目は、OPPの情報サービスセンターにあるマイクロフィルムやスキャンした画像に保存されているオリジナルの報告書のコピーへの「ポインタ」として機能する。

IDS には職業上及び非職業上の両方の事例が含まれているが、報告された事例の 大部分は非職業/住居のシナリオに関するものである。全米から報告された事例 のほとんどは製品情報(EPA登録番号など)の記載に関連している。IDS はほと んどが FIFRA 6(a)(2)報告要件に基づき農薬登録者から提供された情報で構成さ れているため、政府機関は特定の製品を識別することに対して比較的高い信頼性 を持っている。重大さの程度の順位付けは、各事例について(CFR §159.184 で 規定されている)記載されている。症状情報は、事例の解説部分に含まれること があるが、この情報は通常、医療専門家による検証/確認を受けていない。IDS には、ばく露シナリオやハザード情報の解説情報も含まれている。多くの企業は、 標準化された、業界で開発された自主的な事例報告フォームを使用している。

OPP は IDS からデータを収集・評価し、農薬ばく露による健康影響の程度や重 大さの程度に関して潜在的なパターンを識別している。IDS の報告書は範囲が広 く、場合によっては詳細な情報を含むが、システムは必ずしも職業上ばく露状況 や医学的転帰などの発現事象に関する詳細な情報を一貫して把握しているわけで はない。

また、IDS に入る症例データのほとんどは、中毒管理センターへの電話からの収 集もあるが、確認・照合されない。とはいえ、事例情報は、製品の初期登録後、 製造販売後の重要なフィードバックループを政府機関に提供できる。重大な性質 の IDS 事例や、それほど重大ではない事例の間で示唆されたパターンや傾向は、 特定の化学物質や製品をさらに調査するように政府機関に求めることができる。 IDS はこのように広範囲をカバーしているため、一時的な傾向情報を提供し、リ スク軽減によって潜在的な農薬ばく露が減少し、IDS に報告された潜在的な事例 の数が減少したかどうかを判断するのに役立つ可能性がある。全体的に見ると、 IDS は農薬の全国的な傾向と事例の頻度に関する良好な情報を提供し、農薬のハ ザード及び/またはばく露の可能性について有用な予測を提供することができる。

全米中毒データシステム(NPDS)(以前は毒性影響監視システム(TESS)と 呼ばれていた)は、米国中毒管理センター協会(AAPCC)によって維持されて おり、いくつかの連邦政府機関からの資金援助を受けている。NPDSはコンピュ ータ化された情報システムで、地理的に特定されたほぼリアルタイムの報告が可 能である。中毒管理センターの主な任務は、緊急事態に対応する通報者を支援す ることであるが、NPDSのデータは、化学製品の安全性に関する新たな問題を特 定するのに役立つ。全国にある 61 の PCCのホットラインは、365 日 24 時間年 中無休で開設されており、特別な訓練を受けた看護師、薬剤師及びその他の臨床 医療専門家が中毒情報を提供している。コンピュータによるデータ入力、標準化 されたプロトコル、厳格なデータ入力基準を使用して、現地の通報者が事例を報 告する。これらの報告された事例は現地で保存され、AAPCCによって維持され ている全国データベースに要約された形で更新される。情報通報は個別に集計さ れ、事例としてはカウントされない。PCCシステムは、ほぼすべての米国とそ の領土をカバーしており、2001年以来、コンピュータの大幅な強化が行われて きた。

NPDSには主に非職業上の事例が含まれている。NPDSには解説情報は含まれて おらず、製品情報は完全ではない。NPDSは、訓練を受けた専門家が指定/記録 した重大さの程度の順位と症状情報を提供しており、政府機関はこれらの情報に 対して比較的高い信頼性を持っている。NPDSはまた、報告されたばく露による 健康影響の可能性に関する情報も提供している。全体として、NPDSは全国的な 傾向、農薬の事例発生頻度、特定の農薬の潜在的ハザードについての良好な情報 を提供している。しかし、情報源の制限により、毎年発行される AAPCCのサマ リーレポート(例:http://www.aapcc.org/annual-reports/を参照)にしかアク セスできず、これらのレポートは他の情報源を補完する役割を果たしているため、 NPDSはより完全なアクセスが可能となっている。

## □ National Pesticide Information Center (NPIC)

(http://npic.orst.edu/index.html)は、EPA の資金提供を受けており、客観的で科 学的根拠に基づいた農薬情報を提供し、問い合わせに対応したり、事例に対応し たりすることを目的としている。NPIC は平日の営業時間内に全国的に機能して おり、オレゴン州立大学(現在)と EPA との間で協力している。中毒管理セン ターと同様に、NPIC の主な目的は事例データの収集ではなく(NPIC の年間の 問い合わせの約 10%は「事例」に関連していると考えられている)、むしろ幅 広い農薬に関する情報を問い合わせ者に提供し、農薬での事例の調査や緊急処置 のための他の情報源に誘導することである。とはいえ、NPIC では問い合わせ者 からの事例情報(年間約 4000 件)を収集し、データベースに記録している。 NPIC は全国の事例の情報源であるが、一般的に IDS に比べて報告件数は少ない。

特定の農薬や製品が IDS で高頻度に観察された場合でも、NPIC は全国のデータ セット間の整合性を判断する上で有用な情報源を提供している。

IDSやPCCと同様に、NPICの事例は主に非職業上のものである。NPICの事例 には、通報者が情報を提供する際に解説情報や製品情報が含まれている。対象範 囲は全国であるが、NPIC に報告される事例の数は NPDS や IDS に比べてかな り少ない。一方、提供される情報量はかなり多く、必要に応じて政府機関がカス タムレポートを要求することも可能である。ハザード情報には、重大さの程度の 順位付け、ばく露経路、症状などが含まれており、これらは訓練を受けた担当者 によって記録される。また、ばく露と有害影響の関連性がどの程度であるかにつ いての情報も提供している(これを確実性指数と呼んでいる)。また、NPIC は 年次報告や解析結果を公開しており、有用な情報源となっている。

□ 疾病管理予防国立産業衛生研究所(CDC/NIOSH)は、SENSOR(Sentinel Event Notification System for Occupational Risk) - Pesticides と題した農薬監 視プログラムとデータベースを管理している<sup>23</sup>。このデータベースには、1998年 から 2013 年までの 12 の州における農薬による疾病症例報告が含まれている。参 加している州は、カリフォルニア州、フロリダ州、アイオワ州、ルイジアナ州、 ミシガン州、ネブラスカ州、ニューメキシコ州、ニューヨーク州、ノースカロラ イナ州、オレゴン州、テキサス州、ワシントン州である。ある年の参加州は、州 と連邦政府が農薬監視のために資金を提供しているかどうかによって異なる。

SENSOR-農薬データベース内の農薬関連の疾病症例は、様々な情報源(現地の 中毒管理センターからの報告、医師によって報告された際に農業従事者の補償請 求の州労働局からの報告、州農業局からの報告及び州保健局への医師の報告)か ら把握されている。職業上及び非職業上の事例の両方がデータベースに含まれて いるが、SENSOR 責任者は、主に職業上の農業従事者事例に関する追跡症例調 査の成果に焦点を当てている。州保健局の SENSOR 責任者は、症例を追跡し、 ばく露シナリオ、症状、重大さの程度、健康影響を確認するために、カルテを取 得する。標準化されたプロトコルと症例の定義を使用して、州保健局の SENSOR 責任者は、症例、カルテ、医師と患者によって提供された事例インタ ビュー解説を SENSOR データシステムに入力する。

すべての SENSOR-Pesticides の症例は、EPA が事例の解析に使用する集合デー タベースに含まれるために、最低2つの健康影響を報告しなければならない。

<sup>&</sup>lt;sup>23</sup> SENSOR-Pesticidesのホームページ: http://www.cdc.gov/niosh/topics/pesticides/overview.html

各症例のエビデンスは、NIOSH の症例分類マトリックスに基づいて、ばく露と 疾病との因果関係について評価される。SENSOR-Pesticides の症例の 98%は、 確定、可能性高い、または可能性ありに分類され、2%は疑わしい症例に分類さ れている。可能性の低い症例、無症候性の症例、無関係の症例及び情報が不十分 な症例は、SENSOR-Pesticides のデータベースには含まれ いない。

全体的には、職業上及び非職業上の事例について非常に有用な情報を提供してお り、時には農薬のハザードやばく露の可能性についての有用な予想を提供してい る。また、SENSOR-Pesticides は独自のデータの解析を行い、Morbidity and Mortality Weekly に公表している。前述のデータベースとは異なり、非職業上/ 住居上と職業上の両方の事例が含まれているが、SENSOR'S は伝統的に農業従 事者での事例に焦点を当てており、その情報を提供する上で特に価値のあるもの となっている。1998 年から 2011 年までの SENSOR-Pesticides のデータはオン ラインで入手可能である: http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides。

□ カリフォルニア州農薬疾病サーベイランスプログラム (PISP) は、カリフォル ニア州によって管理されている。このデータベースは、農薬に関連した疾病や傷害を記録している。症例報告は、医師からの報告と農業従事者補償記録を介して行われる。現地の郡農業委員会がばく露の状況を調査する。カルテと調査結果は、カリフォルニア州農薬規制局 (DPR)の技術専門家によって評価され、疾病の登録簿に登録される。カリフォルニア州の PISP プログラムで報告されたすべての農薬による疾病は、郡の農業委員会によって調査され、DPR は報告書を評価してデータベースにまとめ、農業従事者やその他の人々を農薬ばく露の悪影響から保護するための州のプログラムを改善するために使用される(http://apps.cdpr.ca.gov/calpig/)。

現在、OPP は化学物質別にヒトの事例データを評価している。各データベースからの事例は、 ハザードポテンシャル(死亡者数、より重大な事例の頻度、報告された症状のパターン/傾向) とばく露ポテンシャル(事例の頻度/経時的傾向、ばく露シナリオのパターン/傾向、ばく露に 影響を与える要因のパターン/傾向、または製品のパターン/傾向)について解析されている。 上記のデータベースからのヒトでの事例データを評価する際、OPP はいくつかの一般的な基準 を考慮している。OPP は症状の相対的な重大さの程度と頻度を考慮している。さらに、OPP は 一般的に、時間的な関連付けが確認できる報告を、あるいは少なくとも妥当と思われる報告を、 より信頼性の高いものとしている。最後に、ヒトの事例データを評価するために使用される他の 要因には、ばく露反応の関連付けのエビデンス、報告された健康影響の一貫性、報告された健康 影響の生物学的妥当性、医薬品の使用などの健康影響の代替原因の排除及び観察された症状や健 康影響の特異度が含まれる。さらに、より重大な事例の解説は、ばく露の時間と報告された影響 の間に時間的な関連付けがあるかどうかを評価し、関連付けが状況によって裏付けられているか どうかを判断することが多い。

例えば、屋内での農薬散布後3か月に発生した高齢者の心臓発作は、因果関係があるとは判断されない。一方で、農薬種類に応じた既知の症状と一致する症状を伴うばく露時またはばく露後間 もなく発生した重大な事例は、事前の病歴がなく発生した場合、因果関係の推測がより正当化されることを示唆している。

以上のことから、医学的文献やヒトの毒性学的文献に掲載されている医学的症例報告/症例シ リーズと、EPA が維持、資金提供またはアクセスしているデータベースから得られる中毒サー ベイランスデータの両方からなる事例データは、実社会での農薬のリスクを評価する際に OPP を支援する有用で補完的な情報を提供することが可能である。

#### VI. 要約と結論

このフレームワークでは、疫学とヒトでの事例データをレビューする際の重要な要素を説明し、 そのようなデータを農薬のヒト健康リスク評価に組み込むために提案されている WOE 解析につ いて説明している。OPP では、複数のエビデンスと、*in vitro、in vivo*及び *in silico*の情報源か ら得られた入手可能な最善のデータを使用する。OPP では、複数の情報源からのデータを統合 する際に、系統化されたツールとして修正された Bradford Hill 基準を用いて、品質、一貫性、 関連性、整合性、生物学的妥当性を考慮するため、WOE アプローチを使用している。WOE 解 析の適用は、リスク評価ガイドラインに概説されている科学的解析に従って EPA が日常的に使 用している統合的かつ解釈的なプロセスである。WOE 解析はまた、結合されたデータセットの 質も評価し、特定の科学的評価に適切である成果と複雑性のレベルと一致している(U.S.EPA、 2002 年)。OPP は、21 世紀の毒性試験の新たなビジョンの実施に向けて、現在、毒性学とリス ク評価が変革期にあることを認識している。これらの変革に伴い、OPP はこのアプローチを適 宜更新して行くであろう。

# VII. 参考文献

American Statistical Association. 2016 "AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND P-VALUES" March 7. Available at: <u>https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf</u>

Ankley, GT, Bennett RS, Erickson RJ et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29(3):730-741.

Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, and Guyatt GH. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401-6. doi: 10.1016/j.jclinepi.2010.07.015. Epub 2011 Jan 5.

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W, Steen W, Dosemeci M, and Alavanja M. 2002. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Ann Epidemiol. Oct 1;10(7):478.

Borenstein M, Hedges LV, Higgins JPT, and Rothstein HR. 2009. Introduction to Metaanalysis. John Wiley and Sons, Chichester, UK.

Boyes WK, Moser VC, Geller AM, Benignus VA, Bushnell PJ, and Kamel F. 2007. Integrating epidemiology and toxicology in neurotoxicity risk assessment. Hum. Exp. Toxicol. 26(4):283-93.

Calderon RL 2000. Measuring risks in humans: the promise and practice of epidemiology. Food and Chemical Toxicology. 38:S59-S63.

Carlile DJ, Zomorodi, K , and Houston, JB. 1997. Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab. Dispos. 25(8):903-911.

Checkoway H, Pearce, N, and Kriebel D. 2004. Research Methods in Occupational Epidemiology, 2nd Edition. Oxford University Press, New York.

Clark LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologicallybased pharmacokinetic models for use in safety or risk assessment. Risk Anal. 24(6):1697-1717.

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

Glymor, MM and Greenland, S. 2012. "Causal Diagrams" in Rothman, KJ, Greenland, S, Poole, C, Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 183-212.

Gordis, L. 2009. Epidemiology. 4th Edition. Saunders-Elsevier, New York.

Greenland, S. 1998. "Basic Methods for Sensitivity Analysis and External Adjustment" in Rothman, KJ and Greenland, S. Modern Epidemiology. 2nd ed. Lippencott-Raven Publishers, Philadelphia. pp.343-357.

Greenland, S. and Lash T. 2012. "Bias Analysis" in Rothman KJ, Greenland S, Poole C, Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 345-380.

Greenland, S and O'Rourke, K. 2012. "Meta-analysis" in Rothman, KJ, Greenland S, Poole C, and Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 652-682.

Grimes, DA and Schultz, KF. 2005. Compared to What? Finding controls for case-control studies. Lancet 365: 1429-1433.

Gustafson P1, and McCandless LC. 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. Int J Environ Res Public Health. 2010 Apr;7(4):1520-39. doi: 10.3390/ijerph7041520..

Hartung T. 2010. Evidence-based toxicology - the toolbox of validation for the 21st century? ALTEX. 2010;27(4):253-63.

Hertz-Picciotto I. 1995. Epidemiology and quantitative risk assessment: a bridge from science to policy. American Journal of Public Health. 85(4): 484-491.

Hill AB. 1965. The Environment and Disease: Association or Causation? President's Address. Proceedings of the Royal Society of Medicine 58:293-300

Hoppin JA, Yucel F, Dosemeci M, and Sandler DP. 2002. Accuracy of self-reported pesticide use duration information for licensed pesticide applicators in the Agricultural Health Study. Journal of Exposure Analysis and Environmental Epidemiology, 12: 313-318.

IPCS (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration response assessment. Harmonization Project Document 2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.

Ioannidis JP. 2008. Why Most Discovered True Associations Are Inflated. Epidemiology. 19(5): 640-8.

Jurek AM, Maldonado G, Greenland G, and Church TR. 2006. Exposure-measurement Error is Frequently Ignored When Interpreting Epidemiological Study Results. Europ. J. Epid. 21: 871-876.

Kelsey JL, Whittemore AS, Evans AS, and Thompson WD. 1996. Methods in Observational Epidemiology. 2nd ed. Oxford University Press, New York.

LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, and Weisel CP. 2014. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environ Int. Dec;73:195-207. doi: 10.1016/j.envint.2014.07.011.

Lash, TL, Fox, MP, and Fink, AK. 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, and Greenland S. 2014. Good practices for quantitative bias analysis. International Journal of Epidemiology p. 1-17. Lilienfeld AM and Lilienfeld D. 1979. Foundations of epidemiology, 2nd ed. Oxford University Press, New York.

Mausner JS and Kramer S. 1985. Epidemiology, 2nd ed. W.B. Saunders, Philadelphia.

Meek, ME, Bucher, JR, Cohen, SM et al. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33:591-653.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

Muñoz-Quezada MT1, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, and Vega C. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology. Dec;39:158-68. doi: 10.1016/j.neuro.2013.09.003.

Needham LL, Calafat AM, and Barr DB. 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210: 229-238.

Nieuwenhuijsen MJ. 2003. Exposure Assessment in Occupational and Environmental Epidemiology. Oxford University Press, New York.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC.

NRC (National Research Council). 1991. Environmental Epidemiology, Volume 1: Public Health and Hazardous Wastes. National Academy Press, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 1997. Environmental Epidemiology, Volume 2: Use of the Gray Literature and Other Data in Environmental Epidemiology. National Academy Press, Washington, DC.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press, Washington, DC.

NRC (National Research Council). 2009: Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. National Academies Press, Washington, DC. http://www.nap.edu/catalog/13142.html

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) process. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record\_id=18764

Ntzani EE, Chondrogiori MNG, Evangelou E and Tzoulaki I. 2013. Literature review of epidemiological studies linking exposure to pesticides and health effects. External Scientific Report. EFSA supporting publication 2013-EN-497. 159 pp. Available online at www.efsa.europa.eu/publications.

Organisation for Economic Co-operation and Development. 2013. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, ENV/JM/MONO(2013)6, April 17, 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6 &doclanguage=en

Paddle GM, and Harrington JM. 2000. Environmental epidemiology--strengths and weaknesses. Int Arch Occup Environ Health. 73:7-14.

Porta MJM. 2014. A Dictionary of Epidemiology. 6th ed. Oxford University Press, New York.

Purdue Pesticides Programs. 2003. Pesticides and Epidemiology: Unraveling Disease Patterns. Purdue University Cooperative Extension Service. http://www.btny.purdue.edu/Pubs/PPP/PPP-43.pdf.

Rothman KJ and Greenland S. 2012. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

Rothman, KJ, Greenland S, Poole C, and Lash TL. 2012a. "Causation and Causal Inference" in Rothman, KJ, Greenland S, Poole C, and Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 5-31.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. Jul;122(7):711-8. doi: 10.1289/ehp.1307972.Seed, J., E.W.

Carney, RA, Corley, et al. 2005. Overview: Using mode of action and lifestage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672.

Schultz, KF and Grimes DA. 2002. Case-control studies: research in reverse. Lancet: 359:431-434.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Sterne JAC, Higgins JPT, and Reeves, BC. on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for non-randomized studies of interventions (ACROBAT-NRSI), Version 1.0. 0, 24 September 2014." *www. riskofbias. info* (2015)

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Bennie D, Moher D, Becker BJ, Sipe TA, and Thacker SB for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. 2000. J. American Medical Association 283(15): 2008-2012.

Szklo M and Nieto FJ. 2004. Epidemiology: Beyond the Basics. Jones and Bartlett Publishers, Boston, MA.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Science Policy Council Handbook: Risk Characterization. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/100/B-00/002. Available at <u>http://www.epa.gov/iris/backgr-d.htm</u>.

U.S. EPA (U.S. Environmental Protection Agency). 2001. General Principles For Performing Aggregate Exposure And Risk Assessments Washington, DC. Available at <a href="https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf">https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf</a>

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). 2002a. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity for Information Disseminated by the Environmental Protection Agency. Office of Environmental Information, Washington, DC. EPA/260/R-02/008. Available at

 $\underline{http://www.epa.gov/quality/informationguidelines/documents/EPA\_InfoQualityGuidelines.pdf.$ 

U.S. EPA (U.S. Environmental Protection Agency). 2002b. A Review of the Reference Dose and Reference Concentration Processes. December. Risk Assessment Forum. Washington, DC. EPA/630/P-02/002F.

U.S. Environmental Protection Agency. 2002c. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"; January 14, 2002. U.S. EPA (U.S. Environmental Protection Agency). 2004. An Examination of EPA Risk Assessment Principles & Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science Advisor. U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at http://www.epa.gov/raf.

U.S. EPA. (U.S. Environmental Protection Agency). 2006a. Harmonization in Interspecies Extrapolation: Use of BW3/4 as Default Method in Derivation of the Oral RfD (External Review Draft). U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-06/001. Available at <u>http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525</u>.

U.S. EPA (U.S. Environmental Protection Agency). 2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Scientific Issues Associated with Field Volatilization of Conventional Pesticides. U.S. Environmental Protection Agency, Washington, DC. OPP Regulatory Public Docket EPA-HQ-OPP-2009-0687.

U.S. EPA (U.S. Environmental Protection Agency). 2010. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. EPA (U.S. Environmental Protection Agency). 2012. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment U.S. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014a. Framework for Human Health Risk Assessment to Inform Decision Making. https://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. <u>http://www2.epa.gov/osa/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and</u>

U.S. EPA (U.S. Environmental Protection Agency). 2015. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA.

 $https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8CC/\$File/ISA_PREAMBLE_FINAL2015.PDF$ 

van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A, and Goldbohm A. 2002. The contribution of epidemiology. Food Chem Toxicol. Feb-Mar;40(2-3): 387-424.

Vandenbroucke JP, Van Elm E, Altman DG, Gotzsche PC, Mulroew CD, Pockock SJ, Pool C, Schlesseman JJ, and Egger, M. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. Ann. Int. Med. Vol 147(8): W163-193

Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, and Vandenbroucke JP. 2014. The Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. Int. J. Surgery 12: 1495-99.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992a. Selection of Controls in Case-Control Studies I. Principles. American J. Epid. 135(9): 1019-1028.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992b. Selection of Controls in Case-Control Studies II. Types of Controls. American J. Epid. 135(9): 1029-1041.

Wacholder, S, McLaughlin, JK, Silverman, DT, and Mandel, JS. 1992c. Selection of Controls in Case-Control Studies III. Design Options. American J. Epid. 135(9): 1042-1050.

Woodruff TJ1 and Sutton P. 2014. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

Zartarian V., Bahadori T, and McKone T. 2005. Adoption of an official ISEA glossary. Journal of Exposure Analysis and Environmental Epidemiology 15:1-5

Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides

December 28, 2016

Office of Pesticide Programs US Environmental Protection Agency



農薬のリスク評価において ヒトでの疫学データと事例データを導入するための 農薬プログラム局のフレームワーク(基本概念)

2016年12月28日

農薬プログラム局 米国環境保護庁

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目次

## I. PURPOSE & SCOPE

The Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) is a licensing program regulating pesticides in the U.S under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). As part of this program, OPP evaluates a substantial body of toxicology and exposure data to assess the effects of pesticides on human health and the environment. In evaluating human health, EPA looks first for information directly evaluating the potential for effects to people, including epidemiological data. Historically, however, few epidemiology studies have been available to inform the potential toxicity of pesticide chemicals. As such, OPP has in the past primarily relied on toxicology studies in laboratory animals to assess the hazard potential and to estimate human health risk. With the publication of numerous papers from the Agricultural Health Study<sup>1</sup> and from the National Institute of Environmental Health Sciences (NIEHS)/EPA Children's Centers<sup>2</sup>, among others, the availability of epidemiology studies conducted on U.S.-relevant exposures to pesticides is increasing. Nevertheless, since the number of pesticides for which quality epidemiology data either exist or are being developed remains relatively low in the near term, experimental laboratory data will likely continue to be the primary source of data for use in quantitative risk assessment for most pesticides.

OPP's goal is to use such information -- when available -- in a scientifically robust and transparent way. To accomplish this, OPP has developed a general epidemiologic framework, as described in this document, that outlines the scientific considerations that OPP will weigh in evaluating how such studies and scientific information can be more fully integrated into risk assessments of pesticide chemicals. The current document is neither a binding regulation nor is it intended to be or serve as a reviewer's guide or manual or as a Standard Operating Procedure for assessing or using epidemiology data. Nor is it intended to be a full treatise on more modern or advanced epidemiological methods or to adequately convey the nuances and complexity that is important for interpreting these types of studies. As such, it does not discuss (or does not discuss in any detail) such important epidemiological topics as causal inference and causal diagrams (Rothman et al., 2012a; Glymor and Greenland, 2012); more recent approaches to confounder identification, assessment, and control; meta-analysis and heterogeneity and its assessment/evaluation (Borenstein et al., 2009: Greenland and O'Rourke, 2012): or sensitivity/quantitative bias analysis for epidemiologic data (Lash et al., 2009; Lash et al., 2014; Ioannidis, 2008; Greenland and Lash, 2012; Jurek et al., 2007). All these topics, concepts, and issues can and do apply to epidemiology studies concerning pesticides, but are not covered in this OPP framework document. Instead, this document provides overall conceptual considerations concerning the evaluation and use of epidemiology studies on pesticides in

#### I.目的と範囲

米国環境保護庁(EPA)の農薬プログラム(OPP)は、連邦殺虫剤・殺菌剤・殺鼠剤法 (FIFRA)及び連邦食品・医薬品・化粧品法(FFDCA)に基づき、米国内の農薬を規制する認 可プログラムである。このプログラムの一環として、OPPは毒性とばく露の多くの主要データ を評価し、農薬がヒトの健康及び環境に及ぼす影響を評価している。ヒト健康影響を評価する際、 EPAは、まず疫学データを含めたヒトへの影響の可能性を直接評価する情報に注目する。しかし、 これまでは農薬の潜在的な毒性を報告した疫学研究はほとんどない。したがって、OPPはハザ ードの可能性を評価し、ヒトの健康リスクを推定するため、これまでは実験動物を用いた毒性試 験に主に依存してきた。Agricultural Health Study<sup>1</sup>や National Institute of Environmental Health Sciences (NIEHS) / EPA Children's Centers<sup>2</sup> などから数多くの論文が発表されたこ とで、米国で実施された農薬へのばく露に関する疫学研究の有用性が高まっている。とはいえ、 質の高い疫学データが存在する、あるいは充実しつつある農薬の数は、今後短期的には比較的少 ないので、ほとんどの農薬の定量的リスク評価に用いるためのデータの主な情報源は試験データ であることがおそらく継続するだろう。

OPP の目標は、そのような情報が入手可能な場合には、科学的に正確で明白な方法で利用す ることである。これを達成するために OPP は、この文書に記載されているように総合的な疫学 的フレームワークを開発した。このフレームワークでは、そのような研究や科学的情報を農薬の リスク評価にどのようにして一層完全に統合することができるかを評価する際に OPP が検討す る科学的に考慮すべき要点を述べている。現在の文書は、拘束力のある規則ではなく、疫学デー タを評価または使用するためのレビュアー向けのガイドやマニュアル、標準作業手順書であるこ とを意図したものでも、その役割を果たすことを意図したものでもない。また、より最新で進歩 した疫学的方法の完全な専門書となることも、この種の研究を解釈するために重要なニュアンス や複雑さを適切に伝えることも意図していない。したがって、因果推論及び因果関係図 (Rothman ら、2009 年; Greenland and O'Rourke、2012 年)、交絡因子の特定・評価・制御 に対する最新の研究法、メタ分析と異質性の評価 (Borenstein ら、2009 年; Greenland and O'Rourke、2012 年)、または疫学データの感度/定量的バイアス解析(Lash ら、2009 年; Lash ら、2014年; Joannidis、2008年; Greenland and Lash、2012年; Jurek ら、2007年)と いった重要な疫学の話題は(詳細に)論じていない。これらの話題、概念及び問題点のすべては、 農薬に関する疫学研究に応用できるが、この OPP フレームワーク文書では取り上げていない。 代わりに、この文書では、OPP の FIFRA 及び FFDCA の機能支援のためのヒト健康リスク評価 の文脈において農薬の疫学研究の評価と使用に関する全体的な概念を提供している。

<sup>&</sup>lt;sup>1</sup> https://aghealth.nih.gov/

 $<sup>^2\</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers$ 

<sup>&</sup>lt;sup>1</sup> <u>https://aghealth.nih.gov/</u>

<sup>&</sup>lt;sup>2</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-diseaseprevention-research-centers

the context of human health risk assessments to support OPP's FIFRA and FFDCA activities. An earlier version of this document was reviewed favorably by the FIFRA Scientific Advisory Panel (SAP) in February, 2010 (USEPA, 2010; FIFRA SAP, 2010). This document incorporates improvements recommended by the SAP, public comments, and the experience gained since 2010 conducting assessments on several pesticides for which epidemiological data were available, and should be considered a document that will be updated from time-to-time as we progress and on as-needed basis

#### II. INTRODUCTION

Two reports by the National Research Council (NRC) of the National Academy of Science (NAS), "Toxicity Testing in the 21st Century: A Vision and A Strategy (2007)" and "Science and Decisions (2009)," together provide new directions in toxicology and risk assessment. These two NRC reports advocate far reaching changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made. Specifically, the 2007 report on 21st century toxicity testing advocates a shift away from the current focus of using apical toxicity endpoints to using toxicity pathways<sup>3</sup> to inform toxicity testing, risk assessment, and ultimately decision making. This approach is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function in human cells. The goal for the new toxicity testing paradigm is to determine how exposure to environmental agents can perturb these pathways, thereby causing a cascade of subsequent key events leading to adverse health effects. Human information like that found in epidemiology studies, human incident databases, and biomonitoring studies, along with experimental toxicological information are expected to play a significant role in this new approach. Specifically, these types of human information provide insight into the effects caused by actual chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. In addition, epidemiologic and human incident data can guide additional analyses or data generations (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies), identify potentially susceptible populations, identify new health effects, or confirm the existing toxicological observations.

This new vision of toxicity testing and risk assessment will involve data from multiple levels of biological organization ranging from the molecular level up to population-based surveillance with a goal of considering chemical effects from their source to the ultimate health outcome and effects on populations. Such data will come from *in vitro* and *in vivo* experimental studies along with *in silico* and modeled data. OPP's framework for incorporating epidemiology and incident data is conceptually consistent with the 2007 NRC report on 21st century toxicity testing in that both emphasize the use of the best available information from multiple data sources are compiled in a weight of the evidence (WOE) analysis.

本文書の以前のバージョンは、2010年2月にFIFRA科学諮問パネル(SAP)によって好意的に レビューされた(USEPA、2010年、FIFRA SAP、2010年)。この文書は、SAPが推奨する改 善点、パブリックコメント及び2010年以降に疫学データが利用可能であった農薬の評価から得 られた知識が盛り込まれており、進捗及び必要に応じて随時更新される文書と考えるべきである。

#### II. 序章

全米科学アカデミー (NAS) の米国研究評議会 (NRC) による 2 つの報告書、「21 世紀の毒 性試験:ビジョンと戦略(2007年)」と「科学と政策調査(2009年)」は、共に毒性学とリス ク評価の新たな方向性を示している。これら 2 つの NRC 報告書は、毒性試験の実施方法、デー タの解釈方法、そして最終的に規制上の政策調査の方法に大きな変更を提唱している。具体的に は、21世紀の毒性試験に関する 2007年の報告書では、毒性試験、リスク評価、そして最終的な 政策調査に情報を提供するために、現在の先端毒性エンドポイントの使用に焦点を当てたものか ら、毒性発現経路3を使用するようにシフトすることを提唱している。このアプローチは、遺伝 子、タンパク質、低分子がどのように相互作用してヒト細胞の機能を維持する分子経路を形成し ているかという急速に進化する科学的理解に基づいている。新しい毒性試験のパラダイムの目標 は、環境因子へのばく露がこれらの経路をどのように乱すかを明らかにし、それによって有害な 健康影響につながる重要な事象を連鎖的に引き起こす原因を明らかにすることである。この新し いアプローチでは、疫学研究、ヒトでの事例データベース、バイオモニタリング研究に見られる ようなヒトの情報と実験毒性学的情報が重要な役割を果たすことが期待されている。具体的には、 このようなヒト情報は、実際の化学物質ばく露による影響についての見識を提供し、問題の定式 化やハザード/リスクの特性評価に貢献することができる。さらに、疫学データやヒトでの事例 データは、追加の解析やデータ生成(例えば、in vitro試験や目標とする in vivo試験で使用する ための用量やエンドポイントの選択)の指針となり、影響を受けやすい集団を特定したり、新た な健康影響を特定したり、既存の毒性学的結果を確認したりすることができる。

この毒性試験とリスク評価の新しいビジョンには、生物学的体系の多種多様なレベル(分子レベルから化学物質の影響をその発生源から最終的な健康影響や集団への影響まで考慮することを 目的とした集団を基にした調査レベルまで)のデータが含まれる。このようなデータには、 *in vitro*試験や *in vivo*試験から得られるデータのほかに、*in silico*モデルデータがある。疫学デ ータと事例データを取り入れる OPP のフレームワークは、21 世紀の毒性試験に関する 2007 年 の NRC の報告書と概念的に一致しており、どちらも多種多様なデータソースから得られる最良 の情報の用途はエビデンスの重み付け (WOE) 解析にまとめて使用することを強調している。

<sup>&</sup>lt;sup>3</sup> Toxicity pathways are cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects.

<sup>3</sup> 毒性発現経路とは、十分に撹乱された場合に有害な健康影響を及ぼすことが予想される細胞応 答経路のことである。

As a general principle, occupational and environmental epidemiology studies are conducted only on widely used pesticides; these pesticides also tend to have to be wellstudied in the scientific literature. Thus, OPP expects in many cases where epidemiologic data are available, a significant body of literature data on toxicology, exposure, pharmacokinetics (PK), and mode of action/adverse outcome pathway information (MOA/AOP) may also be available. Human incident data are available on a broader range of chemicals, some of which have robust databases and others which do not. In those situations, where there are significant human incident cases and little is known about the MOA/AOP or PK of a particular pesticide, the WOE analysis can be used to identify areas of new research.

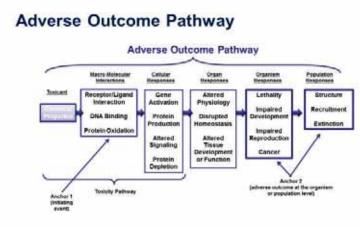
OPP's approach in this framework for incorporating epidemiology and human incident data is not a new or novel approach. Instead, this approach is a reasonable, logical extension of existing tools and methods. This document relies on existing guidance documents and frameworks (Table 1) as the starting point for reviewing and evaluating epidemiology and human incident data for use in pesticide risk assessment. This framework on using epidemiology and incident data in human health risk assessment is consistent with the recommendations of the NRC in its 2009 report on *Science and Decisions*, and with the agency's recent Human Health Risk Assessment Framework (USEPA, 2014a) with respect to emphasizing the use of problem formulation as a tool for scoping, planning, and reviewing available, particularly in the context of risk management needs.

Similarly, OPP's framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety MOA/human relevance framework, which highlights the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al., 2014). The MOA/HR framework begins with identifying the series of key events that are along the causal path, that are established on weight of evidence, using principles like those described by Bradford Hill, taking into account factors such as dose-response and temporal concordance, biological plausibility, coherence and consistency (Hill, 1965). Using this analytic approach, epidemiologic findings can be evaluated in the context of other human information (including human incident findings) and experimental studies and for identifying areas of uncertainty and future research. However, it is noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. As the agency continues to move forward in implementing the transformative approach in the 2007 and 2009 NRC reports and as OPP gains experience in integration of epidemiology and human incident information. OPP will re-evaluate and update this framework as appropriate.

一般的な原則として、産業環境疫学研究は広く使用されている農薬のみを対象に実施されてお り、これらの農薬も科学的文献で十分に研究されている必要がある。したがって、疫学データが 利用可能な多くの場合、毒性、ばく露、薬物動態(PK)、作用機序/有害性発現経路(MOA/ AOP)の情報に関する主要な文献データも利用可能であるとOPP は予想している。ヒトでの事 例データは、より広範囲の化学物質について入手可能であるが、中には正確なデータベースを持 つものと持たないものがある。ヒトでの重大な事例があり、特定の農薬の MOA/AOP や PK に ついてほとんど知られていない場合には、WOE 解析を用いて新たな研究分野を特定することが できる。

疫学データとヒトでの事例データを組み込むための当該フレームワークにおける OPP のアプ ローチは、初めてのものもしくは新しいものではない。むしろ、このアプローチは、既存のツー ルや方法を合理的かつ論理的に拡張したものである。当該文書は、農薬リスク評価に使用する疫 学データ及びヒトでの事例データを検討するための出発点として、既存のガイダンス文書及びフ レームワーク(表 1)に依存している。ヒトの健康リスク評価における疫学データ及びヒトでの 事例データの使用に関する当該フレームワークは、2009 年の「科学と政策調査」に関する報告 書での NRC の提言や、特にリスク管理の必要性の文脈で、利用可能なものを調査、企画、検討 するためのツールとして問題の定式化の使用を強調する点に関して、政府機関の最新のヒト健康 リスク評価フレームワーク(USEPA、2014 年 a) と一致している。

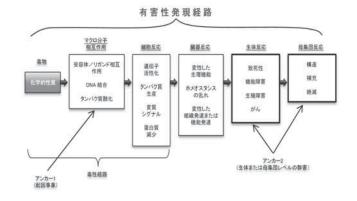
同様に、OPP のフレームワークは、問題の定式化の重要性と生物学的体系の多種多様なレベルでの情報統合の必要性を強調した「世界保健機関/化学物質の安全性に関する国際プログラムMOA/ヒト関連(MOA/HR)フレームワーク」の最新版と一致している(Meek 6、2014 年)。 MOA/HRフレームワークは、用量反応や時間的一致、ならびに生物学的な妥当性、整合性及び 一致性などの要素を考慮に入れて、Bradford Hill によって記述されたような原則を使用して、 エビデンスの重み付けに基づいて確立された因果経路に沿った一連の重要な事象を特定すること から始まる(Hill、1965年)。この解析アプローチを用いることで、疫学的知見は、他のヒト情 報(ヒトでの事例所見を含む)や実験的研究の文脈で評価することができ、不確実分野や今後の 研究分野を特定することができる。しかし、完全に解明された MOA/AOP があることが、ヒト 健康リスク評価に疫学研究を利用するための要件ではないことは注目に値する。政府機関が 2007 年及び 2009 年の NRC 報告書にある変化するアプローチの実施を引き続き進め、OPP が疫 学情報とヒトでの事例情報の統合の経験を積む中で、OPP は当該フレームワークを適切に再評 価し、更新していく予定である。 Figure 1. Schematic of the adverse outcome pathway. Adapted from Ankley *et al.* (2010).



# Table 1. Key guidance documents and frameworks used by OPP

	1983: Risk Assessment in the Federal Government: Managing the Process	
	1994: Science and Judgment	
NAS	2007: Toxicity Testing in the 21st Century	
	2009: Science and Decisions: Advancing Risk Assessment	
	2011: NAS report on Formaldehyde	
	2014: Review of EPA's Integrated Risk Information System (IRIS) Process	
	2001-2007: Mode of Action/Human Relevance Framework	
WHO/IPCS	2005: Chemical Specific Adjustment Factors (CSAF)	
	2014: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis.	

# 有害性発現経路



## 表 1. OPP が使用している主要なガイダンス文書とフレームワーク

	1983: 連邦政府におけるリスクアセスメント:プロセスの管理
	1994: 科学と判断
NAS	2007:21 世紀の毒性試験
NAD	2009:科学と政策調査:リスクアセスメントの推進
	2011: ホルムアルデヒドに関する NAS 報告書
	2014: EPA の統合リスク情報システム(IRIS)プロセスの見直し
	2001~2007: 作用機序/ヒト関連のフレームワーク
WHO/IPCS	2005: 化学物質固有の補正係数(CSAF)
	2014: 作用機序/種のコンコーダンス解析に関する WHO/IPCS フレー ムワークの進化と応用における新たな展開

	1991-2005: Risk Assessment Forum Guidances for Risk Assessment (e.g., guidelines for carcinogen, reproductive, developmental, neurotoxicity, ecological, and exposure assessment, guidance for benchmark dose modeling, review of reference dose and reference concentration processes) <sup>4</sup>	
ЕРА	2000: Science Policy Handbook on Risk Characterization	
	2006b. Approaches for the Application of Physiologically Based Pharmacokinetic	
	(PBPK) Models and Supporting Data in Risk Assessment 2014a. Framework for Human Health Risk Assessment to Inform Decision Making.	
	2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation	
	2001: Aggregate risk assessment	
OPP	2001 and 2002: Cumulative risk assessment	
OECD	2013: Organisation for Economic Co-operation and Development Guidance Document On Developing And Assessing Adverse Outcome Pathways	

Although there are other sources of human information, the focus of this framework is on interpreting and using *epidemiology* and *human incident data* in human risk assessment; other sources of human information are not addressed in this document in any depth. Specifically, this document does not extensively discuss research with pesticides involving intentional exposure of human subjects<sup>5</sup> or on studies done to measure dermal or inhalation exposures in agricultural workers as they perform their activities<sup>6,7</sup>.

	1991~2005: リスクアセスメントのためのリスクアセスメントフォーラム 指針(発がん性、生殖毒性、発生毒性、神経毒性、生態毒性及びばく露 評価のための指針、ベンチマーク用量モデリングのための指針、基準用 量と基準濃度プロセスのレビューなど) 4 2000: リスクの判定に関する科学政策ハンドブック
環境保護庁	2006b. 生理的薬物動態(PBPK)モデルのリスク評価への応用のための アプローチとその裏付けとなるデータ 2014a. 政策調査に情報提供するためのヒト健康リスク評価のフレームワ
	2014b. 異種間・同種間の推定のためのデータ由来の推定係数を開発する ための定量的データの適用ガイダンス
1991 - 1997	2001: 総合的なリスク評価
農薬プログラム	2001 と 2002 年: 累積リスク評価
経済協力開発機構	2013: 有害性発現経路の開発と評価に関する経済協力開発機構 (Organisation for Economic Co-operation and Development) ガイダン ス文書

ヒトの情報源は他にもあるが、このフレームワークの意図は、ヒトのリスク評価における*換*  **学データ**と**とトでの事例データ**の解釈と利用にあり、他にもあるヒトの情報源については当該 文書で深く触れていない。特に、当該文書では、意図的なヒトを対象としたばく露に関する農 薬の研究<sup>5</sup>や、農業従事者の活動に伴う経皮や吸入によるばく露量を測定するために行われた 研究<sup>6,7</sup>については、広く論じていない。

(<u>https://www.extension.purdue.edu/extmedia/PPP/PPP.48.pdf</u>で入手可能) を参照のこと。 7ページ

<sup>&</sup>lt;sup>4</sup> https://www.epa.gov/osa/products-and-publications-relating-risk-assessment-produced-office-science-advisor

<sup>&</sup>lt;sup>5</sup> Both the conduct of such research and OPP's reliance on data from such research are governed by EPA's Rule for the Protection of Human Subjects of Research (40 CFR Part 26.) Among other things, these rules forbid research involving intentional exposure of pregnant or nursing women or of children, require prior review of proposals for new research by EPA-OPP and by the Human Studies Review Board (HSRB), and require further review by EPA-OPP and the HSRB of reports of completed research.

<sup>&</sup>lt;sup>6</sup> In the last several years, OPP has extensively evaluated existing observational studies with agricultural workers in efforts to improve the data and approaches used in worker exposure assessment; those evaluations can be found elsewhere (http://www.epa.gov/scipoly/sap/meetings/2007/010907\_mtg.htm) <sup>7</sup> For additional information on how such worker exposure studies are conducted and used by OPP, see PPP-48 "Pesticides and human Health Risk Assessment: Policies, Processes, and Procedures "available at <u>https://www.extension.purdue.edu/extmedia/PPP/PPP-48.pdf</u>.

<sup>&</sup>lt;sup>4</sup> https://www.epa.gov/osa/products-and-publications-relating-risk-assessment-produced-officescience-advisor

<sup>&</sup>lt;sup>5</sup> このような研究の実施及び OPP がこのような研究から得られたデータに依存することは、 EPA の研究対象者保護規則(40 CFR Part 26)によって管理されている。その他に、この規 則は妊婦、授乳婦、子供への意図的なばく露に関する研究を禁じ、EPA-OPP やヒト研究評価 委員会(HSRB)による新規研究計画の事前評価、ならびに研究成果報告の評価を求めてい る。

<sup>6</sup> 過去数年間に OPP は、農業従事者のばく露評価に使用されるデータとアプローチを改善する ために、農業従事者を対象とした既存の観察研究を広範囲に評価してきた。それらの評価は (http://www.epa.gov/scipoly/sap/meetings/2007/010907\_mtg.htm) で閲覧できる。

<sup>7</sup> OPP が農業従事者ばく露研究をどのように実施し、成果を利用するかについての追加情報は、 PPP-48「農薬とヒト健康リスク評価: Policy, Processes, and Procedures」

#### III. SYSTEMATIC REVIEW IN PESTICIDE RISK ASSESSMENT: EPIDEMIOLOGY

In recent years, the NRC has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making (NRC 2011, 2014). The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies" (NRC, 2014). Consistent with NRC's recommendations, the Office of Chemical Safety and Pollution Prevention (OCSPP) employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting our decisions.

According to the NRC, systematic reviews "have several common elements: transparent and explicitly documented methods, consistent and critical evaluation of all relevant literature, application of a standardized approach for grading the strength of evidence, and clear and consistent summative language (NRC, 2014)." In recent years, several groups (Rooney et al., 2014; Woodruff and Sutton, 2014; Hartung, 2010) have published systematic review approaches for use in environmental health sciences. The OCSPP approach to systematic review is consistent with the principles articulated in the Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine and with the principles of the Grading of Recommendations Assessment, Development and Evaluation (GRADE). GRADE guidelines used by systematic review approaches for environmental health sciences developed by the National Institute of Environmental Health Sciences (NIEHS) Office of Health Assessment and Translation (OHAT) (Rooney et al., 2014) and University of California, San Diego (Woodruff and Sutton, 2014). According to the *Cochrane Handbook*, the key characteristics of a systematic review are:

- a clearly stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings from the identified studies;
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

Each approach mentioned above share common themes and workflow starting with a statement of scientific context (e.g., problem formulation or protocol) followed by literature review with explicit search strategy methods, analysis of study quality (often called risk of bias), evaluation of the quality of the totality of the evidence (e.g., integration) and ultimately leading to a conclusion(s). Each approach recommends transparent and pre-determined criteria for inclusion/exclusion of scientific literature, evaluation of study quality (e.g., high, medium, low). Each approach recommends a pre-stated tool for data integration that provides the foundation for the conclusion(s).

#### III. 農薬のリスク評価におけるシステマティックレビュー: 疫学

近年、NRC は、規制上の政策調査に情報を提供するための化学物質固有のリスク評価をサポ ートする科学的文献レビューの明白性を高めるために、システマティックレビュープロセスに移 行するよう政府機関に奨励している (NRC、2011 年、2014 年)。NRC はシステマティックレ ビューを「特定の疑問に焦点を当て、明確で事前に指定された科学的方法を用いて、類似してい るが別個の研究の結果を特定、選択、評価、要約する科学的調査」と定義している (NRC、 2014 年)。NRC の勧告に沿って、化学物質安全・公害防止局 (OCSPP) は、政策調査を支え る科学的データの収集、評価、統合のための標準的な方法に依存する、目的にかなったシステマ ティックレビューを採用している。

NRC によると、システマティックレビューには、"明白性があり明確に文書化された方法、関 連するすべての文献の一貫した厳しい評価、エビデンスの強さを評価するための標準化されたア プローチの適用、明白で一貫性のある総括的用語 (NRC、2014 年) "といういくつかの共通要素 がある。近年、いくつかの集団 (Rooney 6、2014 年; Woodruff and Sutton、2014 年; Hartung、2010 年) が、環境保健科学で使用するためのシステマティックレビューのアプロー チを発表している。システマティックレビューでの OCSPP のアプローチは、Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine に明記されて いる原則と、Grading of Recommendations Assessment, Development and Evaluation (GRADE) の原則と一致している。GRADE ガイドラインは、国立環境保健科学研究所 (NIEHS) 健康評価・翻訳局 (OHAT) (Rooney 6、2014 年) とカリフォルニア大学サンデ ィエゴ校 (Woodruff and Sutton、2014 年) が開発した環境保健科学のためのシステマティック レビューアプローチで使用されているものである。*コクラン・ハンドブックに*よると、システマテ ィックレビューの主な特性は以下の通りである。

・目的が明確に示されており、研究の適格性基準があらかじめ定義されていること。

- ・明示的で再現性のある方法論。
- ・適格性基準を満たすすべての研究を特定するための系統的検索。
- 特定された研究から得られた知見の妥当性の評価。
- ・収録された研究の特性と知見を体系的に提示し、総合的にまとめたもの。

上記の各アプローチは共通のテーマとワークフローを共有しており、科学的な文脈(例えば、 問題の定式化やプロトコル)の記述から始まり、明確な検索戦略の方法を用いた文献レビュー、 研究の質の解析(しばしばバイアスのリスクと呼ばれる)、エビデンス全体の質の評価(例えば、 統合)、そして最終的に結論に至るまでの流れを示している。それぞれのアプローチでは、科学 文献の包含/除外、研究の質の評価、研究の質(例:高、中、低)の報告のための透明性のある 事前に設定された基準を推奨している。各アプローチでは、結論の基礎となるデータ統合のため の事前に定められたツールを推奨している。 So far, no single nomenclature has been agreed upon by the risk assessment community for systematic review and OCSPP expects terminology to evolve over time as more broad experience is gained. OCSPP considers its systematic review process and workflow as starting with problem formulation followed by data collection, data evaluation, data integration, and summary findings with critical data gaps identified. Scientific analysis is often iterative in nature as new knowledge is obtained.

#### A. Problem Formulation

In the NRC report *Science and Decisions-Advancing Risk Assessment*, the National Academy of Sciences (NAS) recommended to EPA that risk assessments and associated scientific analyses be developed to be useful to policy makers; in order to attain this goal, the NRC recommended that the agency more broadly use problem formulation in developing its risk assessments. In response to the NRC, the agency published the Human Health Risk Assessment Framework (USEPA, 2014) which highlights the importance of problem formulation. Problem formulation entails an initial dialogue between scientists and risk managers and provides the regulatory context for the scientific analysis and helps define the scope of an analysis. Problem formulation draws from regulatory, decision-making and policy context of the assessment, informs the technical approach to the complexity and scope of each systematic review will vary among the different risk assessment contexts. In other words, an OCSPP systematic review is conducted as "fit-for-purpose" (NRC, 2009) based on the pre-determined scope and purpose determined from problem formulation.

The problem formulation involves consideration of the available information along with key gaps in data or scientific information. OPP uses problem formulation as a tool to identify exposure pathways and potential health outcomes along with the appropriate methods, data sources, and approaches for the scientific analysis. If missing data are critical to the assessment, options are discussed as to how best to obtain that information (e.g., required testing, research). The peer review process is identified and the timeline for completing the assessment is defined.

Systematic review provides a transparent tool for organizing available information and identifying gaps in information for the regulatory purpose for the analysis. As such, in problem formulation, the regulatory context of a scientific analysis is described which in turn defines the scope of and purpose for collection and evaluation of scientific literature. Some considerations in problem formulation may be related to population or life-stage, exposure pathways (e.g., route, duration, frequency), and/or health outcomes of interest identified from *in vitro* or *in vivo* laboratory studies along with epidemiology or human incident studies along with resources available and regulatory timeframe. In the context of considering epidemiology and human incident information, an initial evaluation of the study quality, study design, and uncertainties are considered. これまでのところ、リスクアセスメントに関係する人々の間では、システマティックレビュー のための単一の命名法は合意されておらず、OCSPP は、より広範な経験が積まれるにつれて用 語が進化していくことを期待している。OCSPP は、システマティックレビューのプロセスとワ ークフローを、問題の定式化から始まり、データ収集、データ評価、データ統合、そして重要 なデータギャップを特定した結果の要約と考えている。

科学的な解析は、新しい知識が得られるたびに、繰り返し行われることが多い。

#### A. 問題の定式化

NRCの報告書「Science and Decisions Advancing Risk Assessment (科学と意思決定・リス ク評価の促進)」において、全米科学アカデミー(NAS)は EPA に対して、政策立案者に有用 なリスク評価と関連する科学的解析を開発するよう勧告した。この目標達成のために NRC は、 政府機関に対してリスク評価の開発において一層幅広く問題の定式化を用いるよう勧告した。 NRCの勧告を受けて、USEPAは「ヒト健康リスク評価フレームワーク」(USEPA、2014年) を公表し、問題の定式化の重要性を強調している。問題の定式化は、科学者とリスク管理者の間 の初期対話を伴うものであり、科学的解析のための規制上の背景を提供し、解析の範囲を定義す るのに役立つ。問題の定式化は、評価の規制、政策調査及び政策の背景から導き出され、評価の 技術的なアプローチの情報を提供し、考慮すべき主要な要因を体系的に特定するものである。こ のように、各システマティックレビューの複雑さと範囲は、異なるリスク評価の文脈によってさ まざまである。言い換えれば、OCSPPのシステマディックレビューは、問題設定から事前に設 定された範囲と目的に基づいた「目的にかなったもの」(NRC、2009年)として実施される。

問題の定式化には、利用可能な情報とともにデータや科学的情報の主要なギャップを考慮する ことが含まれる。OPP は、ばく露経路と潜在的な健康影響を特定するためのツールとして問題 の定式化を用いており、適切な方法、情報源及び科学的解析のアプローチとともに使用している。 欠損データが評価において重要な場合、情報(例えば、必要とされる試験)を得るための最良の 選択肢が議論される。ピアレビュープロセスが特定され、評価を完了するためのタイムラインが 定義される。

システマティックレビューは、利用可能な情報を整理し、解析に対する規制目的に関する情報 のギャップを特定するための明白なツールを提供する。このように、問題の設定では、科学的解 析の規制上の文脈が説明され、それによって科学的文献の収集と評価の範囲と目的が定義される。 問題の設定における考慮すべき事項は、集団またはライフステージ、ばく露経路(例:経路、期 間、頻度)及び/または in vitro または in vivo の実験室での研究、疫学またはヒトの事故によ る研究から特定された目的の健康結果、ならびに利用可能な資源及び規制の時間枠に関すること であろう。疫学情報及びヒトでの事例情報を考慮する文脈では、試験の質、試験デザイン及び不 確実性の初期評価が考慮される。 Key scientific issues related to hazard assessment considered in problem formulation include: What are the effects associated with exposure? What are the MOA/AOPs associated with these effects? What are the temporal aspects of the effects? Are there susceptible populations and if so, who are they and what factors contribute to susceptibility? Are there differences in PK or pharmacodynamics (PD) between laboratory animals and humans? Exposure information is also evaluated in problem formulation. Key scientific issues related to exposure assessment considered in problem formulation include: How is the pesticide used? What are all of the relevant use sites of exposure? To what chemical substances will people be exposed? What are the routes, durations, and frequencies of exposures? Who may be exposed? Does the exposure pose different risks to different groups (e.g., due age or activity patterns?) In the specific case of epidemiology data, this review considers a variety of factors including, but not limited to, research hypothesis, study design (i.e., sample size, sufficient controls, quality of measurements, etc.), exposure dose/concentration, statistical analysis, and conclusions.

#### B. Data Collection

The data collection phase of systematic review is the collection of available information from various published and unpublished sources, such as the open scientific literature and submitted studies for pesticide registration. OPP reviews data collected under the Organisation for Economic Cooperation and Development (OECD) test guidelines, OCSPP harmonized test guidelines, and other pesticide (OPP guidelines). These guideline studies are collected primarily from in-house databases of submitted studies and are found through searches of such internal databases.

In the case of epidemiology, most studies are expected to be found in the open scientific literature. Although in some cases supplemental analyses or information may be available, dialogue with the researchers may provide additional, important information not published in the original paper in understanding and interpreting epidemiology studies. The sources of human incident information are summarized in Section IV.

Open literature search strategies use specified criteria to retrieve health effects information from the open scientific literature and unpublished sources. After identifying and selecting the most appropriate sources/databases and determining the most resource effective strategy utilizing classification codes, medical subject headings, and/or keywords, a search is conducted of the literature. Depending on the complexity of the scientific evaluation, support from a reference librarian may or may not be needed. The goal of a human health literature search is to perform a reliable and reproducible literature search by providing proper documentation of the literature search process. The following steps are conducted to retrieve relevant studies:

- The purpose of the scientific analysis and inclusion criteria are established.
- Combinations of terms/key words and/or MeSH (Medical Subject Heading) terms and their Boolean combinations (AND; OR; NOT) are used and documented.

問題の設定で考慮されるハザード評価に関連する主な科学的課題は以下の通りである。ばく露 に関連する影響とは何か?これらの影響に関連する MOA/AOPs はどのようなものか?影響の 時間的側面は?影響を受けやすい集団があるのか、あるとすれば、その集団は誰であり、どのよ うな要因によって影響を受けやすいのか。実験動物とヒトの間で PK や薬力学 (PD) に違いは あるか?また、ばく露情報は問題の設定においても評価される。問題の設定で考慮されるばく露 評価に関連する主な科学的課題は以下の通りである。農薬はどのように使用されるのか?ばく露 の関連する使用場所のすべてはどのようなものか?ヒトはどのような化学物質にばく露されるの か?ばく露の方法、期間、頻度はどのようなものか?とトはどのような化学物質にばく露されるの か?ばく露の方法、期間、頻度はどのようなものか?ほどのようれる可能性があるか?ばく露 は異なる集団 (例えば、年齢や行動パターンのため)に異なるリスクをもたらすのか?疫学デー タの具体的なケースでは、このレビューでは、研究仮説、研究デザイン(すなわち、サンブルサ イズ、十分な対照、測定方法の質など)、ばく露量/ばく露濃度、統計解析及び結論を含むが、 これらに限定されない様々な要因を考慮して検討する。

# B. データ収集

システマティックレビューのデータ収集段階では、公開科学文献や農薬登録のために提出され た研究論文など、様々な公開・未公開の情報源から利用可能な情報を収集する。OPP では、経 済協力開発機構(OECD)試験ガイドライン、OCSPP 調和試験ガイドライン及び農薬ガイドラ イン(OPP ガイドライン)に基づいて収集されたデータをレビューしている。これらのガイド ライン研究論文は、主に提出された研究の社内データベースから収集され、そのような社内デー タベースの検索によって発見される。

疫学の場合、ほとんどの研究は公開されている科学文献に掲載されていることが予想される。 場合によっては補足的な解析や情報が得られることもあるが、研究者との対話によって、疫学研 究を理解し解釈する上で、原著論文には掲載されていない追加の重要な情報が得られることもあ る。ヒトでの事例の情報源はセクション IV にまとめられている。

公開文献検索戦略では、特定の基準を用いて、公開されている科学文献や未発表の情報源から 健康影響情報を検索する。最も適切な情報源/データベースを特定して選択し、分類コード、医 学的主題の見出し及び/またはキーワードを利用した最もリソース効率の良い戦略を調査した後、 文献の検索が行われる。科学的評価の複雑さに応じて、文献管理責任者のサポートが必要な場合 もあれば、必要でない場合もある。ヒトの健康に関する文献検索の目標は、文献検索プロセスの 適切な文書を提供することにより、信頼性が高く再現性のある文献検索を行うことである。有用 な研究を検索するには、以下の手順で行う。

- 科学的解析の目的と包括基準が確立されている。
- 用語/キーワードや MeSH (Medical Subject Heading) 用語の組み合わせと、それらの ブール考案の組み合わせ(AND; OR; NOT)を使用し、文書化する。

Advanced Search and Field Search by author, title, keywords or subject heading may also be performed as needed. Knowledge of database structure, and using a separate search strategy for a specific database is helpful in retrieving relevant studies. In addition to an initial comprehensive search, periodic searches may be conducted to update the literature list.

- The search strategy is documented, including the date(s) of the search(es) to ensure that all the searches of all the databases are reproducible.
- Reference lists of retrieved articles are examined<sup>2</sup> for additional background and to look for articles that were not discovered in the initial search.
- After combining the retrieved articles from different databases and removing duplicates, the available titles and abstracts are screened. For some of the articles where relevance could not be determined from the title and the abstract, the article is retrieved for further review.
- Following the initial screening, articles that were not relevant (exclusion criteria) such as opinion articles, studies not in English, and those consisting only of abstracts are excluded. Additional exclusion criteria can be identified on a case by case basis. All exclusion criteria are documented. The rest of the articles, even those that found no adverse health effects, are included for review and evaluation.

## C. Data Evaluation

In the data evaluation phase, data quality is reviewed and conclusions are made about the utility of such data. Study quality reflects the overall confidence that reports findings are correct (Balshem et al., 2011). As such, study quality can include:

- reporting quality (how well or completely a study is reported);
- how credible the findings are based on the design and conduct of the study;
- and how well the study addresses the topic under review (Rooney et al., 2014).

Study quality is first considered on an individual study basis, and the quality is judged. For example, one may have stronger confidence in a well conducted case control study than a poorly conducted cohort study. Credibility of the scientific findings, often called risk of bias, is evaluated using pre-determined criteria for specific domains related to study design and conduct (See Table 2).

OPP initially developed a guidance on using the open scientific literature considerations called the "Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment" (USEPA, 2012) and generally continues to follow this guidance. However, with the acceleration of systematic review in risk assessment, some aspects of the literature guidance may need updating in the future.

必要に応じて、著者名、タイトル、キーワード、主題見出しによる高度な検索やフィー ルド検索を行うこともできる。データベースの構造を知り、特定のデータベースに対し て別の検索戦略を使用することは、有用な研究論文を検索する際に役立つ。最初の包括 的な検索に加えて、文献リストを更新するために定期的な検索を行うこともある。

- すべてのデータベースのすべての検索が再現可能であることを保証するために、検索戦略は検索の日付を含めて文書化されている。
- 検索された論文のリファレンスリスト<sup>2</sup>は、追加の文脈を調べたり、最初の検索では発見 されなかった論文を探したりするために調査されている。
- 異なるデータベースから検索された論文を組み合わせて重複を除去した後、利用可能な タイトルと要約を選別する。タイトルと要約から関連性が判断できなかった論文につい ては、その論文を検索し、さらなる再検討を行う。
- 最初の選別の後、有用性のない論文(除外基準)・例えば、持論の論文、英語で記述されていない研究論文、要約のみで構成された論文は、除外される。追加の除外基準は個別的に特定される。すべての除外基準は文書化されている。残りの論文は、有害な健康影響が認められなかったものであっても、検討と評価のために含まれる。

#### C. データ評価

データ評価段階では、データの質が検討され、そのようなデータの有用性について結論が出さ れる。研究の質は、報告された結果が正しいという全体的な信頼性を反映する(Balshem ら、 2011 年)。このように、研究の質には以下のようなものがある。

- 報告の質(研究の報告がどの程度に十分であるか、または完全であるか)。
- 試験のデザインと実施に基づいて、試験結果がどの程度に信頼性のあるものか。
- さらに、その研究が評価下の主題にどの程度に十分な対応しているか(Rooney ら、2014 年)。

研究の質は、まず個々の研究基盤で検討され、その質が判断される。例えば、不完全に実施さ れたコホート研究よりも、適切に実施された症例対照研究の方が信頼性は高い場合がある。科学 的知見の信頼性は、しばしばバイアスのリスクと呼ばれ、試験のデザインと実施に関連する特定 の分野について、あらかじめ定められた基準を用いて評価される(表2参照)。

OPP は当初、「ヒトの健康リスク評価を支援するための公表されている科学文献の毒性研究 の検討と使用に関するガイダンス」(Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment) (USEPA、2012 年)と呼ばれ る公表されている科学文献の考えの使用に関するガイダンスを作成し、一般的にはこのガイダン スに従っている。しかし、リスク評価におけるシステマティックレビューの促進に伴い、この文 献ガイダンスの一部の側面は将来的に更新が必要になる可能性がある。 Conclusions about the quality of the data are made and can be described in conclusion statements or categories (e.g., acceptable/not acceptable; low, medium, high).

Specific considerations used in evaluating epidemiology studies on pesticide chemicals are provided in Section III.C below. As part of the data review, a concise written review of the study is developed. This written review describes the study design, results, conclusions, and the strengths and weaknesses of the study. The quality of the epidemiologic exposure assessment is an important factor in determining what role epidemiologic data will play in the risk assessment. As such, it is important to fully characterize the assumptions used in the epidemiologic exposure assessment and the degree to which these assumptions affect the interpretation and generalizability of the epidemiologic findings. The evaluation of the epidemiologic exposure assessment may include a consideration of past and present exposure patterns (e.g., exposed populations, pathways, routes, and levels of exposure) and may include significant changes in use patterns (e.g., risk mitigation actions or new use patterns). With regard to evaluating meta-analyses, reporting guidelines for Meta-analysis Of Observational Studies in Epidemiology (MOOSE) have been developed by Stroup et al., (2000) that are useful in evaluating the quality and interpreting meta-analysis.

# D. Data Integration: Weight of Evidence (WOE)

OPP's human health characterizations involve the consideration of all available and relevant data, including but not limited to human studies/epidemiology, biomonitoring data, *in vitro* and *in vivo* experimental laboratory toxicological studies, MOA/AOP information, pharmacokinetic studies, and structure-activity relationships (SAR). Once the different types of hazard data are collected and a full evaluation of each relevant study is conducted and documented, the next step is to integrate multiple lines of evidence.

Data integration is based on the principle of reaching a judgment of the totality of the available negative and positive data for relevant hazards. OPP uses a WOE analysis for evaluating epidemiology and human incident data, such conclusions are made on the preponderance of the information rather than relying on any one study. OPP uses the modified Bradford Hill criteria like those in the MOA/human relevance framework as a tool for organizing and integrating information from different sources (Hill, 1965; U.S. EPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003; Seed et al., 2005; OECD AOP Wiki Users Handbook<sup>8</sup>). It is important to note that the Hill Criteria are not intended as a check box approach but instead are points to consider when evaluating the totality of evidence. In addition, the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. However, even in the absence of a fully developed MOA/AOP, collection and evaluation of mechanistic data may provide support for biological plausibility and help explain differences in tissue sensitivity, species, gender, life-stage, or other factor. The MOA/human relevance framework is a flexible tool which provides a foundation for organizing information without rigidity. It is this

データの質についての結論が出され、結論の記述またはカテゴリー(例えば、許容可/許容不 可;低、中、高)で記述される。

農薬に関する疫学研究の評価に用いられる具体的な考えは、以下のセクション III.C に記載さ れている。データレビューの一環として、研究の簡潔なレビュー文書を作成する。このレビュー 文書には、研究のデザイン、結果、結論と、研究の長所と短所が記載されている。疫学的ばく露 評価の質は、疫学的データがリスク評価においてどのような役割を果たすかを調査する上で重要 な要素である。そのため、疫学的ばく露評価で使用された仮定と、その仮定が疫学的知見の解釈 と一般化の可能性にどの程度影響を与えるかを十分に特性評価することが重要である。疫学的ば く露評価には、過去及び現在のばく露パターン(例えば、ばく露集団、ばく露経路、ばく露方法 及びばく露レベル)の検討が含まれ、また、使用パターンの大幅な変化(例えば、リスク軽減措 置や新たな使用パターン)が含まれている場合がある。メタ分析の評価に関しては、Stroup ら (2000 年)によって報告された「疫学における観察研究のメタアナリシス」Meta-analysis Of Observational Studies in Epidemiology (MOOSE)のガイドラインが、メタ分析の質の評価や 解釈に有用である。

# D. データ統合:エビデンスの重み付け(WOE)

OPPのヒト健康影響評価では、ヒトでの調査/疫学、バイオモニタリングデータ、in vitroの 毒性試験及び in vivoの毒性試験、MOA/AOP 情報、薬物動態試験、構造活性関係(SAR) な どを含む(但し、これらに限定されない)利用可能なすべての関連データを考慮する必要がある。 さまざまな種類のハザードデータを収集し、関連する各試験の完全な評価を実施して記録した後、 次のステップは複数のエビデンスを統合することである。

データ統合は、関連するハザードについて利用可能なネガティブデータとポジティブデータを 総合的に判断するという原則に基づいている。OPP は、疫学データ及びヒトでの事例データの 評価に WOE 解析を使用しているが、結論は、特定の研究に依拠するのではなく、情報の優越性 に基づいて出される。OPP は、異なる情報源からの情報を整理して統合するためのツールとし て、MOA/HR のような修正された Bradford Hill 基準を使用している (Hill、1965 年; U.S. EPA、1999 年、2005 年; Sonich-Mullin ら、2001 年; Meek ら、2003 年; Seed ら、2005 年; OECD AOP Wiki Users Handbook<sup>8</sup>)。 Hill 基準は、チェックボックス方式ではなくて、エビデ ンスの総合的評価の検討を示唆していることに注目することが重要である。また、MOA/AOP が完全に解明されていることは、ヒト健康リスク評価に疫学研究を利用するための必須条件では ない。しかし、完全に解明された MOA/AOP がない場合でも、メカニズムデータの収集と評価 は、生物学的な妥当性の裏付けとなり、生体組織の感受性、生物種、性別、ライフステージ、ま たはその他の要因での違いを説明するのに役立つ可能性がある。MOA/HR は、厳密ではない情 報を整理するための基盤を提供する柔軟なツールである。

<sup>&</sup>lt;sup>8</sup> https://aopwiki.org/wiki/index.php/Main\_Page#OECD\_User\_Handbook

<sup>&</sup>lt;sup>8</sup> https://aopwiki.org/wiki/index.php/Main\_Page#OECD\_User\_Handbook

flexibility that makes it a useful tool for a variety of purposes such as evaluating causality, integrating information across multiple lines of scientific evidence, and identifying data gaps and areas of future research. In this analysis, epidemiologic findings and human incident data can be evaluated in the context of other human information and experimental studies to evaluate biological plausibility, to identify areas of uncertainty and areas of further research. To describe how Bradford Hill aspects are considered in the WOE evaluations, OPP has used some definitions of terms as outlined in EPA's Preamble to the Integrated Science Assessments (ISAs) which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS). (USEPA, 2015).

- Key events. In cases where the MOA/AOP are established for a particular health outcome, a clear description of each of the key events (i.e., measurable parameters) that underlie the MOA/AOP are given. Data to inform the key events may come from a combination of *in vitro* or *in vivo* data sources (human or animal). These key events can be a combination of PK and PD events. However, it noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment.
- Biological Gradient/Exposure-Response/Dose-Response Concordance & Relationships. The Preamble to the ISAs notes that "In the context of epidemiology, a well-characterized exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times) (USEPA, 2015)." When the MOA/AOP is known, dose-response relationships are identified for each key event. Dose-response relationships are compared among key events. In some cases, the earlier key events may be more sensitive than later key events. In other cases, key events may share similar dose-response curves.
- Temporal association. Evidence of a temporal sequence between the introduction of an agent and appearance of the effect constitutes another argument in favor of causality (USEPA, 2015). The Preamble to the ISAs notes that "Strong evidence for causality can be provided through 'natural experiments' when a change in exposure is found to result in a change in occurrence or frequency of health."

This analysis considers key events which occur rapidly (e.g., metabolism to an active metabolite which could occur within minutes of exposure) and those which occur after longer durations (e.g., development of a tumor) to ensure coherence of the effects. Specific to considering epidemiology data, the temporal relationship between the exposure and health outcome may be considered.

これは因果関係の評価、複数の科学的根拠に基づく情報の統合、データのギャップや今後の研究 分野の特定などの様々な目的に有用なツールとなる柔軟性を備えている。この解析では、疫学的 知見やヒトでの事例データは、生物学的な妥当性を評価するために、他のヒト情報や試験の文脈 で評価され、不確実分野や今後の研究分野を特定することができる。Bradford Hill の側面が WOE 評価でどのように考慮されるかを説明するために、OPP は、EPA の全米環境大気質基準 (NAAQS)のレビューのための科学的基盤となる統合科学評価(ISA)の EPA 前文に記載され ている用語の定義を使用している(USEPA、2015年)。

- 重要事象。特定の健康影響について MOA/AOP が確立されている場合には、MOA/AOP の基礎となる重要事象(すなわち、測定可能なパラメータ)をそれぞれ明確に記述する。重要事象を示すデータは、*in vitro*または *in vivo*のデータソース(ヒトまたは動物)の組み合わせから得られる可能性がある。これらの重要事象は、PK 事象と PD 事象の組み合わせである。しかし、完全に解明された MOA/AOP を利用できることは、ヒト健康リスク評価に 疫学研究を利用するための必須条件ではないことは注目に値する。
- 生物学的勾配/ばく露・反応/用量・反応の一致と関係。ISAsの前文では、「疫学の文脈では、よく特徴づけられたばく露・反応関係(例:ばく露量が多いほど影響が増加する)は、特にばく露期間(例:ばく露時間が長くなると影響が増加する)についても因果関係を強く示唆する」(USEPA, 2015)と指摘している。MOA/AOPが明らかである場合、各重要事象について用量反応関係が特定される。用量反応関係は、重要事象間で比較される。場合によっては、初期の重要事象の方が後期の重要事象よりも感度が高い場合がある。他の場合では、重要事象は類似した量反応曲線を共有しているかもしれない。
- ・時間的関連付け。薬剤の投与と影響の出現との間に時間的な順序があるというエビデンスは、 因果関係を支持するもう1つの根拠となる(USEPA、2015年)。ISAsの前文では、「ば く露の変化が健康影響の発現または発生頻度の変化をもたらすことが判明した場合には、 『自然実験』を通じて因果関係を示す強固なエビデンスを提供することができる」と指摘し ている。

この解析では、影響の整合性を確保するために急速に発現する重要事象(例えば、ばく露後数 分以内に発現する活性代謝物への代謝)と長い継続期間の後に発現する重要事象(例えば、腫瘍 の発生)を考慮している。疫学データを検討する際には、ばく露と健康影響の間の時間的関係が 考慮される。

## • Strength, consistency, and specificity.

**Consistency:** An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. Statistical significance is not the sole criterion by which the presence or absence of an effect is determined. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered (USEPA, 2015).

Consistency of findings across studies is informed by the repeated observation of effects or associations across multiple independent studies. Further support is provided by reproducibility of findings in different populations under different circumstances. However, discordant results among independent investigations may be explained by differences in study methods, random errors, exposure, confounding factors, or study power, and thus may not be used to rule out a causal connection (USEPA, 2015).

*Strength of the observed association:* The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may or may not represent a substantial effect in a population (USEPA, 2015).

*Specificity of the observed association:* Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, do environmental exposures invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes (USEPA, 2015).

### Biological plausibility and coherence.

*Coherence:* An inference of causality from one line of evidence (e.g., epidemiologiccontrolled human exposure, animal, or ecological studies) may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. There may be coherence in demonstrating effects from evidence across various fields and/or across multiple study designs or related health endpoints within one scientific line of evidence (USEPA, 2015).

When animal and human data show a similar toxic profile, both quantitatively and qualitatively, there is high confidence in the human health risk assessment. Whereas in other cases, animal and human data may show a qualitatively similar toxic profile but quantitative differences are observed. For example, a particular chemical exhibits the same MOA/AOP in animals and humans but there may be species differences in dose-response characteristics. These dose-response differences could be due to tissue dosimetry (i.e., PK) or from different response characteristics (i.e., PD). In contrast, animal and human data can, in some instances, show qualitatively dissimilar outcomes. This situation highlights the need to fully and objectively evaluate all available information in a

# • 強固性、一致性、特異度。

- **致性。**複数の独立した研究でリスクの上昇が観察された場合に、因果関係の推測は強固になる。結果の再現性は因果関係を示す最も強力な根拠の一つである。統計的な有意性は、効果の有無を判断する唯一の基準ではない。研究間で不一致な結果がある場合は、ばく露の違い、交絡因子、研究の検出力などの理由が考えられる(USEPA、2015年)。

複数の研究における結果の一貫性は、複数の独立した研究における効果または関連付けの繰り 返しの観察から得られる。さらに、異なる状況下での異なる母集団における結果の再現性によっ ても支持される。しかし、独立した調査の間での結果の不一致は、調査方法の違い、ランダムエ ラー、ばく露、交絡因子、または研究の検出力の違いによって説明される可能性があり、因果関 係を否定するために使用することはできない(USEPA、2015年)。

**観察された関連付けの強固性。**大規模で正確なリスクの発見は、関連付けが偶然、偏りまたは その他の要因によるものではない可能性が高いという確信を得られる。しかし、影響の推定が小 さい場合は、母集団における実質的な影響を表す場合もあれば、そうでない場合もあることに注 意が必要である(USEPA、2015 年)。

*観察された関連付けの特異度。*特定の結果をばく露に結びつけるエビデンスは、因果関係を示 す強い根拠となる。しかし、環境ばく露が結果の発生を必ず予測できることは稀であり、結果に は複数の原因がある可能性を認識しなければならない(USEPA、2015年)。

# 生物学的な妥当性と整合性。

**整合性。**1 つのエビデンス(例えば、疫学的に管理されたヒトのばく露研究、毒性試験、また は生態毒性試験)からの因果関係の推測は、関連付けの因果関係の解釈を支持する他のエビデン スによって強化されることがある。1 つの科学的エビデンスグループの中で、さまざまな分野に わたるエビデンス、複数の試験デザインにわたるエビデンス、または健康影響に関連するエビデ ンスから、効果を示す一貫性があるかもしれない(USEPA、2015年)。

動物でのデータとヒトでのデータが、定量的にも定性的にも同様の毒性プロファイルを示してい る場合、ヒト健康リスク評価の信頼性は高い。一方、動物でのデータとヒトでのデータが定性的 には類似した毒性プロファイルを示していても、定量的な相違が観察される場合もある。例えば、 ある化学物質が動物とヒトでは同じMOA/AOPを示していても、用量反応特性に種差がある場 合がある。このような用量反応性の相違は、生体組織薬量測定(すなわちPK)に起因するか、 または異なる反応特性(すなわちPD)に起因する可能性がある。一方、動物でのデータとヒト でのデータは、場合によっては質的に異なる結果を示すことがある。このような状況から、種、 性別及びライフステージの違いや潜在的な感受性などの要因を考慮するために、入手可能なすべ ての情報を透明性のある包括的な方法で十分かつ客観的に評価する必要性が明らかになった。

transparent and comprehensive manner to consider factors such as species, gender, and life-stage differences and potential susceptibilities along with study design considers and exposure potential.

**Biological plausibility:** An inference of causality is strengthened by results from experimental studies or other sources demonstrating biologically plausible mechanisms. A proposed mechanism, which is based on experimental evidence and which links exposure to an agent to a given effect, is an important source of support for causality (USEPA, 2015).

Similarly, information on MOA/AOP for a chemical, as one of many structural analogs, can inform decisions regarding likely causality. Structure activity relationships and information on the agent's structural analogs can provide insight into whether an association is causal (USEPA, 2015).

EPA's Cancer Guidelines (2005) indicate:

" evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies. [p. 39]."

However, The Cancer Guidelines further state that *"lack of mechanistic data, however, is not a reason to reject causality* [p. 41]." As such, lack of established MOA/AOP is not necessary knowledge when using epidemiology data and epidemiology associations may still be valid even in the absence of an established MOA/AOP and may also provide insight into potential MOA/AOP.

Uncertainties. Uncertainties are discussed in the WOE transparently and objectively.

# E. Overall conclusions, recommendations for risk assessment, statement of areas of confidence and uncertainty

It is important to document a summary of the evidence, the procedures or methods used to weigh the evidence, the basis for the WOE conclusion or recommendation, any uncertainties and areas for further research. Recommendations are made on the role of the epidemiologic or human incident data in the risk assessment. Generally, OPP does not use human incident information for quantitative risk assessment but instead to inform risk assessment/risk management activities such as indicating a potential need for a new risk assessment or new risk management measures, evaluating the success of risk mitigation actions after they are implemented, and targeting possible enforcement activities. In **生物学的妥当性。**因果関係の推測は、生物学的に妥当なメカニズムを示す試験またはその他の 情報源からの結果によって強化される。提案されたメカニズムは、実験的エビデンスに基づいて おり、ある物質へのばく露を所定の効果に結びつけるもので、因果関係を支持する重要な情報源 である(USEPA、2015 年)。

同様に、多くの構造的類似物の1つとしての化学物質の MOA/AOP に関する情報は、因果関係の可能性が高いかどうかの判断に役立てることができる。構造活性相関及び薬剤の構造的類似物に関する情報は、関連性が因果関係にあるかどうかについての洞察が提供される(USEPA、2015年)。

EPA のがんガイドライン(2005年)は以下の通りに指摘している。

「疫学研究で観察された関連付けの生物学的妥当性の評価は、潜在的作用機序(MOA)の 特定に関連する毒性学的エビデンスとばく露関連因子の両方を考慮したものである。同様に、 疫学的文献で報告された健康影響の関連付けの整合性を考慮することは、毒性試験や疫学研 究で評価された生物学的マーカーの特性に関する情報を幅広く考慮することを示している [p.39]。」

しかし、このガイドラインはさらに、「しかしながら、メカニズムデータの欠如は因果関係を 否定する理由にはならない[p.41]」と述べている。このように、確立された MOA/AOP の欠 如は、疫学データを使用する際に必要な知識ではなく、確立された MOA/AOP がなくても疫学 的関連付けは有効である可能性があり、また、潜在的な MOA/AOP について洞察も得られる可 能性がある。

• 不確実性。不確実性は WOE で明白かつ客観的に述べられている。

# E. 全体的な結論、リスク評価の推奨事項、信頼性と不確実性の分野の陳述

エビデンスの要約、エビデンスの重み付けに使用した手順または方法、WOE の結論または勧 告の根拠、不確実性及び更なる研究分野を文書化することが重要である。また、リスク評価にお ける疫学的データまたはヒト由来のデータの役割についても推奨されている。一般的に、OPP はヒトでの事例情報を定量的リスク評価には使用しないが、その代わりに、新たなリスク評価や 新たなリスク管理措置の必要性を示すこと、リスク軽減措置が実施された後の成功度を評価する こと及び可能性のある施行活動を対象とすることなど、リスク評価/リスク管理の活動を対象と する。 contrast to more limited role of human incident data, epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to estimate a risk metric quantitatively. Alternatively, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and animal studies to evaluate the human relevance of animal findings (Hertz-Picciotto, 1995) and may be useful in assessing the biological plausibility of epidemiologic outcomes. In the final portion of the proposed WOE analysis, the overall conclusions along with statement of areas of confidence and uncertainty. This section also identifies areas of additional research. This section recommends the source of data for regulatory values and the appropriate approach for extrapolating between species (if necessary) and among humans.

# IV. REVIEWING EPIDEMIOLOGY STUDIES FOR USE IN PESTICIDE RISK ASSESSMENT

#### A. Introduction

Epidemiology is a science that seeks to identify and evaluate relationships between exposure to chemical, physical or biological agents, and the health status of populations (Boyes et al., 2007). It has been defined as the "study of how disease is distributed in populations and the factors that influence or determine this distribution" (Gordis, 2009). More broadly, it is considered as "the study of the occurrence and distribution of healthrelated events, states, and processes in specified populations, including the study of the determinants influencing such processes and the application of this knowledge to control of relevant health problems" (Porta, 2014). The objective of much epidemiologic research is to obtain a valid and precise estimate of the effect of a potential cause on the occurrence of disease. A key objective of epidemiology, like other sciences, is determining cause and effect or - said differently - of identifying the etiology of a disease or health outcome and the risk factors with which it might be associated. Calderon (2000) described four major uses of such studies: 1) describe the health status of a population and discover important time trends in disease and exposure frequency: 2) explain the occurrence of diseases by identifying factors that are associated with specific diseases or trends; 3) predict the number of disease occurrences and the distribution of health states in specific populations; and 4) improving the health status of the population by identifying factors that affect environmental or human health. In the case of pesticides, epidemiology focuses on the relation between exposure and adverse health effects in the general population and in specific sub-populations, such as occupationally exposed workers or applicators.

Epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to quantitatively estimate risk or an appropriate risk surrogate such as an odds ratio or risk ratio. However, many epidemiology studies that deal with pesticides and pesticide exposure suffer some limitations in size, scope, exposure assessment, or data analysis which prevent or otherwise impede their full use in quantitative risk assessment

ヒトでの事例データの役割がより限定的であるのとは対照的に、疫学研究は様々な方法でリス ク評価の複数の構成要素に情報を提供するのに役立つ可能性を持っている。正確なばく露評価を 行った質の高い研究は、リスク指標を定量的に推定するために使用することができる。

また、疫学的研究で報告された結果は、*in vitro* 試験や動物試験で見られた結果と定性的に比較して動物試験の結果のヒトへの関連性を評価することもでき(Hertz-Picciotto、1995年)、 疫学的結果の生物学的妥当性を評価することにも有用である。提案されている WOE 解析の最後 の部分では、全体的な結論と信頼性と不確実性の分野の記述が行われる。このセクションでは、 追加研究の分野も特定している。本セクションでは、規制値の情報源及び(必要に応じて)種間 及びヒトの間での外挿法での推定のための適切なアプローチを推奨している。

# IV. 農薬のリスク評価に用いるための疫学研究のレビュー A. 緒言

疫学は、化学的、物理的、または生物学的要因へのばく露と集団の健康状態との関係を特定し、 評価することを目指す科学である(Boyes ら、2007 年)。疫学とは、「集団における疾病の分 布と、その分布に影響を与えたり、調査したりする要因の研究」と定義されている(Gordis、 2009年)。より広義には、「特定の集団における健康に関連する事象、状態、プロセスの発生 と分布の研究であり、そのようなプロセスに影響を与える調査要因の研究と、この知識を関連す る健康問題の制御に応用することを含む」(Porta、2014 年)と考えられている。多くの疫学研 究の目的は、病気の発生に対する潜在的な原因の影響を有効かつ正確に推定することである。疫 学の主な目的は、他の科学と同様に、原因と結果を明らかにすることであり、別の言い方をすれ ば、病気や健康影響の病因とそれに関連する可能性のあるリスク因子を特定することである。 Calderon(2000年)は、このような研究の4つの主要な用途を説明している。1)集団の健康状 態を記述し、疾病やばく露頻度の重要な時間的傾向を発見すること、2)特定の疾病や傾向に関 i車する因子を特定することで疾病の発生を説明すること、3)特定の集団における疾病の発生数 や健康状態の分布を予測すること、4)環境やヒト健康に悪影響を及ぼす因子を特定することで 集団の健康状態を改善すること、である。農薬の場合、疫学は、一般集団と、職業上ばく露され る農業従事者や農薬散布者などの特定の小集団におけるばく露と健康への悪影響との関係に焦点 を当てている。

疫学研究は、様々な方法でリスク評価の複数の要素に情報を提供するのに役立つ可能性がある。 正確なばく露評価を行った質の高い研究は、リスクを定量的に推定するために、あるいはオッズ 比やリスク比のような適切なリスク・サロゲート(代理)を用いることができる。しかし、農薬 や農薬ばく露を扱う疫学研究の多くは、規模、範囲、ばく露評価、またはデータ解析になんらか の制限があり、それが定量的リスク評価への十分な利用を妨げている(Ntzani ら、2013 年)。

(Ntzani et al., 2013). Pesticide use in the US has changed significantly over the last few decades. As the use changes, so does the exposure to workers. Changes in pesticide use have occurred due to risk mitigation actions by EPA, resistance management activities, introduction of new chemistries, and increased use of genetically modified crops. These significant changes in exposure have to be taken into account when interpreting epidemiology studies and, ultimately, the decision to use such studies in quantitative risk assessment. Even so, epidemiology studies may be used to compare with evidence from experimental animal studies to characterize assumptions used in deriving such values. In other cases, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and laboratory animal studies to evaluate biological plausibility or human relevance of animal findings (Hertz-Picciotto, 1995). Human information like that found in epidemiology studies are expected to potentially play a significant role in the new vision of toxicity testing recommended by the NRC (2007). Specifically, epidemiology studies can provide insight on health outcomes that may arise from real-world chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. Human information may guide additional studies (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies); and identify novel health effects or host susceptibilities which can be investigated with future research.

When laboratory data from animal studies provide the primary source of information for hazard characterization, one potential source of uncertainty is the relevance of animal models to humans. In the absence of data to support the contrary, animal findings are assumed to be relevant to humans. Furthermore, EPA assumes that humans are more sensitive than laboratory animals in the absence of data to support the contrary. In actuality, humans may be more or less sensitive to pesticides than other animal species. Epidemiology and human incident data can provide scientific information and support to inform uncertainties associated with species extrapolation. With respect to population variability, epidemiology studies better characterize potential variability than do animal studies. Specifically, epidemiologic data include the genetic diversity, and variability inherent in human populations and thus can better account for and represent actual population response to environmental chemicals than laboratory animals (Calderon, 2000).

With respect to dose-response characterization, animal toxicology studies have the benefit that studies can be designed to cover a broad range of exposure levels. However, animal toxicology studies generally use exposures which are much larger (sometimes orders of magnitude) than those that occur in the environment. These high exposure levels in animal studies dictate the need for extrapolation from high to low doses. This extrapolation introduces added uncertainty into the risk assessment. Epidemiology studies and human incident data involve actual real-world exposures and thus high dose extrapolation may in many cases not be needed. Epidemiology studies conducted over a range of exposures (from low to high) are most useful.

Animal studies do not replicate the length, magnitude, duration, routes of exposure and variability in exposure experienced by humans (Calderon, 2000). Human exposure often occurs through multimedia exposure pathways, including food, water, air, and indoor and outdoor environments. In contrast, controlled laboratory studies typically use a single

米国での農薬使用は、ここ数十年で大きく変化した。使用が変化すると、農業従事者へのばく 露も変化する。農薬使用の変化は、EPA によるリスク軽減措置、抵抗性管理活動、新しい化学 物質の導入、遺伝子組換え作物の使用量の増加によって生じている。疫学研究を解釈し、最終的 には定量的リスク評価にそのような研究を使用するかどうかを調査する際には、ばく露における これらの大きな変化を考慮しなければならない。そうであっても、疫学研究は、実験的動物研究 から得られたエビデンスと比較して、そのような値を導き出す際の仮定を特徴づけるために使用 することが可能である。また、疫学研究で報告された結果を *in vito* 試験や動物試験で得られた 結果と定性的に比較して、動物実験で得られた結果の生物学的妥当性や人間との関連性を評価す る場合もある(Hertz-Picciotto、1995年)。疫学研究で得られたようなヒトでの情報は、NRC (2007年)が推奨する毒性試験の新しいビジョンにおいて重要な役割を果たす可能性があると 期待されている。具体的には、疫学研究は、ヒトの実社会での化学物質ばく露から生じる可能性 のある健康影響についての知見を提供し、その結果、問題の設定やハザード/リスクの特性評価 に貢献することができる。ヒトでの情報は、追加的な研究(例えば、*in vitro* 試験や目標とする *in vivo*試験で使用するための用量やエンドボイントの選択)の指針となり、今後の研究で調査さ れる新たな健康影響や感受性を特定することができる。

動物実験から得られた実験データがハザードの特性評価のための主要な情報源となる場合、潜 在的な不確実性の原因の一つとして、動物モデルのヒトへの関連性が挙げられる。これに反する データがない場合には、動物の結果はヒトとの関連性があると想定される。さらに EPA はヒト は実験動物よりも感受性が高いと仮定しているが、それを裏付けるデータがない。実際には、ヒ トは他の動物種よりも農薬に対して感受性が高い場合も低い場合もある。夜学データ及びヒトで の事例データは、科学的な情報を提供し、外挿法での推定に関連する不確実性を伝えるための裏 付けとなり得る。母集団の変動性に関しては、動物試験よりも疫学研究の方が潜在的な変動性を の特徴をよく表している。具体的には、疫学データは、ヒトの集団に固有の遺伝的多様性と変動 性を含むため、実験動物よりも環境化学物質に対する実際の集団での反応をよりよく説明し、代 表することができる(Calderon、2000年)。

用量反応の特性評価に関して、動物の毒性試験には、広範囲のばく露レベルをカバーするよう に試験デザインできるという利点がある。しかし、動物の毒性試験では、一般的に環境中でのば く露レベルよりもはるかに高い(時には桁違いの)ばく露レベルが用いられている。動物試験で は、このような高いばく露レベルがあるため、高用量から低用量の外挿法での推定が必要である。 この外挿法での推定はリスク評価に不確実性をもたらす。疫学研究データやヒトでの事例データ は、ヒトの実社会でのばく露を含んでいるため、多くの場合、高用量の外挿法での推定は必要な いと考えられる。疫学研究は低用量から高用量までの広い範囲のばく露が最も有用である。

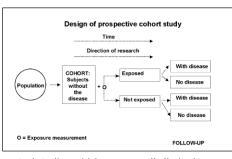
動物試験では、ヒトが経験するばく露の時間、程度、期間、ばく露経路及びばく露の変動性を 再現するものではない(Calderon、2000年)。ヒトでのばく露は、食品、水、空気、屋内外の 環境を含む多岐のばく露経路を介して行われることが多い。対照的に、制御された実験室での研 究では、通常、単一のばく露経路である。 route of exposure. In addition, humans may experience exposure to multiple chemicals and/or non-chemical stressors simultaneously, whereas most animal studies involve a single chemical stressor. On one hand, this multi-chemical exposure in epidemiology studies can provide a challenge when attempting to attribute epidemiologic outcomes to a single pesticide chemical. On the other hand, epidemiologic research considers real-world exposures and may help, when considered along with experimental approaches, address questions associated with multiple chemical exposures which can be difficult to evaluate in an experimental setting.

## B. Types of Epidemiology Studies

The major types of observational epidemiologic studies are described briefly below with consideration of their strengths and weaknesses (Lilienfeld and Lilienfeld, 1979; Mausner and Kramer, 1985; Kelsey et al., 1996; Rothman and Greenland, 2012; Paddle and Harrington, 2000; USEPA, 2005; Purdue Pesticide Programs, PPP-43).

**Cohort studies** begin with a group of people that share common characteristics—the cohort—and evaluate their health over an extended follow-up time period during which the occurrence of disease is recorded (see figure box from van den Brandt et al. (2002)). The common characteristic is often the presence vs. absence of "risk factors" (such as

exposures)<sup>9</sup>. In such studies, differences in disease occurrence between the "exposed" and "nonexposed" individuals are identified and studied over time to determine differences in the rate of disease<sup>10</sup>. This difference in the rate of disease occurrence is then investigated to determine if the rate of disease differs between the exposed and non-exposed groups. Cohort studies have the ability to simultaneously evaluate multiple disease outcomes



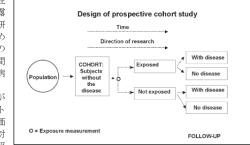
under study (which is not true for case-control studies, which are generally limited to evaluating only a single (pre-specified) disease outcome, discussed below). Cohort studies can also be performed either prospectively, like the Agricultural Health Study (AHS, <u>http://aghealth.nci.nih.gov/</u>), or retrospectively from historical records. A prospective cohort design focuses on a group of people from a current point in time through a future point in time. A retrospective cohort design focuses on a group exposed at some point in the past, and compares disease rates after exposure occurred (generally through existing さらに、ほとんどの動物試験では単一の化学的ストレス因子を用いているのに対し、ヒトでは複 数の化学物質及び/または非化学的ストレス因子へのばく露を同時に受けている可能性がある。 一方で、疫学研究における複数の化学物質へのばく露は、疫学研究の結果が単一の農薬に帰属さ せようとする場合に問題となり得る。一方で、疫学研究は実社会のばく露を考慮しており、実験 的アプローチと併せて考慮することで、実験環境では評価が困難な複数の化学物質ばく露に関連 した問題に対処するのに役立つ可能性がある。

#### B. 疫学研究の種類

観察疫学研究の主なタイプを、その長所と短所を考慮しながら以下に簡単に説明する (Lilienfeld and Lilienfeld、1979 年; Mausner and Kramer、1985 年; Kelsey ら、1996 年; Rothman and Greenland、2012 年; Paddle and Harrington、2000 年; USEPA、2005 年; Purdue Pesticide Programs, PPP-43)。

**コホート研究**では、共通の特性を共有する人々の集団(コホート)から始まり、病気の発生が記録されている長期の追跡期間にわたって健康状態を評価を行う(van den Brandt ら (2002 年)

から引用の図を参照)。共通の特性 は、しばしば「リスク因子」(ばく露 など)<sup>9</sup>の有無である。このような研 究では疾病発生率の違いを調べるため に、「ばく露者」と「非ばく露者」の 間の疾病発生率の違いを特定し、時間 をかけて調査する<sup>10</sup>。次に、この疾病 発生率の差を調査して、「ばく露群」 と「非ばく露群」の間で疾病発生率が 異なるかどうかを判断する。コホート 研究は、複数の疾病発生を同時に評価 する能力を持っている(これは症例対 照研究では当てはまらない。症例対照



研究では、一般的には後述するように単一の(事前に指定された)疾病発生のみを評価すること に限定される)。

コホート研究は、Agricultural Health Study (AHS\_http://aghealth.nci.nih.gov/) のように前 向きで実施することもできるし、過去の記録から後ろ向きに実施することもできる。前向きコホ ート研究は、現在の時点から将来の時点までの人々の集団に焦点を当てている。後ろ向きコホー ト研究は、過去のある時点でばく露された集団に焦点を当て、ばく露が発生した後の疾病率を比 較するものである(一般的には、一人(個人)単位で利用できる既存のばく露データベース(ま たは記録)を利用)。

9コホート研究では、ばく露はしばしばばく露者と非ばく露者に二分されるが、ばく露は定量的 尺度(例えば、連続的尺度または定量値)で測定することも可能である。

10 コホート研究では、一般的に疾病発生率の違いを研究しているが、これらの研究には、出生時の体重、知能、血圧など、関係のある他の限局的な結果も含まれる。

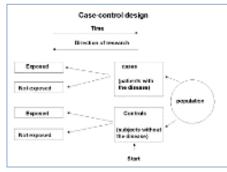
<sup>&</sup>lt;sup>9</sup> While exposure is often dichotomized on an exposed vs. non-exposed basis in cohort studies, exposure can also be measured on a quantitative scale (e.g., by a continuous measure or by quantiles)
<sup>10</sup> Cohort studies commonly study differences in rates of disease, but these can also include other focal

outcomes of interest such as birth weight, mental abilities, blood pressure, etc.

available exposure databases (or records) available on a person-by-person (individual) basis). Prospective cohort studies can be relatively lengthy and expensive to conduct, particularly for rare diseases, and require a large number of subjects to be under study. Importantly, significant resources and professional staff are required for a long period of time to collect high quality data.

**Case-control studies** are studies in which groups of individuals with (cases) and generally without (controls) a given disease are identified and compared with respect to (generally past<sup>11</sup>) exposure to determine whether those with the disease of interest are

more likely or no more likely to have been exposed to the agent(s) or factor(s) of interest. That is, the analysis of case-control studies contrasts the frequency of exposure of the agent or factor in the cases with those in the controls to determine if these differ and, thus, whether there is a differential association. In case-control studies, determination of the disease status (i.e., cases with the disease; controls without) generally precedes determination of the exposure status (see figure box from van den Brandt et



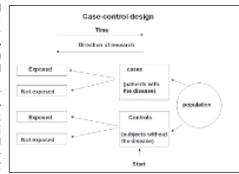
al. (2002)) Because disease has already occurred at the time of selection into the casecontrol study, this study design is particularly useful in studying uncommon diseases or diseases with long latency and can be utilized to evaluate the relation between many different exposures and a specific (pre-specified) disease outcome of interest. And because case-control studies begin with individuals who have the disease, the studies can involve fewer subjects than cohort studies and can be completed in a comparatively shorter time frame. Challenges in case-control investigations include the selection of an appropriate control group and the assessment of exposures which may have occurred long before the disease was diagnosed (Rothman, 2012; Wacholder et al. 1992a; Wacholder et al. 1992b; Wacholder et al. 1992c; Shultz and Grimes, 2002; Grimes and Schultz, 2005). Case-control studies can be particularly susceptible to "recall bias" in which diseased individuals may remember exposures or events differently (generally better) than those who serve as the controls and are healthy.

**Nested case-control studies** are an example of a hybrid design and contain the elements of a cohort and a case-control study. These designs can be useful when the analytical costs for determining pesticide exposure are too high for the entire cohort to be studies. For example, a cases that that have developed the disease or health outcome in an

前向きコホート研究は、特に希少疾病の場合、実施に比較的時間がかかり、費用がかかることが あり、研究対象となる被験者の数が多いことが必要である。重要なことは、質の高いデータを収 集するためには、長期間にわたって多大な資源と専門スタッフが必要であるということである。

**症例対照研究**は、特定の疾病を持つ(症例)個人と、そうでない(対照)個人の集団を特定し、 ばく露(一般的には過去<sup>11</sup>のばく露)に関して比較することで、関係ある疾病を持つ人が、関係 ある薬剤あるいは要因にばく露されていたか否かを判断する研究である。すなわち、症例対照研

究の解析では、症例において関係ある薬剤 または要因のばく露頻度と、対照群におけ るばく露頻度を対比させて、これらが異な るかどうか、要するに関連があるかどうか を判断する。症例対照研究では、一般的 に、疾病状態(すなわち、疾病を持つ症例 と疾病を持たない対照群)を決定すること が、通常、ばく露状態の調査に先立って行 われる(van den Brandt ら、(2002年)か ら引用の図を参照)。この研究デザインは 症例対照研究に選択された時点ですでに疾 病が発生しているので、希な疾病や潜伏期 間の長い疾病の研究で特に有用であり、多 くのばく露と関係ある特定の(事前に指定 された)疾病発生との関連性を評価するた



めに利用することができる。また、症例対照研究は、その疾病に罹患している個人を対象として いるので、コホート研究に比べて被験者数が少なく、比較的短い期間で終了することができる。 症例対照研究の問題は、適切な対照群の選択と、疾病が診断されるずっと前に発生した可能性の あるばく露の評価である(Rothman, 2012 年; Wacholder ら、1992 年 a; Wacholder ら、1992 年 b; Wacholder ら、1992 年 c; Shultz and Grimes、2002 年; Grimes and Schultz、2005 年)。症 例対照研究は、特に"想起バイアス"の影響を受けやすい。これは、罹患した人は、対照あるいは 健康人と比べてばく露や出来事を違った形で(一般的にはよく)覚えている可能性がある。

**コホート内症例対照研究**は、ハイブリッドデザインの一例であり、コホート研究と症例対照研 究の要素を含んでいる。この研究デザインは、農薬ばく露を調査するための解析コストが、高す ぎて、コホート全体を調査することができない場合に有用である。

<sup>11</sup>時間をかけて症例を研究に登録した状況下では研究開始後まで発症していない症例で前向きに 症例対照研究を実施することは可能である。

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<sup>&</sup>lt;sup>11</sup> It is possible for case-control studies to be done prospectively in which the cases have not yet developed the disease until after the study begins under which circumstance the cases are enrolled in the study over time.

ongoing cohort study can be matched with appropriate controls from the study that have not yet developed the disease or outcome of interest at the time of the analysis. One recognized advantage of the nested case-control study (as opposed to a more standard case-control study) is that the issues of selection bias and recall bias are minimized.

**Cross-sectional studies** focus on the prevalence of disease (e.g., birth defects, smallfor-gestational age or SGA), symptoms, biological/physical and physiologic response measurements (e.g., pulmonary function tests, blood pressure, chest X-ray, clinical examinations, liver and kidney biomarkers). A key feature of such studies is that they are observational studies which focuses on the *prevalence* as a frequency measure, with the presence or absence of disease determined at the time of sampling or over a sampling period. Prevalence is the proportion of individuals in a population that has the disease and can either be determined as a "point prevalence" or as a "period prevalence".12 A prevalence is a proportion not a rate and thus the cross sectional studies do not involve a follow up period. Typically, the exposure status (e.g., exposed or unexposed), disease status/outcome, and demographic characteristics are determined at a point in (or over) time. The major comparison in this study design is a comparison of the prevalence of the outcome in the exposed population vs. the prevalence of that outcome in the non-exposed population, with the risk measure being the prevalence risk ratio or odds ratio. Crosssectional studies are generally used to identify patterns or trends in disease occurrence over time or in different geographical locations, and can be conducted guickly and relatively inexpensively. However, they measure the prevalence of a disease outcome which is affected by both incidence - the rate of occurrence of new cases - and duration of the disease, and it can be difficult in any analysis to sufficiently separate these factors. Thus, they involve "survivor populations" and do not measure, evaluate, or consider those that have left the population of interest because they became ill. Another important limitation of cross-sectional studies is they do not allow one to determine whether exposure precedes the disease. As such cross-sectional studies are unable to establish temporal relationships between disease and exposure and typically require additional studies to confirm a hypothesized causal association suggested by a cross-sectional study.

Ecologic studies examine exposure and disease patterns using information reflecting group or population-level data. In an ecologic study, the unit of analysis is a group and not an individual<sup>13</sup>. Here, groups of subjects are sampled, with the exposure, disease, and potential confounding factors measured at this group (or cluster) level. Groups are generally defined on a geographic, administrative, or organizations unit basis (e.g., districts, towns, counties, schools, workplaces, etc.) with all exposure, disease, or confounder measurements made or summarized at the group level rather than at the level of the individual. An ecological (group-based) study contrasts with an individual-level study in that in the former there is no information on whether the cases are the actual individuals

例えば、現在進行中のコホート研究で疾病または健康影響を生じた症例は、解析時に関係ある疾 病や影響を生じていない研究からの適切な対照群と組み合わせることが可能である。コホート内 症例対照研究(標準的な症例対照研究とは対照的に)の利点として認識されているのは、選択バ イアスと想起バイアスの問題が最小限に抑えられることである。

構断研究では、疾病(例えば、先天性異常、胎児発育遅延(SGA))、症状、生物学的/身体 的及び生理学的反応の測定値(例えば、肺機能検査、血圧、胸部X線、臨床検査、肝臓及び腎臓 のバイオマーカー)の有病率に焦点を当てている。このような研究の主な特徴は、サンプリング 時またはサンプリング期間中に疾病の有無を判定し、有病率を頻度の尺度として重視した観察研 究であるということである。有病率とは、ある集団の中で罹患している人の割合であり、「点有 病率|または「期間有病率|として調査される12。有病率は比率ではなく、割合であるため、横 断研究では追跡調査期間を必要としない。一般的に、ばく露状態(例えば、ばく露または非ばく 露)、疾病の状態/発生、人口統計学的特性は、ある時点(またはその時点以後)で調査される。 この研究デザインにおける主な比較は、ばく露集団における疾病発生の有病率と非ばく露集団に おける疾病発生の有病率の比較であり、リスク尺度は有病率リスク比またはオッズ比である。横 断研究は、一般的に、長年にわたる疾病発生または地理的に異なる場所のパターンや傾向を明ら かにするために使用され、迅速かつ比較的安価に実施することができる。しかし、これらの調査 では、疾病の発生率(新規症例の発生率)と疾病の持続期間の両方に影響される疾病発生の有病 率を測定しているため、これらの要因を十分に分離することは、どのような解析においても困難 な場合がある。したがって、これらの研究は「生存者集団」を対象としており、病気になったた めに関係ある集団から離れてしまった人を測定、評価、考慮することはない。横断研究のもう一 つの重要な限界は、ばく露が疾病に先行しているかどうかを判断できないことである。そのため、 横断研究では疾病とばく露の時間的関係を確立することができず、横断研究で示唆された仮説に 基づく因果関係を確認するために、追加の研究を必要とするのが一般的である。

**生態学的研究**では、グループまたは集団のレベルでのデータを反映した情報を用いてばく露や 疾病のパターンを調べる。生態学的研究では、解析の単位は個人ではなく集団である<sup>13</sup>。ここで は、被験者の集団がサンプリングされ、ばく露、疾病及び潜在的な交絡因子を、この集団(また はクラスター)レベルで測定される。集団は一般的に地理的、行政的、組織的な単位(例えば、 地区、町、郡、学校、職場など)で定義され、すべてのばく露、疾病、交絡因子の測定は、個人 のレベルではなく、集団のレベルで行われたり、まとめられたりする。生態学的(集団ペース) 研究は、個人レベルの研究とは対照的であり、前者では症例がばく露された実際の個人であるか どうかの情報がないのに対し、後者ではばく露情報が個人に結びついている。

<sup>&</sup>lt;sup>12</sup> The former involve measurements at a particular place and/or a particular time while the latter involves determinations of the proportion of cases over a given time period.

<sup>&</sup>lt;sup>13</sup> Some studies can be "partially ecologic" in design in which either the exposure or the disease outcome is measured on a group level but the other variable is measures at an individual level with the researcher making inferences to the individual level.

<sup>&</sup>lt;sup>12</sup>前者は特定の場所及び/または特定の時間での測定であるのに対し、後者は特定の期間における症例の割合の調査である。

<sup>&</sup>lt;sup>13</sup>一部の研究では、ばく露または疾病発生のいずれかが集団のレベルで測定されるが、他の変数 は個人のレベルで測定され、研究者は個人のレベルで推測を行うという「部分的に生態学的」 研究もある。

with the exposure whereas in the latter exposure information is tied to the individual. As an example, a study of disease rates by contaminant levels in water can be ecologic with respect to evaluation of the exposure, but the health outcome or disease status may have determined on an individual basis. In these instances, the term "semi-ecological" can sometimes be used when exposure is determined at the group level but outcome is determined at the level of the individual.

Using this design, it is not possible to know whether all members of the exposed group are individually exposed (or the individual exposure levels) nor is it possible to infer individual-level effects from the group level effects that result. If the intent of the study is to direct inferences to the *group* (rather than the individual), then this is <u>not</u> a concern and these studies can be appropriate, particularly if measurements are constrained or difficult to perform at the individual level and exposures within the group are generally homogenous. If the intent of the study is instead to direct inferences to the individual, then this study design suffers from what is termed the ecological fallacy: the assumption that an observed relationship in an aggregated or grouped data set will reflect what would have been observed had the sampling occurred at the individual level. In addition to this control for confounding variables at the level of the individual as opposed to the group when information on confounding factors is only available at the group level.

In most cases, ecologic studies are considered as hypothesis-generating studies and best used for suggesting research hypotheses for future studies and may contribute to problem formulation. Nevertheless, it is important to assess ecological studies on the basis of the quality of their design, and useful information can be gleaned from an ecologic study if it is well-designed (FIFRA SAP, 2010). Ecologic studies alone generally do not have the ability to establish a causal association. When taken with other these studies can be useful under certain circumstances and should be noted in the hazard characterization. In particular, stable populations, clear exposure contrasts, and large differences in risk can be important factors that might increase the utility of these studies.

#### C. Evaluating epidemiology studies for use in pesticide risk assessment

OPP searches the peer reviewed literature for observational epidemiology studies of potential adverse acute and chronic health effects linked to chemical use. Details regarding literature search protocols and strategies are provided elsewhere. Epidemiologic research utilizing cohort, case-control, or cross-sectional study designs may provide information to OPP to strengthen OPP's understanding of the potential hazards, exposure-response characterization, exposure scenarios. or assessment methods, and – ultimately -- risk characterization (van den Brandt, 2002). In addition, compelling case reports or case series analysis may illumine a health effect or mechanism of action previously unidentified.

Generally speaking, the quality of epidemiologic research, sufficiency of documentation of the study (study design and results), and relevance to risk assessment is considered when evaluating epidemiology studies from the open literature for use in OPP's

例えば、水中の汚染物質レベルによる疾病率の研究は、ばく露の評価に関しては生態学的である が、健康影響や疾病状態は個人で調査されている場合がある。このような場合、ばく露は集団レ ベルで決定されるが、結果は個人レベルで調査される場合に「半生態学的」という用語が使用さ れることがある。

この研究デザインでは、ばく露集団のすべてのメンバーが個別にばく露されているかどうか (または個人のばく露レベル)を知ることはできないし、グループレベルの影響から個人レベル の影響を推測することもできない。研究の目的が(個人ではなく)集団への直接的な推測を目的 としている場合には、これは問題では<u>なく</u>、特に個人レベルでの測定が制約されていたり、困難 であったり、集団内のばく露が一般的に均質であったりする場合には、このような研究が適切で あると考えられる。研究の意図が個人への直接的な推測である場合、この研究デザインは生態学 的誤謬と呼ばれる問題を抱えていることになる。集合またはグループ化されたデータセットで観 察された関係が、個人レベルでサンプリングが行われていたら観察されていたであろうことを反 映するという仮定である。この生態学的誤謬の問題に加えて、交絡因子に関する情報が集団レベ ルでしか得られない場合、集団とは対照的に個人レベルで交絡変数を適切にコントロールできな い結果、追加のバイアスが発生する。

ほとんどの場合、生態学的研究は仮説を生み出す研究と考えられており、今後の研究のための 研究仮説を提案するのに最適であり、問題の提起に貢献する可能性がある。とはいえ、生態学的 研究をデザインの質に基づいて評価することは重要であり、デザインが優れていれば、生態学的 研究から有用な情報を得ることができる(FIFRA SAP、2010 年)。一般的に、生態学的研究だ けでは、因果関係を立証する能力はない。これらの研究を他の研究と併用した場合、特定の状況 下では有用となる可能性があり、ハザード特性評価の際に留意すべきである。特に、安定した母 集団、明確なばく露の対比、リスクの大きな差は、これらの研究の有用性を高める重要な要因と なり得る。

#### C. 農薬のリスク評価に用いるための疫学研究の評価

OPP は、化学物質の使用に関連した潜在的な急性及び慢性の健康影響に関する観察疫学研究 を査読済み文献で検索する。文献検索の手順と戦略に関する詳細は、別の場所で提供されている。 コホート研究、症例対照研究、または横断研究のデザインを利用した疫学研究は、潜在的なハザ ード、ばく露反応の特性、ばく露シナリオ、または評価方法、そして最終的にはリスクの特性評 価に関する OPP の理解を深めるための情報を提供することができる(van den Brandt、2002 年)。さらに、説得力のある症例報告や症例集積解析は、これまで明らかにされていなかった健 康影響や作用機序を明らかにする可能性がある。

一般的には、OPP のリスク評価で用いられる公開文献からの疫学研究を評価する際には、疫 学研究の質、研究の文書化の十分さ(研究デザインと結果)、リスク評価との関連性が考慮され る。 risk assessments. It is important that these criteria are endpoint-specific as various methodological details become more or less important given the endpoint of concern. For example, it is important to understand relevant factors that influence outcome ascertainment (*e.g.*, is there a test or a biomarker available to indicate presence of an effect, or are symptoms gradual and non-specific initially leading to physician diagnosis upon advanced disease state). In addition, for environmental and occupational epidemiology studies, the quality of the exposure assessment is vitally important. Prior consideration must be given to aspects of exposure and confounder measurement to the question under consideration.

When considering individual study quality, various aspects of the design, conduct, analysis and interpretation of the epidemiology studies are important. These include:

- 1. Clear articulation of the hypothesis, even if the study is hypothesis-generating in nature;
- Adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment,
- 3. Reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population),
- 4. Appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias,
- Adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed,
- 6. Overall characterization of potential systematic biases in the study including errors in the selection of participation and in the collection of information; this can include performing sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented (*e.g.*, Greenland's formula)
- 7. Evaluation of the statistical power of the study to observe health effects with appropriate discussion and/or presentation of power estimates,
- 8. Use of appropriate statistical modeling techniques, given the study design and the nature of the outcomes under study

Other Federal and non-Federal entities have offered such guides (*e.g.*, OHAT, Navigation Guide, National Toxicology Program [NTP] Report on Carcinogens [ROC<sup>14</sup>], IRIS, Cochrane ACROBAT-Non-Randomized Studies of Interventions) (Sterne et al., 2015 as well as the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for observational epidemiological studies (see <u>www.strobe-statement.org</u> and Vandenbroucke et al., 2007; Von Elm, 2014) As OPP gains experience with integrating epidemiology studies into human health risk assessment, relevant adjustments to its evaluation approach will be made.

これらの基準は、懸念されるエンドポイントに応じて様々な方法論の詳細が重要になったり、な らなかったりするため、エンドポイントを特定することが重要である。疾病発生の確認に影響を 与える関連因子を理解することが重要である(*例えば、*影響の有無を示す検査やバイオマーカー が利用できるか、あるいは初期段階では非特異的な症状であり、進行した病状では医師の診断に つながるかなど)。さらに、環境及び職域疫学研究では、ばく露評価の質が極めて重要である。 検討中の問題に対して、ばく露と交絡要因の評価の側面を事前に考慮しなければならない。

個々の研究の質を考える際には、疫学研究のデザイン、実施、解析、解釈の様々な側面が重要 である。これらには以下が含まれる。

- 1. 仮説を明確に明示することで、たとえその研究が本質的に仮説生成的なものであったとしても、その仮説を明確に示されていること。
- 2. 健康影響の関連する重大な時期、リスク評価対象集団の関係あるばく露範囲、試験から 得られる用量/ばく露反応の傾向の入手可能性など、ばく露評価の資質の中で、適切な ばく露評価が十分であること。
- 3. 合理的に有効で信頼性の高い結果の確認(研究集団における健康影響の有無を正しく識別されていること)。
- 対象集団を代表するサンプル集団となり、系統的な偏りがない適切な組み入れ基準と除 外基準。
- 5. 観察されたリスク推定値における複数の農薬ばく露、または混合物ばく露の役割の評価 または考察を含む、潜在的な交絡変数の適切な評価及び解析。
- 6. 参加者の選択や情報収集における誤りを含む、研究における潜在的な系統的な偏りの全体的な特性。これには、提示されたリスク推定値に対する系統的誤差の潜在的な影響を調査するための感度分析の実施を含む(例:Greenland's 公式)。
- 7. 健康影響を観察するための研究の統計的検出力の評価と適切な考察及び/または説明。
- 8. 研究デザインと対象となる結果の性質を考慮した適切な統計的モデル化技術の使用。

他の連邦及び非連邦の機関が次のようなガイドを提供している(*例えば*、OHAT、ナビゲーシ ョンガイド、National Toxicology Program [NTP] Report on Carcinogens [ROC<sup>14</sup>]、IRIS、 Cochrane ACROBAT-Non-Randomized Studies of Interventions) (Sterne ら、2015 年)、及 び観察疫学研究に関する STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) 声明 (<u>www.strobe-statement.org</u>, Vandenbroucke ら、2007 年; Von Elm、 2014 年)を参照)。OPP が疫学研究をヒトの健康リスク評価に統合する経験を積むにつれ、評 価アプローチに関連する調整が行われることになる。

<sup>&</sup>lt;sup>14</sup> http://ntp.niehs.nih.gov/pubhealth/roc/index.html

<sup>14</sup> http://ntp.niehs.nih.gov/pubhealth/roc/index.html

Independent study evaluation is performed and documented prior to the development of evidence- tables of detailed summary tables which are informative to hazard identification and exposure response assessment. Table 2 provides a structure to the major considerations evaluated and the associated weight (low, medium, high) for each consideration. Table 2 provides a generic set of considerations and should not be considered a checklist. The specific scientific considerations appropriate for particular science analysis are adjusted on a case by case basis.

The culmination of the study evaluation process would be to provide professional/expert opinion as to the nature of the potential bias that may result from systematic errors in each specific study identified through study specific evaluations, and an assessment of overall confidence in the epidemiological database. In this way, data integration (animal, human, mechanistic, other) would be informed by level of confidence in the human epidemiological studies that inform human health effects of environmental and occupational exposures. 独立した研究評価は、ハザードの特定及びばく露反応評価に有益である詳細な要約表を作成す る前に実施され、文書化されている。表2は、評価された主な考慮事項と、各考慮事項に関連す る重み(低、中、高)の構造を示している。表2は考慮事項の一般的な検討事項を示したもので あり、チェックリストと考えるべきではない。特定の科学的解析に適した特定の科学的考慮事項 は、ケース・バイ・ケースで調整される。

研究評価プロセスの最終段階では、研究固有の評価を通じて特定された各研究における系統的 な誤りから生じる可能性のあるバイアスの性質についての専門家/エキスパートの意見を提供し、 疫学データベースに対する全体的な信頼性の評価を行うことである。このようにして、データ統 合(動物、ヒト、メカニズム、その他)は、環境ばく露や職業上ばく露のヒト健康影響を伝える ヒト疫学研究の信頼度に基づいて行われることになる。

#### 表 2. 研究の質に関する考慮事項 a (Munoz-Quezada ら、2013 年; LaKind ら、2014 年より引用) パラメータ 中 高 低

MOA/AOP に関連し 指定されたマトリックス内 わずかなサロゲート

Exposure assessment	relationship with external exposure, internal dose, or target dose, possibly associated with an MOA/AOP. If questionnaire utilized, questionnaire and/or interview answered by subjects for chemical-specific exposure	Evidence exists for a relationship between biomarker in a specified matrix and external exposure, internal dose, or target dose. Questionnaire and/or interview for chemical- specific exposure answered by subjects or proxy individuals	Low-quality questionnaire and/or interview; information collected for groups of chemicals rather than chemical-specific; no chemical-specific exposure information collected; ever/never use of pesticides in general evaluated
Outcome Assessment	Standardized tool, validated in study population; medical record review/diagnosis confirmation by trained staff; appropriate consideration of prevalence/incidence of cases	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated	Selected sections of test, or maternal report, other; or, maternal/paternal self-report; unclear/no consideration for whether prevalent or incident cases are appropriate
Confounder control	Good control for important confounders relevant to scientific question, and standard confounders	Moderately good control confounders, standard variables, not all variables relevant for scientific question	Multi-variable analysis not performed no adjustments; no stratification, restriction, or matching
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly

Other sources of bias present,

influence magnitude but not

acknowledged but not

direction of estimate

addressed in study, may

#### Table 2. Study Quality Considerations a (Adapted from Munoz-Quezada et al., 2013; LaKind et al., 2014) Parameter High Moderate

Evidence exists for a

Accurate and

precise quantitative

ばく露評価	ている外部ばく露、内 部ばく露量、または目 標ばく露量との正確で 精密な定量関係。 アンケートを利用した 場合は、化学物質固有 のばく露についての調 査対象者からのアンケ ート及び/またはイン タビューの回答。	相定された、トラシラシス内 のバイオマーカーと外部ば く露、内部ばく露量、また は目標ばく露量との間の関 係についてのエビデンス。 調査対象者または代理人が 回答した化学物質固有のば く露に関するアンケート及 び/またはインタビュー。	(代理) 質の低いアンケート及 び/またはインタビュ ー;化学物質固有の情 報ではなく、化学物質 群について収集された 情報:代報が収集の使用 ばく或ない;農薬の使用 経験の有無に関する評 価。
発現事象評価	が確認されている標準 化ツール;訓練を受け たスタッフによるカル	母集団で有効性が確認され ていない標準化ツール、ま たはスクリーニングツー ル;または、方法が明記さ れていないカルテのレビュ 一。	分、または母親の報告、その他;または母親の報親/父親の自己報告;
交絡因子コント ロール	る重要な交絡因子及び	科学的な疑問に関連する交 絡因子、標準変数(すべて の変数ではない)を中程度 に良好に制御。	は実施していない;不
統計解析	が適切であること、適切なサンプル規模に支 えられていること、デ ータを最大限に活用していること、適切に報 告されていること(選 択的ではない)。		注意、比較の不実施、 または不明記。
アスのリスク(選 択、差のある誤	アスの主な原因は、存 在する可能性は低く、 存在するがリスク推定 の規模と方向性に影響	その他のバイアスの原因 は、存在しても研究では取 り上げられていないが、推 定の方向性ではなく、推定 の規模に影響を与える可能 性がある。	存在するが研究では取 り上げられておらず、 研究の結果について他

a パラメータ全体の総合的な評価に基づく総合的な研究の質のランキング。

<sup>a</sup> Overall study quality ranking based on comprehensive assessment across the parameters.

(not selective) Major sources of other

to influence

estimate

magnification, other) direction of the risk

magnitude and

potential biases not

likely present, present

but analyzed, unlikely

Risk of (other) bias

misclassification,

(selection,

differential

effect size

Major study biases

unacknowledged or unaddressed in study,

cannot exclude other

explanations for study

present,

finding

Low

Poor surrogate

#### 1. Exposure Assessment

Exposure assessment can be defined as the "process of estimating or measuring the magnitude, frequency and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, pathways, routes, and the uncertainties in the assessment. (Zartarian et al., 2005)." In environmental epidemiology, exposure assessment poses a unique challenge, particularly for toxicants that are found in low concentrations in environmental media (NRC, 1991; NRC, 1997). Given the complexity of exposure pathways, researchers have developed a number of different approaches to assess exposure, which vary in accuracy, precision, and resource requirements (Niewenhuijsen, 2003). Some of these approaches are not specific to epidemiologic research but may be used to inform exposure assessment in a variety of scientific analyses. These approaches include indirect methods, based on historical records, questionnaires, and environmental monitoring, and direct methods, based on personal monitoring and biomonitoring. A brief description of each method and its strengths and limitations is summarized below.

Approach	Method/Tools	Example	Exposure Estimation
	Historical Records	Estimating proximity to agricultural crops using address information	Dichotomous or ordinal exposure
Indirect	Questionnaires	Determine potential for exposure based on pesticide-use responses	Dichotomous or ordinal exposure
	Environmental Monitoring	Measuring pesticide levels in community water drinking system	Dichotomous or ordinal exposure, although exposure can be estimated using modeling
Direct	Personal Monitoring	Measuring pesticide inhalation and dermal contact	Quantified exposure
	Biomonitoring	Measuring pesticide levels in blood and urine	Quantified internal dose

Table 3. Summary of indirect and direct exposure assessment methods.
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Historical records and questionnaires are used to characterize key characteristics which may be associated with chemical exposure. When used in epidemiologic studies, historical records and questionnaires are not typically used to predict quantitative levels of exposure. Rather, historical record information or questionnaire responses are used to assign categorical levels of exposure. Examples of historical record information that can be used to assign exposure levels includes address in proximity to an agricultural crop and employment history information on job title and history. Similarly, questionnaires can be used to determine if individuals recall using pesticides or identify individuals that perform specific job functions that increase their potential for exposure. While historical records and questionnaires can be cost-effective sources of data on potential exposure, they do have limitations. Data collected from historical records and questionnaires is only a surrogate of exposure. As a result, these

#### 1. ばく露評価

ばく露評価は、「ある物質へのばく露の規模、頻度及び期間を、ばく露された集団の数と特性 とともに推定または評価するプロセス」と定義することができる。理想的には、評価において原 因、経路、方法及び不確実性を記述すると記載(Zartarian 6、2005 年)されている。環境疫学 において、ばく露評価は、特に環境媒体中に低濃度で存在する毒性物質については、独特の課題 となっている(NRC、1991 年; NRC、1997 年)。ばく露経路の複雑さを考慮して、研究者はば く露を評価するための多くの異なるアプローチを開発してきたが、その正確さ、精度及び必要な 資源は様々である(Niewenhuijsen、2003 年)。これらのアプローチのいくつかは、疫学研究 に特化したものではないが、様々な科学的解析におけるばく露評価の情報を提供するために使用 される可能性がある。これらのアプローチには、過去の記録、アンケート、環境モニタリングに 基づく間接的な方法と、個人モニタリングやバイオモニタリングに基づく直接的な方法がある。 それぞれの方法の簡単な説明とその長所と限界を以下に要約する。

#### 表 3. 間接的ばく露評価方法と直接的ばく露評価方法のまとめ

アプローチ	方法/ツール	例	ばく露の推定
	過去の記録	住所情報を利用した農地 への近接推定	二分法または順序のばく露
間接的	アンケート	農薬使用の回答に基づく ばく露の可能性の調査	二分法または順序のばく露
	環境モニタリング	地域の水道における農薬 レベルの測定	モデル化を用いてばく露を 推定することができるが、 二分法または順序のばく露
直接的	個人モニタリング	吸入と経皮でばく露され た農薬の測定	定量化されたばく露
	バイオモニタリング	血中・尿中の農薬の測定	定量化された内部ばく露量

**過去の記録やアンケート**は、化学物質へのばく露に関連する可能性のある主要な特性を評価す るために使用される。疫学研究で使用される場合、過去の記録やアンケートは通常、定量的なば く露レベルを予測するためには使用されない。むしろ、過去の記録情報やアンケートの回答は、 ばく露のカテゴリーレベルを調査するために使用される。ばく露レベルを調査するために使用で きる過去の記録情報の例としては、農地に近接した住所や、職位や職歴に関する雇用履歴情報な どが挙げられる。同様に、アンケートは、個人が農薬を使用したことを思い出すかどうかを調査 するために、またはばく露の可能性を高める特定の職務を行っている個人を特定するために使用 される。過去の記録やアンケートは、潜在的なばく露に関するデータの費用対効果の高い情報源 になり得るが、限界がある。過去の記録やアンケートから収集されたデータは、ばく露のサロゲ ート(代理)に過ぎない。

data sources may be an oversimplification of exposure and not accurately rank individual's exposure potential.

**Environmental monitoring** is used to characterize the levels of contaminants in environmental media, including air, water, soil, food, and home and work environments. Many state and Federal programs collect environmental monitoring data that may be useful in epidemiologic studies. Environmental monitoring is particularly useful for exposure that can be defined by geographic boundaries, such as air pollution and drinking water. As such, many epidemiologic studies have utilized ambient air monitoring data and community drinking water system data to characterize exposure to air pollution and drinking water contamination, respectively. While environmental monitoring data is useful for estimating exposures defined by geographic boundaries, it can be less reliable for the purposes of assigning individual-levels exposures, particularly when individuals live, work, and spend time in many different locations.

**Personal monitoring** is used to characterize exposure at the point of contact of a body boundary. Examples of personal monitoring include the use of dosimeters to assess dermal contact with pesticides, personal air sampling devices to assess inhalation exposure, and collection of duplicate diet samples to determine pesticide levels in food. The advantage of personal monitoring is that it is likely to provide more accurate estimates of individual-level exposure than indirect methods. Personal monitoring also makes it possible to quantify exposure levels that can be useful for prioritizing the relevance of different routes of exposure. Additionally, personal monitoring can also be used to assess longitudinal exposure when repeated measurements are taken over time. While personal monitoring offers many advantages over indirect approaches, it also tends to be labor and resource intensive (Niewenhuijsen, 2003). As a result, it is not typically feasible to conduct large-scale epidemiologic studies that assess exposure using personal monitoring. Furthermore, personal monitoring is highly dependent on the measurement techniques and analytic tools used to obtain samples and it is less likely that information that characterizes exposures during the relevant time period (usually in the past) will be available. In addition, it is unlikely that the full range of exposures over the time period of interest will be captured, and sampling may not be over a sufficient time period to capture peaks and fluctuations As such, it is extremely important to consider the scientific rigor and reliability of personal monitoring methodologies that are used in epidemiologic studies, and such monitoring may need to be supplemented by other monitoring (e.g., environmental, biological, and/or interview/questionnaire data).

**Biomonitoring** is used to characterize exposure by measuring a chemical, its metabolite(s), or reactive product(s) in biological samples, such as blood, urine, saliva, milk, adipose, and other body tissues (Needham et al., 2007). Zartarian et al. (2005) state that "a biomarker/biological marker has been defined as an "indicator of changes or events in biological systems. Biological markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicative of exposure to an agent". Thus, biomarkers can be used to assess exposure or as indicators of health effects (LaKind et al., 2014). Table 4 provides scientific considerations for evaluating the quality and relevance of biomonitoring data

その結果、これらの情報源はばく露の単純化しすぎているため、個人のばく露の可能性を正確 に並べていない可能性がある。

**環境モニタリング**は、大気、水、土壌、食品、家庭環境や職場環境などの環境媒体中の汚染物 質のレベルを特性評価するために使用される。多くの州及び連邦政府のプログラムでは、疫学研 究に有用な環境モニタリングデータを収集している。環境モニタリングは、大気汚染や飲料水な ど、地理的な境界によって定義されるばく露に対して特に有用である。そのため、多くの疫学研 究では、大気汚染と飲料水汚染へのばく露を特性評価するために、大気モニタリングデータと地 域の水道のデータをそれぞれ利用している。環境モニタリングデータは、地理的境界によって定 義されるばく露量を推定するのに有用であるが、個人レベルのばく露量を調査する目的では、特 に個人が多くの異なる場所で生活し、仕事をし、時間を過ごす場合には、信頼性が低くなる可能 性がある。

個人モニタリングは、体の境界の接触点でのばく露を特性評価するために使用される。個人モ ニタリングの例としては、農薬との経皮接触を評価するための線量計の使用、吸入ばく露を評価 するための個人用空気サンプリング装置、食品中の農薬レベルを調査するための対の食品サンプ ルの収集などがある。個人モニタリングの利点は、間接的な方法よりも個人レベルのばく露量を より正確に推定できる可能性が高いことである。また、個人モニタリングは、異なる経路でのば く露の関連性の優先順位付けに有用なばく露レベルの定量化を可能にする。さらに、個人モニタ リングは、時間をかけて測定を繰り返すことで、縦断的なばく露量を評価するためにも使用され る。個人的なモニタリングは間接的なアプローチに比べて多くの利点があるが、労力と資源が必 要とする傾向がある(Niewenhuijsen、2003年)。その結果、個人モニタリングを用いてばく 露を評価する大規模な疫学研究を実施することは一般的には不可能である。さらに、個人的なモ ニタリングは、サンプルを取得するために使用される測定技術や解析ツールに大きく依存してお り、関連する期間(通常は過去)のばく露を特性評価する情報が利用可能になる可能性は低い。 さらに、対象となる期間のばく露の全容が捕捉される可能性は低く、ピークと変動を捕捉するの に十分な期間でのサンプリングがではないかもしれない。そのため、疫学研究で使用される個人 モニタリング手法の科学的厳密さと信頼性を考慮することが非常に重要であり、そのようなモニ タリングは他のモニタリング(例えば、環境データ、牛物学的データ及び/またはインタビュー /アンケートのデータ)で補完する必要があるかもしれない。

**バイオモニタリング**は、血液、尿、唾液、乳、脂肪、その他の生体組織などの生物学的試料中 の化学物質、その代謝物、または反応生成物を測定することであり、ばく露の特性を明らかにす るために使用される(Needham 6、2007年)。Zartarian 6 (2005年)は、「バイオマーカー /生物学的マーカーは、「生物学的システムにおける変化や事象の指標」として定義されてきた」 と述べている。「ばく露の生物学的マーカーとは、組織、細胞、体液などの生物学的媒体から得 られる細胞や生化学的、分析的、または分子的な測定値を指し、ある物質へのばく露指標である」 と述べている。したがって、バイオマーカーは、ばく露を評価に用いられ、また健康影響の指標 として使用される(LaKind 6、2014年)。表4は、疫学研究から収集されたバイオモニタリン グデータの質と関連性を評価するための科学的考察を示している。 collected from epidemiology studies. Assessing exposure using biomonitoring has expanded rapidly as analytical tools have become more cost-effective and more biomarkers are identified. Compared with self-reported questionnaire or interview data, biomonitoring may reduce exposure misclassification and enhance the precision of the risk estimates. Similarly, biomonitoring integrates exposures from different routes and can be used to determine the amount of exposure that is absorbed into the body (Checkoway et al., 2004). Furthermore, knowledge as to the role of the biomarker in the natural history of disease is known in certain instances, such that biomarkers may help resolve temporality of exposure issues.

While biomonitoring has many advantages over others exposure assessment methods, it also has its own limitations. In many studies, biological sample are only taken from a single point in time and may not reflect accurately reflect longitudinal patterns, particularly if exposures are highly variable. Furthermore, evaluation of biomarkers also requires an understanding of degradation and metabolism of chemicals in both the environment and human body. As such, biomarkers of exposure may differ between individuals for reasons other than exposure level. Differences in metabolism, comorbidities such as kidney disease in relation to urinary measurements, uncertainty as to whether the biomarker measures exposure to the active ingredient or the environmental degradates may all account for apparent differences in biomarkers of exposure among individuals, and possibly between comparison groups. 解析ツールがより費用対効果の高いものになり、より多くのバイオマーカーが同定されるよう になったため、バイオモニタリングを用いたばく露評価は急速に拡大してきた。自己申告のアン ケートやインタビューデータと比較して、バイオモニタリングはばく露の誤分類を減らし、リス ク推定の精度を高めることができるかもしれない。同様に、バイオモニタリングは、異なる経路 からのばく露を統合し、体内に吸収されるばく露量を調査するために使用されている (Checkoway 6、2004 年)。さらに、疾病の履歴におけるバイオマーカーの役割が知られるよ うになってきている場合もあり、バイオマーカーはばく露の時間的な問題を解決するのに役立つ かもしれない。

バイオモニタリングは他のばく露評価方法に比べて多くの利点があるが、それ自体にも限界が ある。多くの研究では、生物学的サンプルはある時点での一点からしか採取されず、特にばく露 が大きく変動する場合には、経時的なパターンを正確に反映していない可能性がある。さらに、 バイオマーカーの評価には、環境ととトの両方での化学物質の分解と代謝についての理解も必要 である。そのため、ばく露のバイオマーカーはばく露以外の理由で個人間で異なる場合がある。 代謝の違い、尿の測定値に関連した腎臓病などの併発疾患やバイオマーカーが有効成分のばく露 を測定しているのか、環境中分解物のばく露を測定しているのかといった不確実性が、個人間や 想定される比較集団間におけるばく露のバイオマーカーの見かけ上の違いの原因となっているか もしれない。 Table 4. Considerations of biomonitoring data from environmental epidemiology research (Adapted from LaKind et al. (2014).

Biomarker Consideration	Tier 1	Tier 2	Tier 3
Exposure biomarker with external exposure, internal dose, or target dose.		Biomarker has an unknown quantitative relationship with external exposure, internal dose, or target dose or is poor surrogate (low accuracy and precision) for exposure/dose.	NA
Effect biomarker	Bioindicator of a key event in a MOA/AOP.	Biomarkers of effect for which the relationship to health outcome is understood	Biomarker has undetermined consequences (e.g., biomarker is not specific to a health outcome).
Specificity	Biomarker is derived from exposure to one parent chemical.	Biomarker is derived from multiple parent chemicals with similar toxicities.	Biomarker is derived from multiple parent chemicals with varying types of adverse endpoints.
Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research ouestion.		Frequency of detection too low to address the research hypothesis.	NA
Biomarker stability	Samples with a known history and documented stability data.	Samples have known losses during storage but the difference between low and high exposures can be qualitatively assessed.	Samples with either unknown history and/or no stability data for analytes of interest.
Sample contamination	Samples are contamination-free from time of collection to time of measurement (e.g., by use of	Study not using/documenting these procedures.	There are known contamination issues and no documentation that the issues were addressed

#### 表 4. 環境疫学研究におけるバイオモニタリングデータの考察(LaKind ら、2014 年より引用)

パイオマーカーの考察	ティア1	ティア2	ティア 3
ばく露バイオマーカー		バイオマーカーは、外部ばく露、 内部ばく露量、目標ばく露量との 定量的関係が不明であるか、ばく 露/用量に関してわずかなサロゲ ート[代理](正確さ・精度が低 い)である。	
効果バイオマーカー		健康影響との関係に対する効果の バイオマーカーは明らかである。	バイオマーカーは結論をもたらさ ない(例えば、バイオマーカーは 健康影響を明確にしない)。
特具度		バイオマーカーは、類似の毒性を 持つ複数の親化合物に由来する。	バイオマーカーは、様々な有害エ ンドポイントがある複数の親化合 物に由来する。
手法の感度	検出限界は、研究課題に取り組む のに十分な割合のサンプルから化 学物質を検出するのに十分に低 い。	研究仮説に取り組むには、検出頻 度が低すぎる。	NA
パイオマーカーの安定性	既知の履歴と文書化された安定性 データを持つサンプル。	サンブルは保存中の損失が知られ ているが、低量ばく露と高量ばく 露の違いを定性的に評価すること ができる	は目的の分析物の安定性データが

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Biomarker Consideration	Tier 1	Tier 2	Tier 3
	certified analyte-free collection supplies and reference materials, and appropriate use of blanks both in the field and lab). Research includes documentation of the steps taken to provide the necessary assurance that the study data are reliable.		
Method requirements	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (e.g., GC- HRMS, GC-MS/MS, LC-MS/MS)	Instrumentation that allows for identification of the biomarker with a high degree of confidence and the required sensitivity (e.g., GC-MS, GC- ECD).	Instrumentation that only allows for possible quantification of the biomarker but the method has known interferants (e.g., GC-FID, spectroscopy)
Matrix adjustment	Study includes results for adjusted and non-adjusted concentrations	Study only provides results using one method (matrix- adjusted or not).	NA

FP = false positive: FN = false negative: 6C-FRMS = gas chromatography/filar resolution mass spectrometry: 6C-KS = gas chromatography/mass spectrometry: 6C-ECD = gas chromatography-electron capture detector; 6C-FID = gas chromatography-flame ionization detector], ICC = intra-class correlation coefficient : NA = not applicable: PPP = probability of false positive

パイオマーカーの考察	ティア1	ティア2	ティア3
サンプルの汚染	サンブルは、収集時から測定時ま で汚染されていない(例えば、分 析物の無いことが保証された収集 用品及び参照物質の使用及びフィ ールドとラボの両方でのブランク の適切な使用による)。研究に は、研究データの信頼性を保証す るために必要な手順の文書化が含 まれる。		汚染の問題が知られているが、世 題を取り上げた文書はない。
手法の要件	バイオマーカーの明確な同定と定 量を必要な感度で提供する装置 (例:GC-HRMS、GC-MS/MS、 LC-MS/MS)。		
マトリックス調整	研究には、調整濃度と非調整濃度 の結果が含まれている。	研究では、1 つの方法 (マトリッ クス調整済みかどうか)を用いた 結果のみを提供している。	NA

PP=偽陽性、FN=偽陰性、GC-HRMS=ガスクロマトグラフィー/高分解能質量分析、GC-MS=ガスクロマトグラフィー/質量分析、 GC-ECD=ガスクロマトグラフィー・電子補足検出器;GC-FID=ガスクロマトグラフィー・水素炎イオン化型検出器]、 ICC=クラス内相関係数;NA=該当しない;PFP=偽陽性の確率

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Indirect exposure assessment methods are common in retrospective studies and based on factors that are surrogates of chemical exposure. As described above, indirect exposure data cannot generally be used to estimate quantitative exposure levels without additional modeling. For example, a questionnaire can be used to determine if an individual has ever used a pesticide, but can less reliably collect data on all the environmental and behavioral factors that are needed to calculate that individual's exposure. As such, indirect exposure data are often used to classify exposure using a dichotomous exposure variable (i.e. exposed/unexposed) or ordinal exposure scale. In contrast, direct exposure assessment methods are based on data on actual individual-level exposure through personal monitoring and biomonitoring. Thus, direct methods can be used to estimate individual exposure or internal dose levels. Direct methods are more common in prospective studies, but are also used in retrospective studies when existing biological samples are available from well-defined population groups.

<u>Quantified personal measurements</u>, such as personal monitoring and biomonitoring, are generally considered the best source of data for estimating actual exposure levels (NRC, 1991; NRC, 1997). While this is the case, accurate qualitative measures of exposure (e.g. dichotomous and ordinal exposure metrics) from indirect methods can be just as accurate for the purpose of epidemiology. Moreover, indirect methods are often easier to interpret and may require less additional research and development to demonstrate their utility in exposure assessment.

Regardless of the approach, exposure assessment methods should be able to provide exposure estimates that are reliable and valid. In the context of epidemiology, *reliability* general refers to the ability to reproduce results and *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). When evaluating a particular exposure assessment's reliability and validity, it is important to consider the exposure assessment's strengths and weaknesses in the context of the study's research objectives. Less refined exposure assessment may be suitable for exploratory studies. This is because exploratory studies help raise awareness about potential hazards that can encourage investment in more focused research. Conversely, studies with more focused hypotheses can be greatly strengthened through the use of more refined exposure assessment methods. Therefore, indirect and direct exposure assessment stages of research when exploring exposure-disease relationships.

#### 2. Confounding Factors

Confounding occurs when the relationship between the exposure and disease is to some extent attributable to the effect of a second (confounding) risk factor. This can happen when this second (i.e., confounding) risk factor is an independent, causally-associated risk factor for the disease but is also associated -- causally or non-causally -- with the exposure under analysis and does not also serve as an intermediate variable in the causal pathway between the exposure and the outcome of interest. If not properly measured and accounted

**間接的はく露評価**方法は、後ろ向き研究では一般的であり、化学物質のばく露のサロゲート (代理)となる因子に基づいている。上述したように、間接的なばく露データは、追加のモデル 化を行わない限り、一般的には定量的なばく露レベルを推定するためには使用できない。例えば、 ある個人が農薬を使用したことがあるかどうかを調査するためにアンケートを使用することはで きるが、その個人のばく露量を計算するために必要なすべての環境的・行動的な要因に関するデ ータを確実に集めることは困難である。そのため、間接的ばく露データは、二分法のばく露変数 (すなわち、ばく露/非ばく露)または順序ばく露尺度を用いてばく露を効するために使用さ れることが多い。一方、直接的ばく露評価方法は、個人のモニタリングやバイオモニタリングに よる実際の個人レベルのばく露に関するデータに基づいている。したがって、直接法は前向き研究 で用いられることが多いが、十分に定義された母集団から既存の生物学的サンプルが入手可能な 場合には、後ろ向き研究でも使用される。

定量化された個人評価には、個人モニタリングやバイオモニタリングがあり、一般的に実際の ばく露レベルを推定するための最良のデータソースと考えられている(NRC、1991年;NRC、 1997年)。その一方で、間接的な方法による正確なばく露の質的測定(例えば、二分式や順序 式のばく露測定)も、疫学の目的では同じように正確であると考えられる。さらに、間接法は解 釈が容易であることが多く、ばく露評価における有用性を示すための追加の研究開発が少なくて 済む可能性がある。

どのようなアプローチであっても、ばく露評価方法は信頼性と妥当性のあるばく露推定値を提 供できなければならない。疫学の文脈では、一般的に*信頼性とは*結果を再現する能力を意味し、 *妥当性とは、一*般的にばく露推定値が真のばく露レベルを反映しているかどうかを意味している (Checkoway 6、2004 年)。特定のばく露評価の信頼性と妥当性を評価する際には、研究目的 の文脈でばく露評価の長所と短所を考慮することが重要である。あまり洗練されていないばく露 評価は、探索的研究に適しているかもしれない。これは、探索的研究は潜在的なハザードについ ての認識を高めるのに役立つため、より焦点を絞った研究への専心を促すことができるからであ る。逆に、より焦点を絞った仮説を持つ研究は、より洗練されたばく露評価法は相完的なツールであり、 ばく露と疾病の関係を調査する際に研究の様々な段階で使用することができる。

#### **2. 交絡因**子

交絡因子は、ばく露と疾病との関係がある程度で第二のリスク因子(交絡因子)の影響に起因 している場合に発生する。これは、この第二のリスク因子(すなわち、交絡因子)が、疾病の独 立した因果関係のあるリスク因子でありながら、解析対象のばく露と因果関係(因果関係または 非因果関係)があり、ばく露と関係のある疾病との間の因果経路の中間変数としての役割を果た していない場合に起こりうる。交絡因子は、適切に測定され、考慮されなければ、ばく露と健康 影響との間の推定された関連性の大きさ(そして場合によっては方向性)を変化させる可能があ る。

for, confounders have the ability to change the magnitude (and potentially the direction) of the estimated association between an exposure and health outcome. This can result in an over- or under-estimation of the relationship between exposure and disease because the effects of the two risk factors have not been appropriately separated, or "disentangled". As an example: a given pesticide may be associated with lung cancer in a given study, but this may be due to a confounding effect of farm tractor diesel fumes; here, this second factor farm tractor diesel fumes - would be a confounder if it was causally associated with the disease outcome (here, lung cancer) but also associated with pesticide exposure. Confounding factors may include less intuitive lifestyle exposures such as cigarette smoking, dietary factors (e.g., high energy/calorie laden diet), and physical activity (e.g., lack of physical activity) genetics, comorbidity, medication use, alcohol consumption, etc., all of which may adversely affect health and may be statistically associated with pesticide use. In epidemiological analyses, confounding factors are measured in the study sample and typically "adjusted for" in the final risk estimate in either the design phase of the study or the analysis phase. With respect to the former, the epidemiological researcher can "restrict" the study population to individuals that share a characteristic which the researcher wishes to control; this has the result of removing the potential effect of confounding caused by that (now controlled) characteristic. A second available method also applicable to the design phase of the study -- is for the researcher to control confounding by "matching" individuals based on the confounding variable. This ensures that the confounding variable is evenly distributed between the two comparison groups and effectively controls for this. It is important to note that the relationship between the confounder and the exposure or outcome does not need to be found to be statistically significant in order for it to have an impact on the risk estimate for the main effect<sup>15</sup>.

At the analysis stage, one method by which confounding can be controlled is by stratification. Under this means of control, the association is measured separately under each of the (potentially) confounding variables; the separate estimates are "brought together" statistically -- if determined to be appropriate -- to produce a common odds ratio or other effect size measure by using Mantel-Haenszel approaches which weight the estimates measured in each stratum. Stratification can be difficult if there are multiple potential confounders that need to be controlled simultaneously. In such cases, confounding is typically dealt with by means of statistical modelling. (e.g., logistic regression).

It is important that careful consideration be given to confounders prior to any epidemiological studies being initiated in the field and it is important that any study adequately describe how this was done: epidemiological studies are frequently critiqued for ignoring or paying insufficient attention to potential confounders. For this reason, a sensitivity analysis can be helpful to demonstrate the potential effects that a missing or unaccounted for confounder may have on the observed effect sizes (see Gustafson and

これは、2 つのリスク因子の影響が適切に分離、あるいは「区別」されていないため、ばく露と 疾病の関係が過大または過小に評価される結果となりうる。例えば、ある研究では、ある農薬が 肺がんと関連しているかもしれないが、これは農場のトラクターのディーゼル排気ガスの交絡効 果によるものかもしれない。このように疾病発生(ここでは肺がん)との因果関係が農薬ばく露 とも関連する場合、第二の因子の「農場のトラクターのディーゼル排気ガス」は交絡因子である。 交絡因子には、喫煙、食事因子(例えば、高エネルギー/高カロリーの食事)、身体活動(例え ば、身体活動の欠如)、遺伝子、併存症、薬物使用、アルコール摂取などのあまり直感的でない ライフスタイルでのばく露が含まれることがあり、これらはすべて健康に悪影響を及ぼし、農薬 使用と統計的に関連している可能性がある。疫学的解析では、交絡因子は研究サンプルで測定さ れ、通常、研究のデザイン段階または解析段階のいずれかで最終的なリスク推定値に「調整」さ れる。前者に関しては、疫学研究者は、研究者がコントロールしたい特性を共有する個人に研究 集団を「限定」することができる。これにより、その特性(現在はコントロールされている)に 起因する交絡の潜在的な影響を取り除くことがでる。2 番目に利用可能な方法は、研究のデザイ ン段階にも適用可能な方法で、研究者が交絡変数に基づいて個人を「マッチング」することで交 絡をコントロールすることである。これにより、交絡変数が2つの比較群の間で均等に分布し、 交絡変数を効果的にコントロールすることができる。主効果のリスク推定値に影響を与えるため には、交絡因子とばく露または疾病との関係が統計的に有意である必要はないことに注目するこ とが重要である15。

解析の段階で、交絡因子をコントロールできる方法の1つは、層化がある。この方法では、交 絡変数(の可能性がある)ごとに関連性を測定し、適切であると判断された場合には、個別の推 定値を統計的に「まとめる」ことで、各層で評価された推定値に重みをつける Mantel-Haenszel アプローチを使用して共通のオッズ比または他の効果を算出する。層化は同時にコントロールす る必要のある潜在的な交絡因子が複数存在する場合には困難である。このような場合、交絡因子 は通常、統計的モデル化によって対処される。(例:ロジスティック回帰)。

疫学研究が現場で開始される前に交絡因子を慎重に考慮することが重要であり、どのような研 究でも交絡因子をどのようにして考慮したかを適切に記述することが重要である。疫学研究は、 潜在的交絡因子を無視したり、あるいは十分な注意を払っていないと批評されることか多い。こ のため、感度分析は、欠落している交絡因子や考慮されていない交絡因子が観察された効果量に 与える潜在的な影響を示すのに役立つ(Gustafson and McCandless, 2010 年を参照)。

<sup>&</sup>lt;sup>15</sup> This is why it is generally considered inappropriate to "statistically test" for a confounder to determine whether the confounder needs to be adjusted for. Instead, some consider a change in the effect size of 10% or more after adjustment for (inclusion of) a potential confounder to be sufficient evidence for the confounder to be incorporated into the analysis.

<sup>&</sup>lt;sup>15</sup>これが、一般的に、交絡因子を調整する必要があるかどうかを判断するために交絡因子を「統計的に検定」することが不適切であると考えられている理由である。その代わりに、潜在的な交絡因子を調整した(交絡因子を含む)後の効果量の変化が10%以上であれば、交絡因子を分析に組み込むのに十分なエビデンスであると考える人もいる。

McCandless, 2010). If unmeasured confounders are thought to affect the results, researchers should conduct sensitivity analyses to estimate the range of impacts and the resulting range of adjusted effect measures. Such sensitivity analyses -- generally not uniformly conducted in most published epidemiological studies – can be used when available to estimate the impact of biases and potential confounding by known but unmeasured risk factors.

Depending upon the specific exposure-disease association under study, a factor may or may not be a confounding factor that is necessary to control: in order for a substantial distortion in the effect size estimate to occur due to confounding, the confounder must be not only a relatively strong risk factor for the disease of interest<sup>16</sup>, but also be strongly associated with the exposure of interest. Assessment of potential confounding is made on a study specific basis and - if unmeasured confounders are thought to affect the results -researchers should conduct a sensitivity analysis to estimate the range of impacts and resulting range of adjusted effect measures. When evaluating the guality of observational epidemiology studies, OPP will consider whether relevant confounding factors are properly identified, described, measured and analyzed such that an unbiased estimate of the specific association under study can be made, and, when possible, may consider sensitivity analysis as a potential tool to assist in determining the degree to which such confounding might potentially affect the estimate of the effect size. It should be emphasized that a confounder must be a relatively strong risk factor for the disease to be strongly associated with the exposure of interest to create a substantial distortion in the risk estimate. In such cases, it is not sufficient to simply raise the possibility of confounding: one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be, and how important that impact might be to the interpretation of findings. (p. 23-25, FIFRA SAP Report, 22 April 2010)

Finally, it is important to distinguish between confounding, effect modification, synergy, and other mediating effects of covariates. Confounding is a bias that results from not controlling for a variable that is associated causally with the disease and associated – causally or non-causally -- with the exposure of interest. Epidemiologic researchers seek to minimize this bias. Effect modifiers -- on the other hand -- are variables that differentially affect the magnitude of the effect size, by strata (e.g., age, race/ethnicity, SES status, genetic polymorphisms). Effect modifiers may or may not also be confounders. Typically, they are modelled by either introducing interaction terms in multivariable models or by evaluating effect sizes by strata after stratifying the data by levels of the effect modifier. A study frequently needs to be specifically designed to evaluate effect modifiers in order to have a sufficient sample size in each population strata of interest. Epidemiologic researchers seek to understand effect modifiers (not minimize them, as they do with confounders) because they can be important in evaluating risk differences across population strata, in evaluating the association between exposure and the effect of interest, and in identifying susceptible

測定されていない交絡因子が結果に悪影響を及ぼすと考えられる場合、研究者は感度分析を実施 して、影響の範囲とその結果として得られる調整後の効果測定値の範囲を推定すべきである。こ のような感度分析は、一般的にほとんどの公表されている疫学研究では一様には行われていない が、既知ではあるが評価されていないリスク因子によるバイアスや潜在的な交絡因子の影響を推 定するために、利用可能な場合には使用することができる。

特定のばく露・疾病関連付けに応じて、ある因子はコントロールするために必要な交絡因子で ある場合とそうでない場合がある。交絡因子によって推定された効果量に大きな歪みが生じるた めには、交絡因子は、関係ある疾病が比較的強いリスク因子でなければならない<sup>16</sup>し、関係ある ばく露とも強固に関連付けられていなければならない。潜在的な交絡因子の評価は研究ごとに行 われ、測定されていない交絡因子が結果に悪影響を与えると考えられる場合には、研究者は感度 分析を行い、影響の範囲とその結果として得られる調整された効果評価の範囲を推定すべきであ る。観察疫学研究の質を評価する際、OPP は、関連する交絡因子が適切に同定、記述、評価、 解析されているかどうかを検討し、研究対象となっている特定の関連性を偏りのない推定が可能 であるかどうかを検討する。可能な場合は、交絡因子が推定された効果量の推定値に影響を及ぼ す範囲の判断を助けるツールとして感度分析を考慮する。交絡因子は、疾患の比較的強いリスク 因子でなければならず、リスク推定値に大きな歪みをもたらすためには、対象となるばく露と強 の可能性を提起するだけでは十分ではなく、なぜリスク因子が交絡因子となりうるのか、その影響が結果の解釈にとってどの程度重要なものなのかに ついて、説得力のある議論が必要である。(p.23・25、FIFRA SAP レポート、2010年4月22日)

最後に、共変量の交絡、効果修正、相乗効果、その他の媒介効果を区別することが重要である。 交絡因子とは、疾病と因果関係のある変数及び対象となるばく露と因果関係があるか、否かにか かわらず、関連する変数をコントロールしないことによって生じるバイアスである。疫学研究者 はこのバイアスを最小化しようと努める。一方、効果修飾因子とは、層化(例えば、年齢、人種 /民族、SESの状態、遺伝的多型)によって、効果量に異なる影響を与える変数である。効果修 飾因子は、交絡因子である場合もあればそうでない場合もある。一般的に、効果修飾因子は多変 量モデルに交互作用項を導入するか、効果修飾因子のレベルによってデータを層化した後、層化 に効果量を評価することによってモデル化される。対象となる各集団層において十分なサンプル サイズを確保するためには、効果修飾因子を評価するための特別な研究デザインが必要となるこ とが多い。疫学研究者は、母集団の層化にわたるリスクの違いの評価、ばく露と関係のある効果 の関連付けの評価、影響を受けやすい亜集団の特定が重要であることから、効果修飾因子を理解 することを目指す(交絡因子のように最小化するのではなく、効果修飾因子の理解に努めてい る)。

<sup>&</sup>lt;sup>16</sup> Consideration needs to be given not only to ensuring that the confounding factor is indeed a risk factor on its own but also to ensuring not only related to the exposure of interest. Adjusting for a factor that has an association with the disease of interest wholly or partly because of its association with the exposure of interest will lead to attenuation of the exposure-disease relationship if it truly exists.

<sup>16</sup> 交絡因子がそれ自体が本当にリスク因子であることを確認するだけでなく、関係のあるばく露との関連性だけではないことを確認することも考慮する必要がある。関係のある疾病との関連性を持つ因子の全部または一部を、関係のあるばく露との関連性のために調整することは、ばく露と疾病の関係が本当に存在する場合には、ばく露と疾病の関係を減衰させることにつながる。

subpopulations. Effect modifiers may or may not also be confounders. For example, smoking may be a confounder in a study associating lung cancer with a pesticide often used on tobacco, but it may also be an effect modifier if the risk of exposure to this pesticide is higher among smokers than non-smokers. Synergy is often introduced as a biological or pharmacological/toxicological concept rather than an epidemiological one and relates to the ability of two chemicals, together and acting jointly, to magnify or exaggerate the effect beyond that which would be seen considering the (mathematical) sum of each chemical's effects alone. In epidemiological and statistical terms, this is often expressed as effect modification or interaction.

#### 3. Statistical Analysis

Epidemiologic studies are designed to measure an association between a specific exposure and a disease. When evaluating the quality of pesticide epidemiology studies, OPP will also consider the statistical methods used. Specifically, OPP will consider the extent to which the analytic methods described in the study are appropriate to the research question; the completeness of the description of the statistical methods utilized; the appropriateness of the methods for identification, assessment and adjustment of potentially confounding variables in the exposure-disease relation; and, the description, extent of, and presentation of any sub-group analyses which may have been performed (including whether statistical corrections for multiple comparisons have been made).

Epidemiologic investigations typically utilize statistical modeling to estimate risk (e.g. generalized linear models such as logistic (for odds ratios) or Poisson (for count data) regression. To do so, researchers must consider not only the relevant main exposure and outcome variables, but also consider relevant confounding factors, and whether the association under investigation may differ by level of these factors, i.e., effect modification or interaction (Szklo et al., 2004). Upon identification of a potentially confounding variable -- one that substantively changes the magnitude and/or direction of the association under study -- adjustment through regression modeling can help to isolate the risk estimate of interest, i.e., the association under study. In addition, OPP will evaluate the stratification of statistical interaction. If the magnitude and direction of the association of interest differs greatly by level of a third variable, then the stratified results should be considered primary.

When performing statistical modeling when the outcome is rare or the sample size is relatively small, it is important to be cautious about including too many covariates in the model. Any resulting effect size estimate may be too high or too low and is unlikely to reflect the true estimate of effect. Such issues due to rare events or low sample sizes are also possible when conditional methods are used (e.g., conditional logistic regression when the design includes matching of the comparison group under study): if too few discordant pairs (or discordant sets) are observed, the estimated effect size may also be unreliable. Thus: while controlling for confounders and other covariates is important, the assessor must take care not to over-control or end up with too few degrees of freedom to produce a

効果修飾因子は交絡因子である場合もあればそうでない場合もある。例えば、タバコによく使用 される殺虫剤と肺がんを関連付ける研究では、喫煙は交絡因子になるかもしれないが、この殺虫 剤へのばく露のリスクが非喫煙者よりも喫煙者の方が高い場合には、効果修飾因子になるかもし れない。相乗効果は、疫学的な概念ではなく、生物学的または薬理学的/毒物学的な概念として 紹介されることが多く、2 つの化学物質が一緒になって共同で作用することで、各化学物質の効 果の(数学的な)合計を考慮した場合の効果を超えて、効果を拡大したり、増強したりする能力 に関係している。疫学や統計学の用語では、これは効果の修飾や相互作用として表現されること が多い。

#### 3. 統計解析

疫学研究は、特定のばく露と疾病との関連付けを評価することを目的としている。農薬疫学研 究の質を評価する際、OPP は使用された統計的手法も考慮する。具体的には、研究に記載され ている分析方法が研究課題に適切であるかどうか、使用された統計的方法の記述の完全性、ばく 露と疾病の関係における潜在的な交絡変数の特定、評価、調整のための方法の適切性、実施され た可能性のある小集団解析の説明、範囲、提示(多重比較のための統計的補正が行われているか どうかを含む)が考慮される。

疫学研究では通常、リスクを推定するために統計的モデル(例えば、ロジスティック回帰(オ ッズ比)やポアソン回帰(カウントデータ)のような一般化された線形モデル)を利用する。そ のためには、研究者は関連する主要なばく露変数と転帰変数だけでなく、関連する交絡因子を考 慮しなければならず、また、研究における関連付けがこれらの因子、すなわち、効果の修飾や相 互作用のレベルによって異なるかどうかを考慮しなければならない(Szklo 6、2004年)。交絡 の可能性のある変数(調査対象となる関連性の大きさや方向性を実質的に変化させる変数)が同 定された場合、回帰モデルによる調整を行うことで、目的のリスク推定値、すなわち調査対象と なる関連性を分離することができる。さらに、OPP は、研究における潜在的な効果修飾因子の レベルまたは統計的相互作用の評価による関連付けの層化を評価する。関係のある関連付けの規 模と方向性が第三の変数のレベルによって大きく異なる場合、層化された結果は一次的なものと みなされるべきである。

発現事象がまれな場合やサンプル規模が比較的小さい場合に統計的モデリングを行う場合、モ デルにおける共変量が多すぎないように注意することが重要である。結果として得られる効果量 の推定が高すぎたり、低すぎたりすることがあり、効果の真の推定を反映しているとは考えにく い。稀な事象やサンプル規模が小さいことによるこのような問題は、条件付き手法(例えば、デ ザインに研究における比較群の組み合わせを含む場合の条件付きロジスティック回帰)を使用す る場合にも起こり得る。すなわち不一致ペア(または不一致セット)の観察数が少なすぎると、 推定された効果量もまた信頼性が低くなる可能性がある。このように、交絡因子及びその他の共 変量をコントロールすることは重要であるが、信頼性の高い検定を行うために過度にコントロー ルしたり、自由度が少なくしすぎたりしないように評価者は注意しなければならない。 reliable test. In these cases, it may be more important to seek parsimonious models that adjust for only a smaller number of the most influential confounders and other covariates so that the effective sample size remains adequate.

Finally, it is important in any statistical modeling exercise to consider statistical significance in the context of clinical/biological/scientific significance of the result. It may be that some results are statistically significant but unimportant in a clinical/biological/ scientific context. The reverse can be true: it may be that results are not statistically significant but may be important in a clinical/biological/scientific context. The former may suggest a sample size that is larger than necessary while the latter may suggest one than is smaller than needed. The latter case may be important from a public health perspective and warrant further exploration, especially when the association is strong (despite it being imprecise)

#### 4. Potential Bias in Observational Research

Bias is a systematic error in the design or conduct of a study that gives rise to study results that are systematically different from the (unobserved) true situation. This contrasts with random errors which relate to sampling variability and precision (or, equivalently, confidence bounds) around the effect size measure, but which do not "drive" or "push" the result in one particular direction (e.g., either toward or away from the null).

Bias is a reflection of methodological imperfections in the design or conduct of the study and should be addressed or discussed by researchers as part of their analysis. There are a number of ways that bias can be introduced into a study: studies may be biased in the way in which participants are selected into the study (selection bias), or the way in which information about exposure and disease status is collected (information bias, including recall bias discussed earlier for case-control studies). One example of a common occupational selection bias is the "healthy worker effect" which can create an important bias in occupational epidemiology studies, leading to bias toward the null, and even below (creating the interpretation that the exposure is "protective") No study is totally devoid of bias and one should consider the extent to which authors of published studies described potential bias in the study, and how (if at all) they attempted to address it and characterize it in the study. Bias can result from differential or non-differential misclassification (Greenland, 1998). Differential misclassification (bias) means that misclassification has occurred in a way that depends on the values of other variables, while non-differential misclassification (bias) refers to misclassifications that do not depend on the value of other variables. Misclassification biases - either differential or non-differential - depend on the sensitivity and specificity of the study's methods used to categorize such exposures and can have a predictable effect on the direction of bias under certain (limited) conditions: this ability to characterize the direction of the bias based on knowledge of the study methods and analyses can be useful to the regulatory decision-maker since it may allow the decision maker to determine the extent to which, if any, the epidemiological effect sizes being considered (e.g., OR, RR) are likely underestimates or overestimates of the true effect

このような場合には、有効なサンプルサイズを十分に確保するため、最も影響力のある交絡因子 や他の共変量をより少ない数だけ調整して、より解析的なモデルを求めることがより重要である。

最後に、どのような統計モデリング演習においても、結果の臨床的/生物学的/科学的な意義 の文脈で統計的有意性を考慮することが重要である。統計的には有意だが、臨床/生物学的/科 学的な文脈では重要ではないということもある。逆に、統計的には有意ではないが、臨床的/生 物学的/科学的な文脈では重要な結果である場合もある。前者の場合はサンプルサイズが必要以 上に大きいことを示唆し、後者の場合は必要以上に小さなサンプル規模を示唆しているかもしれ ない。後者の場合は、公衆衛生の観点から重要であり、(不正確であるにもかかわらず)特に関 連付けが強固である場合には、さらなる調査が必要である。

#### 4. 観察研究における潜在的なバイアス

バイアスとは、(観察されていない)真の状況とは系統的に異なる研究結果をもたらす研究の デザインまたは実施における系統的な誤差のことである。これは、効果量の評価におけるサンプ リングの変動や精度(または同義で信頼限界)に関係するランダム誤差とは対照的であるが、結 果をある特定の方向(例えば、帰無仮説に向かって、または帰無仮説から離れて)に「追い込む (drive)」または「押し込む (push)」ようなことはない。

バイアスとは、研究のデザインや実施における方法論的な不完全性の反映であり、研究者は解 析の一部として、それに対処したり、議論したりすべきである。研究にバイアスが導入される方 法はいくつかある。研究は、参加者を研究に選択する方法(選択バイアス)や、ばく露や疾病に 関する情報を収集する方法(情報バイアス、先に説明した症例対照研究の想起バイアスを含む) にバイアスがかかっている可能性がある。一般的な職業選択バイアスの例としては、「健康な労 働者効果」があり、職域疫学研究において重要なバイアスを生じさせ、帰無値に向かってバイア スを生じさせ、さらにはそれ以下のバイアス(ばく露が「保護的」であるという解釈を生じさせ る)につながる。バイアスの全くない研究はなく、公表した研究の著者は研究における潜在的バ イアスどの程度説明したか、研究におけるバイアスを(もしあれば)どのように対処し、研究の 特徴を明らかにしたかを検討すべきである。バイアスは、差異的(differential)または非差異 的 (non-differential) な誤分類から生じる可能性がある (Greenland、1998 年)。差異的誤 分類(バイアス)とは、他の変数の値に依存する方法で誤分類が発生したことを意味し、非差異 的誤分類(バイアス)とは、他の変数の値に依存しない誤分類を意味する。誤分類バイアス(差 異的または非差異的のいずれか)は、そのようなばく露を分類するために使用される試験方法の 感度と特異度に依存し、特定の(限定された)条件の下でバイアスの方向性を予測可能な影響を 及ぼすことがある。研究の方法と解析の知識に基づいてバイアスの方向を特定できるということ は、規制当局の意思決定者にとって有用である。なぜなら、意思決定者は、検討されている疫学 的効果の大きさ(例:OR、RR)が、真の効果の大きさの過小評価または過大評価である可能性 がどの程度あるかを判断することができるからである17。

size<sup>17</sup>. It is not atypical to find degrees of misclassification in the range of 10 to 20 percent and it can be helpful in reviewing epidemiological studies to consider a form of sensitivity (or "what if") analysis which evaluates such a degree of misclassification -- and whether it is differential or non-differential – and the degree to which such misclassification might impact the odds ratio or relative risk with respect to both magnitude and direction<sup>18</sup>. (p.25, FIFRA EPA SAP report, 22 April, 2010). As mentioned earlier with respect to confounding, such quantitative sensitivity analysis is only rarely performed or practiced in published epidemiology studies, with bias instead more typically evaluated in a narrative manner without any quantitative assessment of its potential magnitude and the effect it may have on the epidemiological effect size estimates (Jurek at al., 2006). This may be due – in part -- to a general lack of availability of computational tools for such analysis by epidemiologists or their unfamiliarity with them. Such tools are becoming increasingly available and may be valuable in developing more rigorous quantitative methods for evaluation of potential biases.

#### 5. Interpretation of Null studies

"Null" studies -- or well-conducted studies which report no association between exposure to the pesticide and an adverse health outcome -- will be evaluated carefully for their potential usefulness in human health risk assessment. The study may report a null result either because the investigated association indeed does not in reality exist, or because the study was conducted failed to detect an association at a given predetermined level of significance. This latter result -the failure to detect an association -- should not necessarily be interpreted to mean that no association exists, but rather as simply one was not found in the particular study<sup>19,20</sup>. To evaluate which of these two conditions may be correct when reviewing "null" studies, one should consider other research reported concerning the same or similar research question, the manner in which exposure and outcome were assessed, the extent to which exposure misclassification may have biased the study to the null, the statistical methods used including the identification and analysis of confounding variables in the association, the extent to which the exposure is below a threshold at which an effect would occur or be detected, as well as the power of the study and its ability to detect an effect size of substantive interest. Statistical power refers to the probability that researchers may correctly identify that there is a difference between the two comparison groups, i.e., there is an association between exposure and disease, when in 誤分類の程度が 10~20%の範囲にあることは異例ではなく、このような誤分類の程度(それが 差異的か非差異的か)と、そのような誤分類の大きさと方向性の両方に関してオッズ比または相 対リスクに影響を与える可能性の程度を評価する感度(または「もしも」)解析の形態を検討す ることは、疫学研究をレビューする際に有用であると考えられる<sup>17</sup>。(p.25、FIFRA EPA SAP 報告書、2010年4月22日)。交絡因子に関して前述したように、このような定量的感度分析は、 公表されている疫学研究ではほとんど実施されておらず、パイアスの潜在的な規模や疫学的効果 量推定への影響の定量的な評価は行われずに、むしろ一般的には解説文で評価されている (Jurek at al., 2006)。これは次のような理由によるものである。その理由の一部は、疫学者が このような解析のための計算ツールを一般的に利用できないことや、そのようなツールに慣れて いないことにある。このようなツールは次第に利用可能になりつつあり、潜在的なパイアスを評 価するためのより厳密な定量的手法を開発する上で有用であると考えられる。

#### 5. 帰無研究の解釈

「帰無」研究、すなわち、農薬へのばく露と有害な健康影響との間に関連付けがないと報告す るよく行われている研究は、ヒト健康リスク評価に有用な可能性があるかどうか慎重に評価され る。研究が無効という結果になるのは、調査された関連付けが実際には存在しないか、あるいは、 実施された研究が所定の有意水準で関連付けを検出することができなかったためである。後者の 結果(関連性を検出できなかった)は、必ずしも関連付けが存在しないことを意味するものでは なく、特定の研究で関連付けが検出されなかったと解釈すべきである<sup>18,19</sup>。「帰無」研究をレビ ューする際に、これら 2 つの条件のどちらが正しいかを評価するためには、同一または類似の研 究課題について報告された他の研究、ばく露と発現事象の評価方法、ばく露の誤分類が研究を帰 無に偏らせた可能性の程度、関連付けの交絡変数の同定と分析を含む使用された統計的方法、影 響が発生する、あるいは影響が検出される閾値を下回っているばく露の程度、研究の検出力と実 質的な関係のある効果量を検出する能力を考慮する必要がある。統計的検出力とは、実際に真の 違い(または関連付け)がある場合、研究者が 2 つの比較群間に差があること、すなわち、ばく 露と疾病の間に関連付けがあることを正しく識別できる確率を意味する。

<sup>&</sup>lt;sup>17</sup> The direction of bias that results from the degree of non-differential misclassification will also depend on the categorization of exposure (either dichotomous or polytomous).

<sup>&</sup>lt;sup>18</sup> Such sensitivity analyses might be especially recommended for exposure misclassification biases which in many cases are expected to result in more substantive effects on the effect size estimate than those from confounding.

<sup>&</sup>lt;sup>19</sup> The old adage that "the absence of evidence does should not be interpreted as the evidence of absence" is true here.

<sup>&</sup>lt;sup>20</sup> See also the American Statistical Association's Statement on Statistical Significance and P-values at <u>https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf</u>

<sup>17</sup> このような感度分析は、多くの場合、交絡因子よりも効果量推定に実質的な影響を及ぼすと予想されるばく露の誤分類バイアスに対して特に推奨されるかもしれない。

<sup>&</sup>lt;sup>18</sup>「エビデンスがないからといって、エビデンスがないと解釈すべきではない→エビデンスがないことを、ないことのエビデンスと解釈してはならない」という古い格言は、ここでは真実である。

<sup>&</sup>lt;sup>19</sup>米国統計協会の統計的意義と P 値に関する声明 <u>https://www.amstat.org/asa/files/pdfs/P-</u> ValueStatement.pdf も参照のこと。

fact there is in fact a true difference (or association). Studies that are "low powered" may falsely conclude there is no association, when an association actually exists<sup>21</sup>.

Finally, it is important to consider the effects of publication bias in any systematic review of the literature with respect to interpretation of null studies. The term publication bias refers to the tendency for the available published literature to disproportionately exclude such null studies. Studies that demonstrate such a "null" association between a disease or health outcome can be as equally informative as those that do provided that the study in question meets the quality criteria established as part of the epidemiological review process. These may include such factors as study design; the existence of an *a priori* hypothesis vs. an exploratory analysis; sample size and statistical power to detect an effect size of interest; proper ascertainment of outcome *vis-à-vis* sensitivity and specificity; the quality of the exposure assessment and the potential for differential and non-differential misclassification; adequacy of the measurement of key potential confounders and other forms of bias (information, selection, etc.); and evaluation of effect modifiers; appropriate statistical analyses, including consideration of and possible correction for multiple comparisons that a unsupported by a priori hypotheses, biological plausibility, or other supporting information.

#### 6. External Validity (Generalizability)

As noted above, *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). *External validity*, or *generalizability*, refers to the ability to extend the epidemiologic study results derived from a sample of the population (e.g., pesticide applicators) to other populations (e.g., all agricultural workers). To assess external validity, comparison of characteristics in the sample to the larger population (if known) can be made. Such evaluation should include not only demographic factors, but also whether exposures (e.g., dose, timing, duration) are similar and whether important effect modifiers (e.g., sensitivity of vulnerable populations) were considered. Generalizability is of particular importance because it is important to understand whether and how individual study results may be applied to the larger group or targeted sub-groups in regulatory risk assessment. For example, the AHS has reported statistical associations between some cancer and non-cancer health outcomes for some pesticide chemicals. OPP has an interest in evaluating the extent to which the reported findings may apply to pesticide applicators in states other than North Carolina and Iowa or to farm workers who primarily do post-application activities.

「低検出力」の研究は、関連付けが実際に存在しているのに、関連付けがないと誤って結論を下 すことがある<sup>20</sup>。

最後に、帰無研究の解釈に関しては、文献のシステマティックレビューにおける出版バイアス の影響を考慮することが重要である。出版バイアスとは、利用可能な公表文献が、そのような帰 無研究を不釣り合いに除外する傾向のことである。疾病または健康影響の間にこのような「帰無」 関連付けを示す研究は、研究課題が疫学的レビューブロセスの一部として確立された質の基準を 満たしていれば、その研究と同様に有益な情報を得ることができる。これらには、研究デザイン、 *優先*的仮説と探索的解析の比較、関係のある効果量を検出するためのサンプルサイズと統計的検 出力、感度と特異度に*対する*影響の適切な確認、ばく露評価の質と差異的・非差異的な誤分類の 可能性、主要な潜在的交絡因子とその他のバイアス(情報、選択等)の評価の適切性、効果修飾 因子の評価(優先的仮説による裏付けがない仮設、生物学的妥当性、またはその他の裏付け情報 についての多重比較の検討とその補正を含む適切な統計解析)などの要因が含まれる。

#### 6. 外的妥当性(一般化可能性)

上述したように、妥当性とは、一般的にはばく露推定が真のばく露レベルを反映している程度 を指す(Checkoway 6)。外的妥当性または一般化可能性とは、母集団のサンプル(例:農薬 散布者)から得られた疫学的研究結果を他の母集団(例:すべての農業従事者)に拡張する能力 のことである。外的妥当性を評価するために、サンプルの特性とより大きな集団(既知の場合) との比較を行うことができる。このような評価には、人口統計学的要因だけでなく、ばく露(例 えば、用量、時期、期間)が類似しているかどうか及び重要な影響修飾因子(例えば、脆弱者集 団の感受性)が考慮されているかどうかも含まれるべきである。一般化可能性は特に重要であり、 個々の研究結果が、規制当局のリスク評価において、より大きな集団または対象となる亜集団に 適用できるかどうか、またどのように適用できるかを理解することが重要である。例えば、AHS は、いくつかの農薬について、いくつかのがんとがん以外の健康影響との間に統計的な関連付け があることを報告している。OPP は、報告された知見が、ノースカロライナ州とアイオワ州以 外の州の農薬散布者や、主に散布後の作業を行う農業従事者にどの程度適用できるかを評価する ことに関心が持たれている。

<sup>&</sup>lt;sup>21</sup> Studies that are low-powered but find statistically significant effects may also be subject to the phenomenon of effect size magnification and this can be important to investigate as well. (Ioannidis, 2008).

<sup>&</sup>lt;sup>20</sup> 検出力は低くても統計的に有意な効果が見られる研究は、効果量の拡大という現象である可能 性があり、これを調査することが重要である(Ioannidis、2008年)。

#### V. HUMAN INCIDENT SURVEILLANCE DATA

Generally speaking, epidemiology studies on pesticides such as those described above focus on lower exposures (over a longer time period) that are less likely to result in acute clinical symptoms. OPP is also interested in exposures that are higher and occur over shorter-intervals (often on an acute "one-time" basis). This "human incident," or poisoning data can be useful for evaluating short term, high exposure scenarios that can be readily attributed to the pesticide in question.

OPP uses such "human incident information" for several purposes. Most broadly, the program uses incident data to inform risk assessment/risk management activities; this forms an integral part of our registration review activities under our Pesticide Registration Improvement Act (PRIA) responsibilities. To this end, OPP evaluates human incident data for trends over time and examines patterns in the severity and frequency of different pesticide exposures. In some cases, incident information can indicate need for additional information or additional risk management measures. Incident information can also help assess the success of risk mitigation actions after they are implemented, and incident information is an important part of OPP's performance accountability system to ensure the effectiveness of risk management actions that OPP has taken to protect human health and the environment. Lastly, incident information can be useful in providing real world use information with respect to usage practices and also in potentially targeting enforcement or educational activities, where appropriate.

OPP obtains this information from a variety of sources. Sources of human incident data include both (human) **medical case reports** appearing in the medical and toxicological literature as well as information from a variety of national **toxico-surveillance activities** for acute pesticide poisonings which are considered jointly to aid acute and chronic hazard identification and as an integral part of the risk assessment process.<sup>22</sup>

Medical case reports (first-hand accounts written by physicians) or medical case series (a compendium of medical case reports across individuals that share common source or symptomology) are valuable tools for analyzing all available evidence of health effects, and to complement the findings of animal studies and epidemiological studies. In addition, they can identify unusual or novel occurrences of an adverse health effects plausibly associated with use of a specific pesticide providing "advance notice" to the agency for toxico-vigilance purposes. Published case reports for pesticides typically describe the effects from an atypical (high exposure/dose, illegal, off-label) acute or short-term exposure. The reports are often anecdotal and can be highly selective in nature. They can, however, can be particularly valuable in identifying previously unidentified toxic effects in humans and in learning about the effects, health outcomes, and medical sequelae following high exposures. They frequently have more detailed medical information (including sequelae), detailed follow-up, and generally higher quality and/or quantitative

#### V. ヒトでの事例調査データ

一般的に言えば、上記のような農薬に関する疫学研究は、急性の臨床症状をもたらす可能性が 小さい低量ばく露(より長い期間にわたって)に焦点を当てている。OPP はまた、より高く、 より短い間隔(多くの場合、急性の"一回限り"で)のばく露に関心を持っている。この「ヒトで の事例」または中毒データは、問題となっているに起因すると考えられる短期的で高濃度の高量 ばく露シナリオを評価するのに有用である。

OPP はこのような「ヒトでの事例情報」をいくつかの目的で使用している。最も広い意味で は、OPP はリスク評価/リスク管理の活動に情報を提供するためにヒトでの事例データを使用 している。これは、農薬登録改善法(PRIA)の責任のもと、登録審査活動の重要な一部となっ ている。この目的のために、OPP は人の事例データを評価して経時的な傾向を調べ、さまざま な農薬ばく露の重大さの程度と頻度のパターンを調べている。場合によっては、事例情報は、追 加の情報や追加のリスク管理措置の必要性を示すことができる。また、事例情報は、リスク軽減 措置が実施された後の達成の評価にも役立ち、事例情報は、OPP がヒト健康と環境を保護する ために行ってきたリスク管理措置の有効性を保証するための OPP のパフォーマンス・アカウン タビリティ・システムの重要な部分である。最後に、事例情報は、使用方法に関する実際の使用 情報を提供する上で有用であり、また、必要に応じて、施行や教育の活動の対象となる可能性が ある。

OPP はこの情報を様々な情報源から入手している。ヒトでの事例データの情報源には、医学 的及び毒物学的文献に掲載されている(ヒトの)医学的症例報告や急性及び慢性のハザードの特 定を促進し、リスク評価プロセスに不可欠な共同で検討されている急性農薬中毒の様々な国の中 毒サーベイランス活動からの情報が含まれている<sup>21</sup>。

医学的症例報告(医師によって書かれた直接の証言)や医学的症例集積(共通の原因や症状を 持つ個人の症例報告をまとめたもの)は、健康影響に関する利用可能なすべてのエビデンスを解 析するための貴重なツールであり、動物試験や疫学研究の知見を補完するものでもある。さらに、 特定の農薬の使用に関連すると思われる有害な健康影響の稀な発生や新規の発生を特定すること ができ、中毒警戒の行政機関に「事前通知」を提供できる。公表されている農薬の症例報告は、 通常、非定型的な(高量ばく露/大量投与、違法、適用外)急性または短期のばく露による影響 を記述している。報告書はしばしば個々の事例に基づいており、その性質上、選択性が高い。し かし、これらの報告書は、ヒトにおいてこれまでに確認されていなかった毒性影響を特定し、高 量ばく露後の影響、健康影響及び医学的後遺症を知る上で、特に有用である。これらの研究には、 より詳細な医学的情報(後遺症を含む)や詳細な追跡調査が行われていることが多く、一般的に 用量に関する高い質及び/または定量的な情報が得られている。

<sup>&</sup>lt;sup>22</sup> OPP is aware of efforts by IPSC to consider human incident data in risk assessment. <u>http://www.who.int/ipcs/publications/methods/human data/en/index.html</u>

<sup>&</sup>lt;sup>21</sup> OPP は、IPSC がリスク評価においてヒトでの事例データを考慮する成果を認識している。 http://www.who.int/ipcs/publications/methods/human data/en/index.html

information about dose. If similarities are seen across multiple medical case studies or patterns emerge – in symptoms, exposure scenarios or usage practices -- these can provide valuable information for the risk assessment process and strengthen any findings. Medical case studies and series that include quantitative exposure information can be compared to exposure estimates in the risk assessment (which are based on labeled application rates and surrogate exposure information) to characterize margins of exposure expected from typical use, when appropriate.

The following considerations are evaluated in assessing medical case reports and medical case series:

- A detailed history of exposure (when, how, how much); time of onset of adverse effects; and signs and symptoms of the patient, are reported.
- Information on the product/chemical/pesticide, such as name, pesticide label, registration number, etc.
- Patient information (e.g. age, race, sex); underlying health conditions and use of any medications that can produce similar signs and symptoms; relevant medical history; and the presence of any risk factors.
- Description of events and how the diagnosis was made.
- Management and treatment of the patient, and laboratory data (before, during and after the therapy), including blood levels of pesticides and chemicals.
- Whether the medical report is reliable, reasonable and whether it is consistent with current knowledge, including other research, reviews and guidelines.
- Clinical course of the event and patient outcome (e.g. patient recovered and discharged from hospital; condition of patient after the discharge, any chronic health effects or premature death related to the pesticide or chemical exposure).

In addition to using medical case reports/series as a source of real-world exposure and toxicological information, OPP also engages in toxico-surveillance activities using a variety of pesticide poisoning incident databases are also available. Specifically, OPP has access to the following five human incident data sources: the *OPP Incident Data System* (IDS); the American Association of Poison Control Centers (PCC) summary reports from their *National Poison Data System* (NPDS); data from the EPA-funded *National Pesticide Information Center* (NPIC), currently at Oregon State University; the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health *Sentinel Event Notification System for Occupational Risk-Pesticides* (NIOSH SENSOR-Pesticides) and the *California Pesticide Illness Surveillance Program* (PISP). Each of these are described, in turn below:

OPP Incident Data System (IDS) is maintained by OPP and incorporates data submitted by registrants under FIFRA section 6(a)(2)<sup>23</sup>, as well as other incidents reported directly to EPA. OPP has compiled the pesticide related 複数の医療事例研究で類似点が見られたり、症状、ばく露シナリオ、使用方法などにパターン が見られた場合には、リスク評価プロセスと結果の裏付けに有用な情報を提供することができる。 医学的症例研究や定量的なばく露情報集積は、必要に応じて、典型的な使用から予想されるばく 露マージンを特性評価することで(ラベルに明示された適用率や代替ばく露情報に基づく)リス ク評価のばく露推定値と比較できる。

医学的症例報告や医学的症例集積を評価する際には、以下の点を考慮する。

ばく露の詳細な履歴(いつ、どのように、どのくらい)、健康影響の発現時期、患者の 徴候や症状が報告されている。

商品名、農薬表示、登録番号など、商品・化学品・農薬に関する情報。

患者情報(例:年齢、人種、性別);基本的な健康状態及び同様の徴候や症状をもたら す可能性のある医薬品の使用;関連する病歴及びリスク因子の有無。

疾病の説明と診断に至った経緯。

患者の管理・治療、農薬や化学物質の血中濃度などの検査データ(治療前、治療中、治 療後)。

医学的報告は信頼性があるか、妥当であるか。他の研究、レビュー、ガイドラインを含 む最新の知見と一致しているかどうか。

疾病の臨床経過及び患者の転帰(例:患者の回復及び退院;退院後の患者の状態、農薬 または化学物質へのばく露に関連した慢性的な健康影響または早期死亡)。

実際のばく露情報及び毒物学的情報の情報源として医学的症例の報告/集積を利用することに 加えて、OPP は様々な農薬中毒事例データベースを利用した中毒サーベイランス活動も行って いる。具体的には、OPP は以下の 5 つのヒトでの事例データソースにアクセスできる。OPP Incident Data System (IDS) 、American Association of Poison Control Centers (PCC) の National Poison Data System (NPDS) からの要約報告書、現在オレゴン州立大学にある EPA が資金提供している National Pesticide Information Center (NPIC) からのデータ、疾病管理 予防センター (Centers for Disease Control and Prevention) /National Institute for Occupational Safety and Health for Occupational Risk - Pesticides (NIOSH SENSOR-Pesticides) 及び California Pesticide Illness Surveillance Program (PISP) のデータ。以下、 それぞれについて順番に説明していく。

> OPP Incident Data System (IDS) は OPP が管理しており、FIFRA 第 6 条 (a)(2)<sup>22</sup>項に基づき登録者が提出したデータ及び EPA に直接報告されたその他の 事例を含んでいる。

<sup>&</sup>lt;sup>23</sup> Under FIFRA 6(a) (2), pesticide registrants are required to notify EPA if and when they become aware of "factual information regarding unreasonable adverse effects on the environment of the pesticide."

<sup>&</sup>lt;sup>22</sup> FIFRA 6(a)(2)に基づき、農薬登録者は、「農薬の環境への不適切な悪影響に関する事実情報」を知った場合には、EPA に通知するよう求められている。

incident reports in the IDS since 1992. The IDS includes reports of alleged human health incidents from various sources, including mandatory FIFRA Section 6 (a) (2) reports from registrants, other federal and state health and environmental agencies and individual consumers. IDS include information on incidents involving humans, plants, wild and domestic animals where there is a claim of an adverse effect. The vast majority of IDS reports are received by the agency in paper format. IDS entries act as a "pointers" to copies of original reports retained on microfilm and scanned images in OPP's Information Service Center.

While IDS includes both occupational and non-occupational incidents, the majority of incidents reported relate to non-occupational/residential scenarios The reports are obtained from across the U.S. and most incidents have all relevant product information (such as the EPA Registration Number) recorded. As IDS is populated mostly by information provided by pesticide registrants under their FIFRA 6(a)(2) reporting requirements, the agency has relatively high confidence in the identification of the specific product which is involved. Severity rankings are included for each incident (as specified by CFR §159.184). Symptom information is sometimes included in the narrative portion of the incident, but this information is usually not validated/confirmed by a healthcare professional. IDS also includes narrative information on exposure scenario and hazard information. Many companies use standardized, industry-developed Voluntary Incident Reporting Forms.

OPP collects and evaluates the data from the IDS and identifies potential patterns with respect to the extent and severity of the health effects due to pesticides exposure. While IDS reports are broad in scope and can in some cases contain detailed information, the system does not necessarily consistently capture detailed information about incident events, such as occupational exposure circumstances or medical outcome.

In addition, most cases data going into IDS is not validated or verified, though some reports are collected from calls to contract poison control centers. Nevertheless, incident information can provide an important post-marketing feedback loop to the agency following initial registration of the product: IDS incidents of a severe nature, or a suggested pattern or trend among less severe incidents can signal the agency to further investigate a particular chemical or product. Because IDS has such extensive coverage, it can assist in providing temporal trend information and determining whether risk mitigation has helped reduce potential pesticide exposure and decreased the number of potential incidents reported to IDS. Overall, IDS provides good information about national trends and frequency of incidents for pesticides and can provide valuable insights into the hazard and/or exposure potential of a pesticide.

OPP は 1992 年以来、IDS に農薬関連の事例報告をまとめている。IDS には、登録者、他の連邦・州の保健・環境機関及び個人消費者からの強制的な FIFRA 第6章(a)(2)報告を含む、様々な情報源から主張されたヒトでの健康影響事例の報告が含まれている。IDS には、ヒト、植物、野生動物、家畜への悪影響を主張している事例に関する情報が含まれている。IDS 報告の大部分は、政府機関が紙形式で受け取っている。IDSの項目は、OPPの情報サービスセンターにあるマイクロフィルムやスキャンした画像に保存されているオリジナルの報告書のコピーへの「ポインタ」として機能する。

IDS には職業上及び非職業上の両方の事例が含まれているが、報告された事例の 大部分は非職業/住居のシナリオに関するものである。全米から報告された事例 のほとんどは製品情報(EPA登録番号など)の記載に関連している。IDSはほと んどが FIFRA 6(a)(2)報告要件に基づき農薬登録者から提供された情報で構成さ れているため、政府機関は特定の製品を識別することに対して比較的高い信頼性 を持っている。重大さの程度の順位付けは、各事例について(CFR §159.184 で 規定されている)記載されている。症状情報は、事例の解説部分に含まれること があるが、この情報は通常、医療専門家による検証/確認を受けていない。IDS には、ばく露シナリオやハザード情報の解説情報も含まれている。多くの企業は、 標準化された、業界で開発された自主的な事例報告フォームを使用している。

OPP は IDS からデータを収集・評価し、農薬ばく露による健康影響の程度や重 大さの程度に関して潜在的なパターンを識別している。IDS の報告書は範囲が広 く、場合によっては詳細な情報を含むが、システムは必ずしも職業上ばく露状況 や医学的転帰などの発現事象に関する詳細な情報を一貫して把握しているわけで はない。

また、IDS に入る症例データのほとんどは、中毒管理センターへの電話からの収 集もあるが、確認・照合されない。とはいえ、事例情報は、製品の初期登録後、 製造販売後の重要なフィードバックルーブを政府機関に提供できる。重大な性質 の IDS 事例や、それほど重大ではない事例の間で示唆されたパターンや傾向は、 特定の化学物質や製品をさらに調査するように政府機関に求めることができる。 IDS はこのように広範囲をカバーしているため、一時的な傾向情報を提供し、リ スク軽減によって潜在的な農薬ばく露が減少し、IDS に報告された潜在的な事例 の数が減少したかどうかを判断するのに役立つ可能性がある。全体的に見ると、 IDS は農薬の全国的な傾向と事例の頻度に関する良好な情報を提供し、農薬のハ ザード及び/またはばく露の可能性について有用な予測を提供することができる。 **The National Poison Data System (NPDS)** -- formerly called the Toxic Effects Surveillance System (TESS) -- is maintained by the American Association of Poison Control Centers (AAPCC) and is supported with funding from several federal agencies. NPDS is a computerized information system with geographically specific and near real-time reporting. Although the main mission of Poison Control Centers is in helping callers respond to emergencies, NPDS data can help identify emerging problems in chemical product safety. Hotlines at 61 PCC's nationwide are open 24/7, 365 days a year and are staffed by specially trained nurses, pharmacists, and other clinical health care specialists to provide poisoning information. Using computer assisted data entry, standardized protocols, and strict data entry criteria, local callers report incidents. These reported incidents are retained locally and are updated in summary form to the national database maintained by AAPCC. Information calls are tallied separately and not counted as incidents. The PCC system covers nearly all the US and its territories and has undergone major computer enhancements since 2001.

NPDS includes mainly non-occupational incidents. NPDS does not include narrative information and the product information may not be complete. NPDS provides severity rankings and symptom information that are designated/recorded by trained specialists, and the agency has relatively high confidence in this information. NPDS also provides some information on the likelihood of the adverse effect being a result of the reported exposure. Overall, NPDS provides, as well as the hazard potential for particular pesticides. However, resource limitations permit the agency to only access AAPCC summary reports published each year (e.g., see <a href="http://www.aapcc.org/annual-reports">http://www.aapcc.org/annual-reports</a> ) and these serve as a supplement to other data sources for which the agency has more complete access.

#### □ <u>The National Pesticide Information Center (NPIC)</u>

(http://npic.orst.edu/index.html) is funded by EPA to serve as a source of objective, science-based pesticide information in response to inquiries and to respond to incidents. NPIC functions nationally during weekday business hours and is a cooperative effort between Oregon State University (currently) and EPA; it is intended to serve as a source of objective, science-based pesticide information and to respond to inquiries from the public and to incidents. Similar to Poison Control Centers, NPIC's primary purpose is not to collect incident data (about 10% of NPIC's annual calls are considered "incident" related), but rather to provide information to inquirers on a wide range of pesticide topics, and direct them to other sources for pesticide incident investigation and emergency treatment. Nevertheless, NPIC does collect information about incidents (approximately 4000 incidents per year) from inquirers and records that information in a database. NPIC is a source of national incident information, but generally receives fewer reports than IDS. Regardless, if a high frequency is observed in IDS for a given pesticide or

□ 全米中毒データシステム(NPDS)(以前は毒性影響監視システム(TESS)と 呼ばれていた)は、米国中毒管理センター協会(AAPCC)によって維持されて おり、いくつかの連邦政府機関からの資金援助を受けている。NPDSはコンピュ ータ化された情報システムで、地理的に特定されたほぼリアルタイムの報告が可 能である。中毒管理センターの主な任務は、緊急事態に対応する通報者を支援す ることであるが、NPDSのデータは、化学製品の安全性に関する新たな問題を特 定するのに役立つ。全国にある61の PCCのホットラインは、365日24時間年 中無休で開設されており、特別な訓練を受けた看護師、薬剤師及びその他の臨床 医療専門家が中毒情報を提供している。コンピュータによるデータ入力、標準化 されたプロトコル、厳格なデータ入力基準を使用して、現地の通報者が事例を報 告する。これらの報告された事例は現地で保存され、AAPCCによって維持され ている全国データベースに要約された形で更新される。情報通報は個別に集計さ れ、事例としてはカウントされない。PCCシステムは、ほぼすべての米国とそ の領土をカバーしており、2001年以来、コンピュータの大幅な強化が行われて きた。

NPDSには主に非職業上の事例が含まれている。NPDSには解説情報は含まれて おらず、製品情報は完全ではない。NPDSは、訓練を受けた専門家が指定/記録 した重大さの程度の順位と症状情報を提供しており、政府機関はこれらの情報に 対して比較的高い信頼性を持っている。NPDSはまた、報告されたばく露による 健康影響の可能性に関する情報も提供している。全体として、NPDSは全国的な 傾向、農薬の事例発生頻度、特定の農薬の潜在的ハザードについての良好な情報 を提供している。しかし、情報源の制限により、毎年発行される AAPCCのサマ リーレポート(例:<u>http://www.aapcc.org/annualreports/を参照)</u>にしかアク セスできず、これらのレポートは他の情報源を補完する役割を果たしているため、 NPDSはより完全なアクセスが可能となっている。

#### National Pesticide Information Center (NPIC)

(http://npic.orst.edu/index.html)は、EPA の資金提供を受けており、客観的で科 学的根拠に基づいた農薬情報を提供し、問い合わせに対応したり、事例に対応し たりすることを目的としている。NPIC は平日の営業時間内に全国的に機能して おり、オレゴン州立大学(現在)と EPA との間で協力している。中毒管理セン ターと同様に、NPIC の主な目的は事例データの収集ではなく(NPIC の年間の 問い合わせの約 10%は「事例」に関連していると考えられている)、むしろ幅 広い農薬に関する情報を問い合わせ者に提供し、農薬での事例の調査や緊急処置 のための他の情報源に誘導することである。とはいえ、NPIC では問い合わせる からの事例情報(年間約 4000 件)を収集し、データベースに記録している。 NPIC は全国の事例の情報源であるが、一般的に IDS に比べて報告件数は少ない。 product, NPIC provides a source of information that can prove valuable in determining consistency across national data sets.

As with IDS and PCC, the incidents in NPIC are mainly non-occupational. NPIC incidents include narratives and product information when the caller provides the information. Although the scope is national, there are significantly fewer incidents reported to NPIC than to NPDS or IDS but considerably more information is provided and the agency can request custom reports on an as-needed basis. Hazard information includes severity rankings, route of exposure and symptoms – which are recorded by trained personnel. NPIC also provides information on how likely the link between exposure and adverse effect is (which they call a certainty index). NPIC also publishes annual reports and analyses in the open literature which are valuable resources.

The Center for Disease Control and Prevention National Institute for Occupational Health (CDC/NIOSH) manages a pesticide surveillance program and database entitled the Sentinel Event Notification System for Occupational Risk (SENSOR)-Pesticides.<sup>24</sup> This database includes pesticide illness case reports in 12 states from 1998-2013. Participating states are: California, Florida, Iowa, Louisiana, Michigan, Nebraska, New Mexico, New York, North Carolina, Oregon, Texas and Washington. The participating states for a given year vary depending on state and federal funding for pesticide surveillance.

Cases of pesticide-related illnesses in the SENSOR-Pesticides database are ascertained from a variety of sources, including: reports from local Poison Control Centers, state Department of Labor workers' compensation claims when reported by physicians, reports from state Departments of Agriculture, and physician reports to state Departments of Health. Although both occupational and non-occupational incidents are included in the database, the SENSOR coordinators primarily focus their follow-up case investigation efforts on the occupational pesticide incidents. The SENSOR coordinator at the state Department of Health will follow-up with cases and work to obtain medical records in order to verify exposure scenario, symptoms, severity, and health outcome. Using standardized protocol and case definitions, SENSOR coordinators at state Departments of Health enter the incident interview description provided by the case, medical report, physician and patient into the SENSOR data system.

All SENSOR-Pesticides cases must report a minimum of two health effects in order to be included in the aggregate database that EPA uses for incident

特定の農薬や製品が IDS で高頻度に観察された場合でも、NPIC は全国のデータ セット間の整合性を判断する上で有用な情報源を提供している。

IDSやPCCと同様に、NPICの事例は主に非職業上のものである。NPICの事例 には、通報者が情報を提供する際に解説情報や製品情報が含まれている。対象範 囲は全国であるが、NPICに報告される事例の数は NPDSや IDSに比べてかな り少ない。一方、提供される情報量はかなり多く、必要に応じて政府機関がカス タムレポートを要求することも可能である。ハザード情報には、重大さの程度の 順位付け、ばく露経路、症状などが含まれており、これらは訓練を受けた担当者 によって記録される。また、ばく露と有害影響の関連性がどの程度であるかにつ いての情報も提供している(これを確実性指数と呼んでいる)。また、NPIC は 年次報告や解析結果を公開しており、有用な情報源となっている。

□ 疾病管理予防国立産業衛生研究所(CDC/NIOSH)は、SENSOR(Sentinel Event Notification System for Occupational Risk) - Pesticides と題した農薬監 視プログラムとデータベースを管理している<sup>23</sup>。このデータベースには、1998年 から2013年までの12の州における農薬による疾病症例報告が含まれている。参 加している州は、カリフォルニア州、フロリダ州、アイオワ州、ルイジアナ州、 ミシガン州、ネブラスカ州、ニューメキシコ州、ニューヨーク州、ノースカロラ イナ州、オレゴン州、テキサス州、ワシントン州である。ある年の参加州は、州 と連邦政府が農薬監視のために資金を提供しているかどうかによって異なる。

SENSOR・農薬データベース内の農薬関連の疾病症例は、様々な情報源(現地の 中毒管理センターからの報告、医師によって報告された際に農業従事者の補償請 求の州労働局からの報告、州農業局からの報告及び州保健局への医師の報告)か ら把握されている。職業上及び非職業上の事例の両方がデータベースに含まれて いるが、SENSOR 責任者は、主に職業上の農業従事者事例に関する追跡症例調 査の成果に焦点を当てている。州保健局の SENSOR 責任者は、症例を追跡し、 ばく露シナリオ、症状、重大さの程度、健康影響を確認するために、カルテを取 得する。標準化されたプロトコルと症例の定義を使用して、州保健局の SENSOR 責任者は、症例、カルテ、医師と患者によって提供された事例インタ ビュー解説を SENSOR データシステムに入力する。

すべての SENSOR-Pesticides の症例は、EPA が事例の解析に使用する集合デー タベースに含まれるために、最低2つの健康影響を報告しなければならない。

<sup>&</sup>lt;sup>24</sup> SENSOR-Pesticides webpage: <u>http://www.cdc.gov/niosh/topics/pesticides/overview.html</u>

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<sup>&</sup>lt;sup>23</sup> SENSOR-Pesticides $\mathcal{O}$   $\pi - \Delta \sim - \vec{\mathcal{V}}$ : http://www.cdc.gov/niosh/topics/pesticides/overview.html 41 $\sim - \vec{\mathcal{V}}$ 

analyses. Evidence for each case is evaluated, based on the NIOSH case classification matrix, for its causal relationship between exposure and illness. 98% of SENSOR-Pesticides cases are classified as definite, probable, or possible, and 2% of the cases are classified as suspicious. Unlikely, asymptomatic, and unrelated cases, as well as those with insufficient information, are not included in the SENSOR-Pesticides database.

Overall, SENSOR-Pesticides provides very useful information on both occupational and non-occupational incidents, and sometimes valuable insights into the hazard and/or exposure potential of a pesticide. SENSOR-Pesticides also conducts analyses of its own data and publishes these in the Morbidity and Mortality Weekly. Unlike the aforementioned databases and although it contains both non-occupational/residential and occupational incidents, SENSOR's has traditionally focused on occupational pesticide incidents, and is of particular value in providing that information. SENSOR-Pesticides data from 1998-2011 is available online at: <a href="http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides">http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides</a>.

The California Pesticide Illness Surveillance Program (PISP) is maintained by the State of California. This database documents pesticide-related illnesses and injuries. Case reports are received from physicians and via workers' compensation records. The local County Agricultural Commissioner investigates the circumstances of the exposure. Medical records and investigative findings are then evaluated by California's Department of Pesticide Regulation (DPR) technical experts and entered into an illness registry. All reported pesticide illnesses in the California PISP program are investigated by the county agricultural commissioners, and the DPR evaluates the reports and compiles them into a database, which is used to improve the state's program to protect workers and others from the adverse effects of pesticide exposure (http://apps.cdpr.ca.gov/calpig/).

Currently, OPP evaluates human incident data on a chemical-specific basis. Incidents from each database are analyzed for hazard potential (deaths, frequency of more severe incidents, and patterns/trends of reported symptoms) and exposure potential (frequency of incidents/ trends over time, patterns/trends of exposure scenarios, of factors affecting exposure or of products). When evaluating human incident data from the above databases, OPP considers several general criteria. OPP considers the relative severity and frequency of symptoms. Additionally, OPP generally has greater confidence in reports in which temporal association can be verified or are at least plausible. Lastly, other factors that are used to evaluate human incident data include evidence of an exposure response association, consistency in reported health effects, biological plausibility of reported health effects, elimination of alternative causes of health effects. Additionally, narratives of more severe incidents are often evaluated for any temporal association between time-of-exposure and effects reported to determine whether an association is supported by the circumstances. For example, a heart attack in an elderly individual that occurs three

各症例のエビデンスは、NIOSH の症例分類マトリックスに基づいて、ばく露と 疾病との因果関係について評価される。SENSOR-Pesticides の症例の 98%は、 確定、可能性高い、または可能性ありに分類され、2%は疑わしい症例に分類さ れている。可能性の低い症例、無症候性の症例、無関係の症例及び情報が不十分 な症例は、SENSOR-Pesticides のデータベースには含まれ いない。

全体的には、職業上及び非職業上の事例について非常に有用な情報を提供しており、時には農薬のハザードやばく露の可能性についての有用な予想を提供している。また、SENSOR-Pesticides は独自のデータの解析を行い、Morbidity and Mortality Weekly に公表している。前述のデータベースとは異なり、非職業上/住居上と職業上の両方の事例が含まれているが、SENSOR'S は伝統的に農業従事者での事例に焦点を当てており、その情報を提供する上で特に価値のあるものとなっている。1998 年から 2011 年までの SENSOR-Pesticides のデータはオンラインで入手可能である:http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides。

□ カリフォルニア州農薬疾病サーベイランスプログラム(PISP) は、カリフォル ニア州によって管理されている。このデータベースは、農薬に関連した疾病や傷 害を記録している。症例報告は、医師からの報告と農業従事者補償記録を介して 行われる。現地の郡農業委員会がばく露の状況を調査する。カルテと調査結果は、 カリフォルニア州農薬規制局(DPR)の技術専門家によって評価され、疾病の登 録簿に登録される。カリフォルニア州の PISP プログラムで報告されたすべての 農薬による疾病は、郡の農業委員会によって調査され、DPR は報告書を評価し てデータベースにまとめ、農業従事者やその他の人々を農薬ばく露の悪影響から 保護するための州のプログラムを改善するために使用される (http://apps.cdpr.ca.gov/calpiq/)。

現在、OPP は化学物質別にヒトの事例データを評価している。各データベースからの事例は、 ハザードボテンシャル(死亡者数、より重大な事例の頻度、報告された症状のパターン/傾向) とばく露ボテンシャル(事例の頻度/経時的傾向、ばく露シナリオのパターン/傾向、ばく露に 影響を与える要因のパターン/傾向、または製品のパターン/傾向)について解析されている。 上記のデータベースからのヒトでの事例データを評価する際、OPP はいくつかの一般的な基準 を考慮している。OPP は症状の相対的な重大さの程度と頻度を考慮している。さらに、OPP は 一般的に、時間的な関連付けが確認できる報告を、あるいは少なくとも妥当と思われる報告を、 より信頼性の高いものとしている。最後に、ヒトの事例データを評価するために使用される他の 要因には、ばく露反応の関連付けのエビデンス、報告された健康影響の一貫性、報告された健康 影響の生物学的妥当性、医薬品の使用などの健康影響の代替原因の排除及び観察された症状や健 康影響の特異度が含まれる。さらに、より重大な事例の解説は、ばく露の時間と報告された影響 の間に時間的な関連付けがあるかどうかを評価し、関連付けが状況によって裏付けられているか どうかを判断することが多い。 months following an indoor pesticide application may be determined not to be a likely causal association. On the other hand, a severe incident occurring at or shortly after the time of exposure with symptoms consistent with known symptomology for the pesticide class and that occurs without prior medical history may suggest that causal inference is more justified.

In sum, then, incident data -- consisting of both medical case reports/case series appearing the medical and human toxicological literature and toxico-surveillance data derived from the databases that EPA either maintains, funds, or accesses -- can provide useful, complementary information that assists OPP in evaluating the real-world risks of pesticides.

#### VI. SUMMARY & CONCLUSIONS

This framework describes important factors in reviewing epidemiology and human incident data and describes a proposed WOE analysis for incorporating such data in pesticide human health risk assessment. OPP uses the best available data across multiple lines of evidence and from *in vitro*, *in vivo*, and *in silico* data sources. OPP uses a WOE approach when integrating data from multiple sources to take into account for quality, consistency, relevancy, coherence and biological plausibility using modified Bradford Hill criteria as an organizational tool. Application of WOE analysis is an integrative and interpretive process routinely used by EPA according to in scientific analysis outlined in its risk assessment guidelines. The WOE analysis also evaluates the quality of the combined data set and is consistent with the level of effort and complexity that is appropriate for a particular scientific assessment (U.S. EPA, 2002). OPP acknowledges that toxicology and risk assessment are currently undergoing transformational changes towards implementing the new vision of 21<sup>st</sup> century toxicity testing. As these transformation changes occur, OPP will update this approach as appropriate.

例えば、屋内での農薬散布後3か月に発生した高齢者の心臓発作は、因果関係があるとは判断されない。一方で、農薬種類に応じた既知の症状と一致する症状を伴うばく露時またはばく露後間もなく発生した重大な事例は、事前の病歴がなく発生した場合、因果関係の推測がより正当化されることを示唆している。

以上のことから、医学的文献やヒトの毒性学的文献に掲載されている医学的症例報告/症例シ リーズと、EPA が維持、資金提供またはアクセスしているデータベースから得られる中毒サー ベイランスデータの両方からなる事例データは、実社会での農薬のリスクを評価する際に OPP を支援する有用で補完的な情報を提供することが可能である。

#### VI. 要約と結論

このフレームワークでは、疫学とヒトでの事例データをレビューする際の重要な要素を説明し、 そのようなデータを農薬のヒト健康リスク評価に組み込むために提案されている WOE 解析につ いて説明している。OPP では、複数のエビデンスと、*in vitro、in vivo*及び*in silico*の情報源か ら得られた入手可能な最善のデータを使用する。OPP では、複数の情報源からのデータを統合 する際に、系統化されたツールとして修正された Bradford Hill 基準を用いて、品質、一貫性、 関連性、整合性、生物学的妥当性を考慮するため、WOE アプローチを使用している。WOE 解 析の適用は、リスク評価ガイドラインに概説されている科学的解析に従って EPA が日常的に使 用している統合的かつ解釈的なプロセスである。WOE 解析はまた、結合されたデータセットの 質も評価し、特定の科学的評価に適切である成果と複雑性のレベルと一致している(U.S.EPA、 2002 年)。OPP は、21 世紀の毒性試験の新たなビジョンの実施に向けて、現在、毒性学とリス ク評価が変革期にあることを認識している。これらの変革に伴い、OPP はこのアプローチを適 宜更新して行くであろう。

#### VII. REFERENCES

American Statistical Association. 2016 "AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND P-VALUES" March 7. Available at: https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf

Ankley. GT, Bennett RS, Erickson RJ et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29(3):730–741.

Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, and Guyatt GH. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401-6. doi: 10.1016/j.jclinepi.2010.07.015. Epub 2011 Jan 5.

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W, Steen W, Dosemeci M, and Alavanja M. 2002. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Ann Epidemiol. Oct 1;10(7):478.

Borenstein M, Hedges LV, Higgins JPT, and Rothstein HR. 2009. Introduction to Metaanalysis. John Wiley and Sons, Chichester, UK.

Boyes WK, Moser VC, Geller AM, Benignus VA, Bushnell PJ, and Kamel F. 2007. Integrating epidemiology and toxicology in neurotoxicity risk assessment. Hum. Exp. Toxicol. 26(4):283-93.

Calderon RL 2000. Measuring risks in humans: the promise and practice of epidemiology. Food and Chemical Toxicology. 38:S59-S63.

Carlile DJ, Zomorodi, K , and Houston, JB. 1997. Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab. Dispos. 25(8):903-911.

Checkoway H, Pearce, N, and Kriebel D. 2004. Research Methods in Occupational Epidemiology, 2<sup>nd</sup> Edition. Oxford University Press, New York.

Clark LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologicallybased pharmacokinetic models for use in safety or risk assessment. Risk Anal. 24(6):1697-1717.

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

#### VII. 参考文献

American Statistical Association. 2016 "AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND P-VALUES" March 7. Available at: https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf

Ankley, GT, Bennett RS, Erickson RJ et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29(3):730-741.

Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, and Guyatt GH. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401-6. doi: 10.1016/j.jclinepi.2010.07.015. Epub 2011 Jan 5.

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W, Steen W, Dosemeci M, and Alavanja M. 2002. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Ann Epidemiol. Oct 1;10(7):478.

Borenstein M, Hedges LV, Higgins JPT, and Rothstein HR. 2009. Introduction to Metaanalysis. John Wiley and Sons, Chichester, UK.

Boyes WK, Moser VC, Geller AM, Benignus VA, Bushnell PJ, and Kamel F. 2007. Integrating epidemiology and toxicology in neurotoxicity risk assessment. Hum. Exp. Toxicol. 26(4):283-93.

Calderon RL 2000. Measuring risks in humans: the promise and practice of epidemiology. Food and Chemical Toxicology. 38:S59-S63.

Carlile DJ, Zomorodi, K, and Houston, JB. 1997. Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab. Dispos. 25(8):903-911.

Checkoway H, Pearce, N, and Kriebel D. 2004. Research Methods in Occupational Epidemiology, 2nd Edition. Oxford University Press, New York.

Clark LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologicallybased pharmacokinetic models for use in safety or risk assessment. Risk Anal. 24(6):1697-1717.

FIFRA Scientific Advisory Panel. (2010). February 2 · 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

Glymor, MM and Greenland, S. 2012. "Causal Diagrams" in Rothman, KJ, Greenland, S, Poole, C, Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 183-212.

Gordis, L. 2009. Epidemiology. 4th Edition. Saunders-Elsevier, New York.

Greenland, S. 1998. "Basic Methods for Sensitivity Analysis and External Adjustment" in Rothman, KJ and Greenland, S. Modern Epidemiology. 2<sup>nd</sup> ed. Lippencott-Raven Publishers, Philadelphia. pp.343-357.

Greenland, S. and Lash T. 2012. "Bias Analysis" in Rothman KJ, Greenland S, Poole C, Lash TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 345-380.

Greenland, S and O'Rourke, K. 2012. "Meta-analysis" in Rothman, KJ, Greenland S, Poole C, and Lash, TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 652-682.

Grimes, DA and Schultz, KF. 2005. Compared to What? Finding controls for case-control studies. Lancet 365: 1429-1433.

<u>Gustafson P<sup>1</sup></u>, and <u>McCandless LC</u>. 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. <u>Int J Environ Res Public Health</u>. 2010 Apr;7(4):1520-39. doi: 10.3390/ijerph7041520..

Hartung T. 2010. Evidence-based toxicology - the toolbox of validation for the 21st century? <u>ALTEX</u>. 2010;27(4):253-63.

Hertz-Picciotto I. 1995. Epidemiology and quantitative risk assessment: a bridge from science to policy. American Journal of Public Health. 85(4): 484-491.

Hill AB. 1965. The Environment and Disease: Association or Causation? President's Address. Proceedings of the Royal Society of Medicine 58:293-300

Hoppin JA, Yucel F, Dosemeci M, and Sandler DP. 2002. Accuracy of self-reported pesticide use duration information for licensed pesticide applicators in the Agricultural Health Study. Journal of Exposure Analysis and Environmental Epidemiology, 12: 313-318.

IPCS (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration response assessment. Harmonization Project Document 2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.

Ioannidis JP. 2008. Why Most Discovered True Associations Are Inflated. Epidemiology. 19(5): 640-8.

Glymor, MM and Greenland, S. 2012. "Causal Diagrams" in Rothman, KJ, Greenland, S, Poole, C, Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 183-212.

Gordis, L. 2009. Epidemiology. 4th Edition. Saunders-Elsevier, New York.

Greenland, S. 1998. "Basic Methods for Sensitivity Analysis and External Adjustment" in Rothman, KJ and Greenland, S. Modern Epidemiology. 2nd ed. Lippencott-Raven Publishers, Philadelphia. pp.343-357.

Greenland, S. and Lash T. 2012. "Bias Analysis" in Rothman KJ, Greenland S, Poole C, Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 345-380.

Greenland, S and O'Rourke, K. 2012. "Meta-analysis" in Rothman, KJ, Greenland S, Poole C, and Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 652-682.

Grimes, DA and Schultz, KF. 2005. Compared to What? Finding controls for case-control studies. Lancet 365: 1429-1433.

Gustafson P1, and McCandless LC. 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. Int J Environ Res Public Health. 2010 Apr;7(4):1520-39. doi: 10.3390/ijerph7041520.

Hartung T. 2010. Evidence-based toxicology  $\cdot$  the toolbox of validation for the 21st century? ALTEX. 2010;27(4):253-63.

Hertz-Picciotto I. 1995. Epidemiology and quantitative risk assessment: a bridge from science to policy. American Journal of Public Health. 85(4): 484-491.

Hill AB. 1965. The Environment and Disease: Association or Causation? President's Address. Proceedings of the Royal Society of Medicine 58:293:300

Hoppin JA, Yucel F, Dosemeci M, and Sandler DP. 2002. Accuracy of self-reported pesticide use duration information for licensed pesticide applicators in the Agricultural Health Study. Journal of Exposure Analysis and Environmental Epidemiology, 12: 313-318.

IPCS (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration response assessment. Harmonization Project Document 2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.

Ioannidis JP. 2008. Why Most Discovered True Associations Are Inflated. Epidemiology. 19(5): 640-8.

Jurek AM, Maldonado G, Greenland G, and Church TR. 2006. Exposure-measurement Error is Frequently Ignored When Interpreting Epidemiological Study Results. Europ. J. Epid. 21: 871-876.

Kelsey JL, Whittemore AS, Evans AS, and Thompson WD. 1996. Methods in Observational Epidemiology. 2<sup>nd</sup> ed. Oxford University Press, New York.

LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, and Weisel CP. 2014. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. <u>Environ Int.</u> Dec;73:195-207. doi: 10.1016/j.envint.2014.07.011.

Lash, TL, Fox, MP, and Fink, AK. 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, and Greenland S. 2014. Good practices for quantitative bias analysis. International Journal of Epidemiology p. 1-17.

Lilienfeld AM and Lilienfeld D. 1979. Foundations of epidemiology,  $2^{nd}$  ed. Oxford University Press, New York.

Mausner JS and Kramer S. 1985. Epidemiology, 2<sup>nd</sup> ed. W.B. Saunders, Philadelphia.

Meek, ME, Bucher, JR, Cohen, SM et al. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33:591-653.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

Muñoz-Quezada MT<sup>1</sup>, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, and Vega C. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology. Dec;39:158-68. doi: 10.1016/j.neuro.2013.09.003.

Needham LL, Calafat AM, and Barr DB. 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210: 229-238.

Nieuwenhuijsen MJ. 2003. Exposure Assessment in Occupational and Environmental Epidemiology. Oxford University Press, New York.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC.

Jurek AM, Maldonado G, Greenland G, and Church TR. 2006. Exposure-measurement Error is Frequently Ignored When Interpreting Epidemiological Study Results. Europ. J. Epid. 21: 871-876.

Kelsey JL, Whittemore AS, Evans AS, and Thompson WD. 1996. Methods in Observational Epidemiology. 2nd ed. Oxford University Press, New York.

LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, and Weisel CP. 2014. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environ Int. Dec;73:195-207. doi: 10.1016/j.envint.2014.07.011.

Lash, TL, Fox, MP, and Fink, AK. 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, and Greenland S. 2014. Good practices for quantitative bias analysis. International Journal of Epidemiology p. 1-17. Lilienfeld AM and Lilienfeld D. 1979. Foundations of epidemiology, 2nd ed. Oxford University Press, New York.

Mausner JS and Kramer S. 1985. Epidemiology, 2nd ed. W.B. Saunders, Philadelphia.

Meek, ME, Bucher, JR, Cohen, SM et al. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33:591-653.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

Muñoz-Quezada MT1, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, and Vega C. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology. Dec:39:158-68. doi: 10.1016/j.neuro.2013.09.003.

Needham LL, Calafat AM, and Barr DB. 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210: 229-238.

Nieuwenhuijsen MJ. 2003. Exposure Assessment in Occupational and Environmental Epidemiology. Oxford University Press, New York.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC.

NRC (National Research Council). 1991. Environmental Epidemiology, Volume 1: Public Health and Hazardous Wastes. National Academy Press, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 1997. Environmental Epidemiology, Volume 2: Use of the Gray Literature and Other Data in Environmental Epidemiology. National Academy Press, Washington, DC.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press, Washington, DC.

NRC (National Research Council). 2009: Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. National Academies Press, Washington, DC. http://www.nap.edu/catalog/13142.html

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) process. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record\_id=18764

Ntzani EE, Chondrogiori MNG, Evangelou E and Tzoulaki I. 2013. Literature review of epidemiological studies linking exposure to pesticides and health effects. External Scientific Report. EFSA supporting publication 2013-EN-497. 159 pp. Available online at <a href="http://www.efsa.europa.eu/publications">www.efsa.europa.eu/publications</a>.

Organisation for Economic Co-operation and Development. 2013. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, ENV/JM/MONO(2013)6, April 17, 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono( 2013)6&doclanguage=en

Paddle GM, and Harrington JM. 2000. Environmental epidemiology--strengths and weaknesses. Int Arch Occup Environ Health. 73:7-14.

Porta MJM. 2014. A Dictionary of Epidemiology. 6th ed. Oxford University Press, New York.

Purdue Pesticides Programs. 2003. Pesticides and Epidemiology: Unraveling Disease Patterns. Purdue University Cooperative Extension Service. http://www.btny.purdue.edu/Pubs/PPP/PPP-43.pdf.

Rothman KJ and Greenland S. 2012. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

NRC (National Research Council). 1991. Environmental Epidemiology, Volume 1: Public Health and Hazardous Wastes. National Academy Press, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 1997. Environmental Epidemiology, Volume 2: Use of the Gray Literature and Other Data in Environmental Epidemiology. National Academy Press, Washington, DC.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press, Washington, DC.

NRC (National Research Council). 2009: Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. National Academies Press, Washington, DC. http://www.nap.edu/catalog/13142.html

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) process. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record\_id=18764

Ntzani EE, Chondrogiori MNG, Evangelou E and Tzoulaki I. 2013. Literature review of epidemiological studies linking exposure to pesticides and health effects. External Scientific Report. EFSA supporting publication 2013-EN-497. 159 pp. Available online at www.efsa.europa.eu/publications.

Organisation for Economic Co-operation and Development. 2013. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, ENV/JM/MONO(2013)6, April 17, 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6 &doclanguage=en

Paddle GM, and Harrington JM. 2000. Environmental epidemiology--strengths and weaknesses. Int Arch Occup Environ Health. 73:7-14.

Porta MJM. 2014. A Dictionary of Epidemiology. 6th ed. Oxford University Press, New York.

Purdue Pesticides Programs. 2003. Pesticides and Epidemiology: Unraveling Disease Patterns. Purdue University Cooperative Extension Service. http://www.btny.purdue.edu/Pubs/PPP/PPP-43.pdf.

Rothman KJ and Greenland S. 2012. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

Rothman, KJ, Greenland S, Poole C, and Lash TL. 2012a. "Causation and Causal Inference" in Rothman, KJ, Greenland S, Poole C, and Lash TL. Modern epidemiology. 3<sup>rd</sup> ed. Lippincott Williams & Wilkins, Philadelphia. pp. 5-31.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. Jul;122(7):711-8. doi: 10.1289/ehp.1307972.Seed, J., E.W.

Carney, RA, Corley, et al. 2005. Overview: Using mode of action and lifestage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672.

Schultz, KF and Grimes DA. 2002. Case-control studies: research in reverse. Lancet: 359:431-434.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Sterne JAC, Higgins JPT, and Reeves, BC. on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for non-randomized studies of interventions (ACROBAT-NRSI), Version 1.0. 0, 24 September 2014." *www. riskofbias. info* (2015)

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Bennie D, Moher D, Becker BJ, Sipe TA, and Thacker SB for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. 2000. J. American Medical Association 283(15): 2008-2012.

Szklo M and Nieto FJ. 2004. Epidemiology: Beyond the Basics. Jones and Bartlett Publishers, Boston, MA.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Science Policy Council Handbook: Risk Characterization. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/100/B-00/002. Available at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2001. General Principles For Performing Aggregate Exposure And Risk Assessments Washington, DC. Available at <a href="https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf">https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf</a>

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Rothman, KJ, Greenland S, Poole C, and Lash TL. 2012a. "Causation and Causal Inference" in Rothman, KJ, Greenland S, Poole C, and Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 5-31.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. Jul;122(7):711-8. doi: 10.1289/ehp.1307972.Seed, J., E.W.

Carney, RA, Corley, et al. 2005. Overview: Using mode of action and lifestage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672.

Schultz, KF and Grimes DA. 2002. Case-control studies: research in reverse. Lancet: 359:431-434.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Sterne JAC, Higgins JPT, and Reeves, BC. on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for non-randomized studies of interventions (ACROBAT-NRSI), Version 1.0. 0, 24 September 2014." *www. riskofbias. info* (2015)

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Bennie D, Moher D, Becker BJ, Sipe TA, and Thacker SB for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. 2000. J. American Medical Association 283(15): 2008-2012.

Szklo ${\rm M}$  and Nieto FJ. 2004. Epidemiology: Beyond the Basics. Jones and Bartlett Publishers, Boston, MA.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Science Policy Council Handbook: Risk Characterization. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/100/B-00/002. Available at <u>http://www.epa.gov/iris/backgr-d.htm</u>.

U.S. EPA (U.S. Environmental Protection Agency). 2001. General Principles For Performing Aggregate Exposure And Risk Assessments Washington, DC. Available at <a href="https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf">https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf</a>

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. U.S. EPA (U.S. Environmental Protection Agency). 2002a. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity for Information Disseminated by the Environmental Protection Agency. Office of Environmental Information, Washington, DC. EPA/260/R-02/008. Available at

http://www.epa.gov/quality/informationguidelines/documents/EPA InfoQualityGuidelines.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 2002b. A Review of the Reference Dose and Reference Concentration Processes. December. Risk Assessment Forum. Washington, DC. EPA/630/P-02/002F.

U.S. Environmental Protection Agency. 2002c. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"; January 14, 2002. U.S. EPA (U.S. Environmental Protection Agency). 2004. An Examination of EPA Risk Assessment Principles & Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science Advisor. U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at <a href="http://www.epa.gov/raf">http://www.epa.gov/raf</a>.

U.S. EPA. (U.S. Environmental Protection Agency). 2006a. Harmonization in Interspecies Extrapolation: Use of BW<sup>3/4</sup> as Default Method in Derivation of the Oral RfD (External Review Draft). U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-06/001. Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525.

U.S. EPA (U.S. Environmental Protection Agency). 2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Scientific Issues Associated with Field Volatilization of Conventional Pesticides. U.S. Environmental Protection Agency, Washington, DC. OPP Regulatory Public Docket EPA-HQ-OPP-2009-0687.

U.S. EPA (U.S. Environmental Protection Agency). 2010. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. EPA (U.S. Environmental Protection Agency). 2012. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment U.S. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2002a. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity for Information Disseminated by the Environmental Protection Agency. Office of Environmental Information, Washington, DC. EPA/260/R-02/008. Available at

http://www.epa.gov/quality/informationguidelines/documents/EPA\_InfoQualityGuidelines.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 2002b. A Review of the Reference Dose and Reference Concentration Processes. December. Risk Assessment Forum. Washington, DC. EPA/630/P-02/002F.

U.S. Environmental Protection Agency. 2002c. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"; January 14, 2002. U.S. EPA (U.S. Environmental Protection Agency). 2004. An Examination of EPA Risk Assessment Principles & Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science

Advisor, U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at http://www.epa.gov/raf.

U.S. EPA. (U.S. Environmental Protection Agency). 2006a. Harmonization in Interspecies Extrapolation: Use of BW3/4 as Default Method in Derivation of the Oral RfD (External Review Draft). U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-06/001. Available at <a href="http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525">http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525</a>.

U.S. EPA (U.S. Environmental Protection Agency). 2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Scientific Issues Associated with Field Volatilization of Conventional Pesticides. U.S. Environmental Protection Agency, Washington, DC. OPP Regulatory Public Docket EPA-HQ-OPP-2009-0687.

U.S. EPA (U.S. Environmental Protection Agency). 2010. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. EPA (U.S. Environmental Protection Agency). 2012. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment U.S. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014a. Framework for Human Health Risk Assessment to Inform Decision Making.

https://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. <u>http://www2.epa.gov/osa/guidance-applying-quantitativedata-develop-data-derived-extrapolation-factors-interspecies-and</u>

U.S. EPA (U.S. Environmental Protection Agency). 2015. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA. https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8C C/\$File/ISA\_PREAMBLE\_FINAL2015.PDF

van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A, and Goldbohm A. 2002. The contribution of epidemiology. Food Chem Toxicol. Feb-Mar;40(2-3): 387-424.

Vandenbroucke JP, Van Elm E, Altman DG, Gotzsche PC, Mulroew CD, Pockock SJ, Pool C, Schlesseman JJ, and Egger, M. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. Ann. Int. Med. Vol 147(8): W163-193

Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, and Vandenbroucke JP. 2014. The Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. Int. J. Surgery 12: 1495-99.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992a. Selection of Controls in Case-Control Studies I. Principles. American J. Epid. 135(9): 1019-1028.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992b. Selection of Controls in Case-Control Studies II. Types of Controls. American J. Epid. 135(9): 1029-1041.

Wacholder, S, McLaughlin, JK, Silverman, DT, and Mandel, JS. 1992c. Selection of Controls in Case-Control Studies III. Design Options. American J. Epid. 135(9): 1042-1050.

<u>Woodruff TJ</u><sup>1</sup> and <u>Sutton P</u>. 2014. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. <u>Environ Health Perspect</u>. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

Zartarian V., Bahadori T, and McKone T. 2005. Adoption of an official ISEA glossary. Journal of Exposure Analysis and Environmental Epidemiology 15:1-5

U.S. EPA (U.S. Environmental Protection Agency). 2014a. Framework for Human Health Risk Assessment to Inform Decision Making. https://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. <u>http://www2.epa.gov/osa/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and</u>

U.S. EPA (U.S. Environmental Protection Agency). 2015. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA.

 $https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8CC/\$File/ISA_PREAMBLE_FINAL2015.PDF$ 

van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A, and Goldbohm A. 2002. The contribution of epidemiology. Food Chem Toxicol. Feb-Mar;40(2-3): 387-424.

Vandenbroucke JP, Van Elm E, Altman DG, Gotzsche PC, Mulroew CD, Pockock SJ, Pool C, Schlesseman JJ, and Egger, M. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. Ann. Int. Med. Vol 147(8): W163-193

Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, and Vandenbroucke JP. 2014. The Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. Int. J. Surgery 12: 1495-99.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992a. Selection of Controls in Case-Control Studies I. Principles. American J. Epid. 135(9): 1019-1028.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992b. Selection of Controls in Case-Control Studies II. Types of Controls. American J. Epid. 135(9): 1029-1041.

Wacholder, S, McLaughlin, JK, Silverman, DT, and Mandel, JS. 1992c. Selection of Controls in Case-Control Studies III. Design Options. American J. Epid. 135(9): 1042-1050.

Woodruff TJ1 and Sutton P. 2014. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

Zartarian V., Bahadori T, and McKone T. 2005. Adoption of an official ISEA glossary. Journal of Exposure Analysis and Environmental Epidemiology 15:1-5

# **SCIENTIFIC OPINION**



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# Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects'

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## Abstract

In 2013, EFSA published a comprehensive systematic review of epidemiological studies published from 2006 to 2012 investigating the association between pesticide exposure and many health outcomes. Despite the considerable amount of epidemiological information available, the quality of much of this evidence was rather low and many limitations likely affect the results so firm conclusions cannot be drawn. Studies that do not meet the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009 are thus not suited for risk assessment. In this Scientific Opinion, the EFSA Panel on Plant Protection Products and their residues (PPR Panel) was requested to assess the methodological limitations of pesticide epidemiology studies and found that poor exposure characterisation primarily defined the major limitation. Frequent use of case-control studies as opposed to prospective studies was considered another limitation. Inadequate definition or deficiencies in health outcomes need to be avoided and reporting of findings could be improved in some cases. The PPR Panel proposed recommendations on how to improve the quality and reliability of pesticide epidemiology studies to overcome these limitations and to facilitate an appropriate use for risk assessment. The Panel recommended the conduct of systematic reviews and meta-analysis, where appropriate, of pesticide observational studies as useful methodology to understand the potential hazards of pesticides, exposure scenarios and methods for assessing exposure, exposure-response characterisation and risk characterisation. Finally, the PPR Panel proposed a methodological approach to integrate and weight multiple lines of evidence, including epidemiological data, for pesticide risk assessment. Biological plausibility can contribute to establishing causation.

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**Keywords:** epidemiology, pesticides, risk assessment, quality assessment, evidence synthesis, lines of evidence, weight-of-evidence

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## Summary

The European Food Safety Authority (EFSA) asked the Panel on Plant Protection Products and their Residues (PPR Panel) to develop a Scientific Opinion on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (Ntzani et al., 2013). This report was based on a systematic review and meta-analysis of epidemiological studies published between 2006 and 2012 and summarised the associations found between pesticide exposure and 23 major categories of human health outcomes. Most relevant significant associations were found for liver cancer, breast cancer, stomach cancer, amyotrophic lateral sclerosis, asthma, type II diabetes, childhood leukaemia and Parkinson's disease. While the inherent weaknesses of the epidemiological studies assessed do not allow firm conclusions to be drawn on causal relationships, the systematic review raised a concern about the suitability of regulatory studies to inform on specific and complex human health outcomes.

The PPR Panel developed a Scientific Opinion to address the methodological limitations affecting the quality of epidemiological studies on pesticides. This Scientific Opinion is intended only to assist the peer review process during the renewal of pesticides under Regulation (EC) 1107/2009 where the evaluation of epidemiological studies, along with clinical cases and poisoning incidents following any kind of human exposure, if available, is a data requirement. Epidemiological data concerning exposures to pesticides in Europe will not be available before first approval of an active substance and so will not be expected to contribute to a draft assessment report (DAR). However, there is the possibility that earlier prior approval has been granted for use of an active substance in another jurisdiction and epidemiological studies. This type of data is more suited for the renewal process of active substances, also in compliance with Regulation (EC) 1141/2010 which indicates that 'The dossiers submitted for renewal should include new data relevant to the active substance and new risk assessments'.

In this Opinion, the PPR Panel proposed a methodological approach specific for pesticide active substances to make appropriate use of epidemiological data for risk assessment purposes, and proposed recommendations on how to improve the quality and reliability of epidemiological studies on pesticides. In addition, the PPR Panel discussed and proposed a methodology for the integration of epidemiological evidence with data from experimental toxicology as both lines of evidence can complement each other for an improved pesticide risk assessment process.

First, the opinion introduces the basic elements of observational epidemiological studies<sup>1</sup> and contrasts them with interventional studies which are considered to provide the most reliable evidence in epidemiological research as the conditions for causal inference are usually met. The major observational study designs are described together with the importance of a detailed description of pesticide exposure, the use of validated health outcomes and appropriate statistical analysis to model exposure–health relationships. The external and internal study validity is also addressed to account for the role of chance in the results and to ascertain whether factors other than exposure can distort the associations found. Several types of human data can contribute to the risk assessment process of pesticides, particularly to support hazard identification. Besides formal epidemiological studies, other sources of human data such as case series, disease registries, poison control centre information, occupational health surveillance data and post-marketing surveillance programmes, can provide useful information for hazard identification, particularly in the context of acute, specific health effects.

However, many of the existing epidemiological studies on pesticides exposure and health effects suffer from a range of methodological limitations or deficiencies (Terms of Reference (ToR) 1). The Panel notes that the complexity of studying associations between exposure to pesticides and health outcomes in observational settings among humans is more challenging than in many other disciplines of epidemiology. This complexity lies in some specific characteristics in the field of pesticide epidemiology such as the large number of active substances in the market (around 480 approved for use in the European Union (EU)), the difficulties to measure exposure, and the frequent lack of quantitative (and qualitative) data on exposure to individual pesticides. The systematic appraisal of epidemiological evidence carried out in an EFSA external scientific report (Ntzani et al., 2013) identified a number of methodological limitations. Poor exposure characterisation primarily defines the major limitation of most existing studies because of the lack of direct and detailed exposure assessment to specific pesticides (e.g. use of generic pesticide definitions). Frequent use of case–control studies as

<sup>&</sup>lt;sup>1</sup> This Opinion deals only with observational studies (also called epidemiological studies) and vigilance data. In contrast, interventional studies (also called experimental studies, such as randomised clinical trials) are outside the scope of this Opinion.



opposed to prospective studies is also a limitation. Inadequate definition or deficiencies in health outcomes, deficiencies in statistical analysis and poor quality reporting of research findings were identified as other limitations of some pesticide epidemiological studies. These limitations are to some extent responsible for heterogeneity or inconsistency of data that challenge drawing robust conclusions on causality. Given the small effect sizes for most of the outcomes addressed by Ntzani et al. (2013), the contribution of bias in the study design can play a role.

The PPR Panel also provides a number of refinements (ToR 2) and recommendations (ToR 3) to improve future pesticide epidemiological studies that will benefit the risk assessment. The quality and relevance of epidemiological research can be enhanced by (a) an adequate assessment of exposure, preferentially by using personal exposure monitoring or biomarker concentrations of specific pesticides (or combination of pesticides) at an individual level, reported in a way that minimises misclassification of exposure and allows for dose-response assessment; (b) a sufficiently valid and reliable outcome assessment (well defined clinical entities or validated surrogates); (c) adequately accounting for potentially confounding variables (including other known exposures affecting the outcomes); (d) conducting and reporting subgroup analysis (e.g. stratification by gender, age, etc.). A number of reporting guidelines and checklists developed specifically for studies on environmental epidemiology are of interest for epidemiological studies assessing pesticide exposures. This is the case for extensions of the modified STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) criteria, among others, which includes recommendations on what should be included in an accurate and complete report of an observational study.

Exposure assessment can be improved at the individual level (direct and detailed exposure assessment to specific pesticides in order to provide a reliable dosimeter for the pesticide of concern that can be supplemented with other direct measures such as biomonitoring). Besides, exposure can be assessed at population level by using registered data that can then be linked to electronic health records. This will provide studies with unprecedented sample size and information on exposure and subsequent disease. Geographical information systems (GIS) and small area studies might also serve as an additional way to provide estimates of residential exposures. These more generic exposure assessments have the potential to identify general risk factors and may be important both informing overall regulatory policies, and for identification of matters for further epidemiological research. The development of -omic technologies also presents intriguing possibilities for improving exposure assessment through measurement of a wide range of molecules, from xenobiotics and metabolites in biological matrices (metabolomics) to complexes with DNA and proteins (adductomics). Omics have the potential to measure profiles or signatures of the biological response to the cumulative exposure to complex chemical mixtures and allows a better understanding of biological pathways. Health outcomes can be refined by using validated biomarkers of effect, that is, a quantifiable biochemical, physiological or any other change that, is related to level of exposure, is associated with a health impairment and also helps to understand a mechanistic pathway of the development of a disease.

The incorporation of epidemiological studies into regulatory risk assessment (ToR 4) represents a major challenge for scientists, risk assessors and risk managers. The findings of the different epidemiological studies can be used to assess associations between potential health hazards and adverse health effects, thus contributing to the risk assessment process. Nevertheless, and despite the large amount of available data on associations between pesticide exposure and human health outcomes, the impact of such studies in regulatory risk assessment is still limited. Human data can be used for many stages of risk assessment; however, a single (not replicated) epidemiological study, in the absence of other studies on the same pesticide active substance, should not be used for hazard characterisation unless it is of high quality and meets the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009. As these 'recognised standards' are not detailed in the Regulation, a number of recommendations should be considered for optimal design and reporting of epidemiological studies to support regulatory assessment of pesticides. Although further specific guidance will be helpful, this is beyond the ToR of this Opinion. Evidence synthesis techniques, such as systematic reviews and meta-analysis (where appropriate) offer a useful approach. While these tools allow generation of summary data, increased statistical power and precision of risk estimates by combining the results of all individual studies meeting the selection criteria, they cannot overcome methodological flaws or bias of individual studies. Systematic reviews and meta-analysis of observational studies have the capacity of large impact on risk assessment as these tools provide information that strengthens the understanding of the potential hazards of pesticides, exposure scenarios and methods for assessing exposure, exposure-response characterisation and risk characterisation. Although systematic reviews



are also considered a potential tool for answering toxicological questions, their methodology would need to be adapted to the different lines of evidence.

Study evaluation should be performed within a best evidence synthesis framework as it provides an indication on the nature of the potential biases each specific study may have and an assessment of overall confidence in the epidemiological database. This Opinion reports the study quality parameters to be evaluated in single epidemiological studies and the associated weight (low, medium and high) for each parameter. Three basic categories are proposed as a first tier to organise human data with respect to risk of bias and quality: (a) low risk of bias and high/medium reliability; (b) medium risk of bias and medium reliability; (c) high risk of bias and low reliability because of serious methodological limitations or flaws that reduce the validity of results or make them largely uninterpretable for a potential causal association. These categories are intended to parallel the reliability and relevance rating of each stream of evidence according to the EFSA peer review of active substances: acceptable, supplementary and unacceptable. Risk assessment should not be based on results of epidemiological studies that do not meet well-defined data quality standards in order to meet the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009.

Epidemiological studies provide complementary data that can be integrated together with data from *in vivo* laboratory animal studies, mechanistic *in vitro* models and ultimately *in silico* technology for pesticide risk assessment (ToR 4). The combination of all these lines of evidence can contribute to a Weight-of-Evidence (WoE) analysis in the characterisation of human health risks with the aim of improving decision-making. Although the different sets of data can be complementary and confirmatory, and thus serve to strengthen the confidence of one line of evidence on another, they may individually be insufficient and pose challenges for characterising properly human health risks. Hence, all four lines of evidence (epidemiology, animal, *in vitro*, *in silico*) make a powerful combination, particularly for chronic health effects of pesticides, which may take decades to be clinically manifested in an exposed human population.

The first consideration is how well the health outcome under consideration is covered by existing toxicological and epidemiological studies on pesticides. When both types of studies are available for a given outcome/endpoint, both should be assessed for strengths and weaknesses before being used for risk assessment. Once the reliability of available human evidence (observational epidemiology and vigilance data), experimental evidence (animal and *in vitro* data) and non-testing data (*in silico* studies) has been evaluated, the next step involves weighting these sources of data. This opinion proposed an integrated approach where all lines of evidence are considered in an overall WoE framework to better support the risk assessment. This framework relies on a number of principles highlighting when one line should take precedence over another. The concordance or discordance between human and experimental data should be assessed in order to determine which data set should be given more weight, regardless of whether the data comes from human or experimental studies. The more challenging situation is when study results are not concordant. In such cases, the reasons for the difference should be considered and efforts should be made to develop a better understanding of the biological basis for the contradiction.

Human data on pesticides can help verify the validity of estimations made based on extrapolation from the full toxicological database regarding target organs, dose–response relationships and the reversibility of toxic effects, and to provide reassurance on the extrapolation process without direct effects on the definition of reference values. Thus, pesticide epidemiological data can form part of the overall WoE of available data using modified Bradford Hill criteria as an organisational tool to increase the likelihood of an underlying causal relationship.

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## 1. Introduction

# **1.1.** Regulatory data requirements regarding human health in pesticide risk assessment

Regulatory authorities in developed countries conduct a formal human risk assessment for each registered pesticide based on mandated toxicological studies, done according to specific study protocols, and estimates of likely human exposure.

In the European Union (EU), the procedure for the placing of plant protection products (PPP) on the market is laid down by Commission Regulation No 1107/2009<sup>2</sup>. Commission Regulations No 283/2013<sup>3</sup> and 284/2013<sup>4</sup> set the data requirements for the evaluation and re-evaluation of active substances and their formulations.

The data requirements regarding mammalian toxicity of the active substance are described in part A of Commission Regulation (EU) No 283/2013 for chemical active substances and in part B for microorganisms including viruses. With regard to the requirements for pesticide active substances, reference to the use of human data may be found in different chapters of Section 5 related to different end-points. For instance, data on toxicokinetics and metabolism that include *in vitro* metabolism studies on human material (microsomes or intact cell systems) belong to Chapter 5.1 that deals with studies of absorption, distribution, metabolism and excretion in mammals; *in vitro* genotoxicity studies performed on human material are described in Chapter 5.4 on genotoxicity testing and specific studies such as acetylcholinesterase inhibition in human volunteers are found in Chapter 5.7 on neurotoxicity studies. Chapter 5.8 refers to supplementary studies on the active substance, and some specific studies, such as pharmacological or immunological investigations.

Although the process of pesticide evaluation is mainly based on experimental studies, human data could add relevant information to that process. The requirements relating to human data are mainly found in Chapter 5.9 'Medical data' of Regulation (EU) No 283/2013. It includes medical reports following accidental, occupational exposure or incidents of intentional self-poisoning as well as monitoring studies such as on surveillance of manufacturing plant personnel and others. The information may be generated and reported through official reports from national poison control centres as well as epidemiological studies published in the open literature. The Regulation requires that 'relevant' information on the effects of human exposure, where available, shall be used to confirm the validity of extrapolations regarding exposure and conclusions with respect to target organs, dose–response relationships, and the reversibility of adverse effects.

Regulation (EU) No 1107/2009 equally states that, 'where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards, epidemiological studies are of particular value and must be submitted'. However, it is clear that there is no obligation for the petitioners to conduct epidemiological studies specific for the active substance undergoing the approval or renewal process. Rather, according to Regulation (EC) No 1107/2009, applicants submitting dossiers for approval of active substances shall provide 'scientific peer-reviewed public available literature [...]. This should be on the active substance and its relevant metabolites dealing with side-effects on health [...] and published within the last ten years before the date of submission of the dossier'.

In particular, epidemiological studies on pesticides should be retrieved from the literature according to the EFSA Guidance entitled 'Submission of scientific-peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009' (EFSA, 2011a), which follows the principles of the Guidance 'Application of systematic review methodology to food and feed safety assessments to support decision-making' (EFSA, 2010a). As indicated in the EFSA Guidance, 'the process of identifying and selecting scientific peer-reviewed open literature for active substances, their metabolites, or plant protection products' is based on a literature review which is systematic in the approach.

The submission of epidemiological studies and more generally of human data by the applicants in Europe has especially previously sometimes been incomplete and/or has not been performed in

<sup>&</sup>lt;sup>2</sup> Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

<sup>&</sup>lt;sup>3</sup> Commission Regulation (EU) No 283/2013, of 1 March 2013, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

<sup>&</sup>lt;sup>4</sup> Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 85–152.



compliance with current EFSA Guidance (EFSA, 2011a). This is probably owing to the fact that a mandatory requirement to perform an (epidemiological) literature search according to specific EFSA Guidance is relatively recent, e.g. introduced for AIR-3 substances (Regulation AIR-3: Reg. (EU) No 844/2012; Guidance Document SANCO/2012/11251 – rev.4).

The integration of epidemiological data with toxicological findings in the peer review process of pesticides in the EU should be encouraged but is still lacking. A recent and controversial example is the one related to the evaluation of glyphosate in which significant efforts were made to include epidemiological studies in the risk assessment, but the conclusion was that these studies provided very limited evidence of an association between glyphosate and health outcomes.

In the case of the peer review of 2,4-D, most of epidemiological data were not used in the risk assessment because it was critical to know the impurity profile of the active substance and this information was not available in the publications (as happens frequently in epidemiological studies). In conclusion, within the European regulatory system there is no example of a pesticide active substance approval being influenced by epidemiological data.

Now that a literature search including epidemiological studies is mandatory and guidance is in place (EFSA, 2011a), a more consistent approach can facilitate risk assessment. However, no framework has been established on how to assess such epidemiological information in the regulatory process. In particular, none of the classical criteria used for the evaluation of these studies is included in the current regulatory framework (e.g. study design, use of odd ratios and relative risks, potential confounders, multiple comparisons, assessment of causality). It follows that specific criteria or guidance for the appropriate use of epidemiological findings in the process of writing and peer reviewing Draft Assessment Reports (DARs) or Renewal Assessment Reports (RAR) is warranted. The EFSA Stakeholder Workshop (EFSA, 2015a) anticipated that the availability of more robust and methodologically sound studies presenting accurate information on exposure would bolster the regulation of pesticides in the EU.

Another potential challenge is synchronisation between the process of renewal of active substances and the output of epidemiological studies. Indeed, the planning, conduct, and analysis of epidemiological studies often require a substantial amount of time, especially where interpretation of data is complex.

## **1.2.** Background and Terms of Reference as provided by the requestor

In 2013, the European Food Safety Authority (EFSA) published an External scientific report 'Literature review on epidemiological studies linking exposure to pesticides and health effects' carried out by the University of Ioannina Medical School (Ntzani et al., 2013). The report is based on a systematic review of epidemiological studies published between 2006 and 2012 and summarises the association between pesticide exposure and any health outcome examined (23 major categories of human health outcomes). In particular, a statistically significant association was observed through fixed and random effect meta-analyses between pesticide exposure and the following health outcomes: liver cancer, breast cancer, stomach cancer, amyotrophic lateral sclerosis, asthma, type II diabetes, childhood leukaemia and Parkinson's disease.

Despite the large number of research articles and analyses (> 6,000) available, the authors of the report could not draw any firm conclusions for the majority of the health outcomes. This observation is in line with previous studies assessing the association between the use of pesticides and the occurrence of human health adverse effects which all acknowledge that such epidemiological studies suffer from a number of limitations and large heterogeneity of data. The authors especially noted that broad pesticides definitions in the epidemiological studies limited the value of the results of meta-analyses. Also, the scope of the report did not allow the in-depth associations between pesticide exposure and specific health outcomes. Nonetheless, the report highlights a number of health outcomes where further research is needed to draw firmer conclusions regarding their possible association with pesticide exposures.

Nevertheless, the outcomes of the External scientific report are in line with other similar studies published in Europe,<sup>5,6</sup> and raise a number of questions and concerns, with regard to pesticide exposure and the associations with human health outcomes. Furthermore, the results of the report

<sup>&</sup>lt;sup>5</sup> France: INSERM report 2013: Pesticides – effets sur la santé.

<sup>&</sup>lt;sup>6</sup> UK: COT report 2011: Statement on a systematic review of the epidemiological literature on para-occupational exposure to pesticides and health outcomes other than cancer, and COT report 2006: Joint Statement on Royal Commission on Environmental Pollution report on crop spraying and the health of residents and bystanders.



open the way for discussion on how to integrate results from epidemiological studies into pesticide risk assessments. This is particularly important for the peer-review team at EFSA dealing with the evaluation of approval of plant protection products for which the peer-review needs to evaluate epidemiological findings according to EU Regulation No 283/2013. The regulation states that applicants must submit 'relevant' epidemiological studies, where available.

For the Scientific Opinion, the PPR Panel will discuss the associations between pesticide exposure and human health effects observed in the External scientific report (Ntzani et al., 2013) and how these findings could be interpreted in a regulatory pesticide risk assessment context. Hence, the PPR Panel will systematically assess the epidemiological studies collected in the report by addressing major data gaps and limitations of the studies and provide related recommendations.

The PPR Panel will specifically:

- collect and review all sources of gaps and limitations, based on (but not necessarily limited to) those identified in the External scientific report in regard to the quality and relevance of the available epidemiological studies.
- 2) based on the gaps and limitations identified in point 1, propose potential refinements for future epidemiological studies to increase the quality, relevance and reliability of the findings and how they may impact pesticide risk assessment. This may include study design, exposure assessment, data quality and access, diagnostic classification of health outcomes, and statistical analysis.
- 3) identify areas in which information and/or criteria are insufficient or lacking and propose recommendations for how to conduct pesticide epidemiological studies in order to improve and optimise the application in risk assessment. These recommendations should include harmonisation of exposure assessment (including use of biomonitoring data), vulnerable population subgroups and/or health outcomes of interest (at biochemical, functional, morphological and clinical level) based on the gaps and limitations identified in point 1.
- 4) discuss how to make appropriate use of epidemiological findings in risk assessment of pesticides during the peer review process of draft assessment reports, e.g. weight-ofevidence (WoE) as well as integrating the epidemiological information with data from experimental toxicology, adverse outcome pathways (AOP), mechanism of actions, etc.

The PRAS Unit will consult the Scientific Committee on the consensual approach to EFSA's overarching scientific areas,<sup>7</sup> including the integration of epidemiological studies in risk assessment.

## **1.3.** Interpretation of the Terms of Reference

In the Terms of Reference (ToR), EFSA requested the PPR Panel to write a scientific Opinion on the follow up of the results from the External Scientific Report on a systematic review of epidemiological studies published between 2006 and 2012 linking exposure to pesticides and human health effects (Ntzani et al., 2013). According to EU Regulation No 283/2013, the integration of epidemiological data into pesticide risk assessment is important for the peer review process of DAR and RAR of active substances for EU approval and their intended use as plant protection products.

In its interpretation of the terms of reference, the PPR Panel will then develop a Scientific Opinion to address the methodological limitations identified in epidemiological studies on pesticides and to make recommendations to the sponsors of such studies on how to improve them in order to facilitate their use for regulatory pesticide risk assessment, particularly for substances in the post-approval period. The PPR Panel notes that experimental toxicology studies also present limitations related to their methodology and quality of reporting; however, the assessment of these limitations is beyond the ToR of this Opinion.

This Scientific Opinion is intended to assist the peer review process during the renewal of pesticides under Regulation 1107/2009 where the evaluation of epidemiological studies, along with clinical cases and poisoning incidents following any kind of human exposure, if available, represent a data requirement. Epidemiological data concerning exposures to pesticides in Europe will not be available before first approval of an active substance (with the exception of incidents produced during the manufacturing process, which are expected to be very unlikely) and so will not be expected to contribute to a DAR. However, there is the possibility that earlier prior approval has been granted for use of an active substance in another jurisdiction and epidemiological data from that area may be considered relevant. Regulation (EC) No 1107/2009 requires a search of the scientific peer-reviewed

<sup>&</sup>lt;sup>7</sup> According to article 28 of Regulation (EC) No 178/2002.



open literature, where it is expected to retrieve existing epidemiological studies. It is therefore recognised that epidemiological studies are more suitable for the renewal process of active substances, also in compliance with the provision of the EC regulation 1141/2010 indicating that 'The dossiers submitted for renewal should include new data relevant to the active substance and new risk assessments to reflect any changes in data requirements and any changes in scientific or technical knowledge since the active substance was first included in Annex I to Directive 91/414/EEC'.

The PPR Panel will specifically address the following topics:

- 1) Review inherent weaknesses affecting the quality of epidemiological studies (including gaps and limitations of the available pesticide epidemiological studies) and their relevance in the context of regulatory pesticide risk assessment. How can these weaknesses be addressed?
- 2) What are potential contributions of epidemiological studies that complement classical toxicological studies conducted in laboratory animal species in the area of pesticide risk assessment?
- 3) Discuss and propose a methodological approach specific for pesticide active substances on how to make appropriate use of epidemiological studies, focusing on how to improve the gaps and limitations identified.
- 4) Propose refinements to practice and recommendations for better use of the available epidemiological evidence for risk assessment purposes. Discuss and propose a methodology for the integration of epidemiological information with data from experimental toxicology.

This Scientific Opinion, particularly Section 2–4, is not intended to address the bases of epidemiology as a science. Those readers willing to deepen into specific aspects of this science are encouraged to read general textbook of epidemiology (e.g. Rothman et al., 2008).

It should be taken into account that this Opinion is focussed only on pesticide epidemiology studies in the EU regulatory context and not from a general scientific perspective. Therefore, the actual limitations and weaknesses of experimental toxicology studies will not be addressed herein.

## **1.4.** Additional information

In order to fully address topics 1–4 above (Section 1.3), attention has been paid to a number of relevant reviews of epidemiological studies and the experience of other National and International bodies with knowledge of epidemiology in general and in applying epidemiology to pesticide risk assessment specifically. Detailed attention has been given to these studies in Annex A and drawn from the experience of the authors that have contributed constructively to understanding in this area. Also Annex A records published information that has been criticised for its lack of rigour showing how unhelpful some published studies may be. The lessons learned from such good (and less-good) practice have been incorporated into the main text by cross-referring to Annex A. In this way, this Scientific Opinion has the aim of clearly distilling and effectively communicating the arguments in the main text without overwhelming the reader with all the supporting data which is nevertheless accessible.

In addition, Annex B contains a summary of the main findings of a project that EFSA outsourced in 2015 to further investigate the role of human biological monitoring (HBM) in occupational health and safety strategies as a tool for refined exposure assessment in epidemiological studies and to contribute to the evaluation of potential health risks from occupational exposure to pesticides (Bevan et al., 2017).

## 2. General framework of epidemiological studies on pesticides

This section introduces the basic elements of epidemiological studies on pesticides and contrasts them with other types of studies. For more details general textbook on epidemiology are recommended (Rothman et al., 2008; Thomas, 2009).

## 2.1. Study design

Epidemiology studies the distribution and determinants of health outcomes in human or other target species populations, to ascertain how, when and where diseases occur. This can be done through observational studies and intervention studies (i.e. clinical trials),<sup>8</sup> which compare study

<sup>&</sup>lt;sup>8</sup> In this opinion, 'human data' includes observational studies, also called epidemiological studies, where the researcher is observing natural relationships between factors and health outcomes without acting upon study participants. Vigilance data also fall under this concept. In contrast, intervention studies (also referred to as experimental studies) are outside the scope of this Opinion, and their main feature is that the researcher intercedes as part of the study design.



groups subject to differing exposure to a potential risk factor. Both types of studies are carried out in a natural setting, which is a less controlled environment than laboratories.

Information on cases of disease occurring in a natural setting can also be systematically recorded in the form of case reports or case series of exposed individuals only. Although case series/reports do not compare study groups according to differing exposure, they may provide useful information, particularly on acute effects following high exposures, which makes them potentially relevant for hazard identification.

In randomised clinical trials, the exposure of interest is randomly allocated to subjects and, whenever possible, these subjects are blinded to their treatment, thereby eliminating potential bias due to their knowledge about their exposure to a particular treatment. This is why they are called intervention studies. Observational epidemiological studies differ from clinical intervention studies in that the exposure of interest is not randomly assigned to the subjects enrolled and participants are often not blinded to their exposure. This is why they are called observational. As a result, randomised clinical trials rank higher in terms of design as they provide unbiased estimates of average treatment effects.

The lack of random assignment of exposure in observational studies represents a key challenge, as other risk factors that are associated with the occurrence of disease may be unevenly distributed between those exposed and non-exposed. This means that known confounders need to be measured and accounted for. However, there is always the possibility that unknown or unmeasured confounders are left unaccounted for, although unknown confounders cannot be addressed. Furthermore, the fact that study participants are often unaware of their current or past exposure or may not recall these accurately in observational studies (e.g. second-hand smoke, dietary intake or occupational hazards) may result in biased estimates of exposure if it is based on self-report. As an example, it is not unlikely that when cancer cases and controls are asked whether they have previously been exposed to a pesticide the cancer cases may report their exposure differently from controls, even in cases where the past exposures did not differ between the two groups.

Traditionally, designs of observational epidemiological studies are classified as either ecological, cross-sectional, case–control or cohort studies. This approach is based on the quality of exposure assessment and the ability to assess directionality from exposure to outcome. These differences largely determine the quality of the study (Rothman and Greenland, 1998; Pearce, 2012).

- **Ecological studies** are observational studies where either exposure, outcome or both are measured on a group but not at individual level and the correlation between the two is then examined. Most often, exposure is measured on a group level while the use of health registries often allows for extraction of health outcomes on an individual level (cancer, mortality). These studies are often used when direct exposure assessment is difficult to achieve and in cases where large contrast in exposures are needed (comparing levels between different countries or occupations). Given the lack of exposure and/or outcome on an individual level, these studies are useful for hypothesis generation but results generally need to be followed up using more rigorous design in either humans or use of experimental animals.
- In **cross-sectional studies**, exposure and health status are assessed at the same time, and prevalence rates (or incidence over a limited recent time) in groups varying in exposure are compared. In such studies, the temporal relationship between exposure and disease cannot be established since the current exposure may not be the relevant time window that leads to development of the disease. The inclusion of prevalent cases is a major drawback of (most) cross-sectional studies, particularly for chronic long-term diseases. Cross-sectional studies may nevertheless be useful for risk assessment if exposure and effect occur more or less simultaneously or if exposure does not change over time.
- **Case–control studies** examine the association between estimates of past exposures among individuals that already have been diagnosed with the outcome of interest (e.g. cases) to a control group of subjects from the same population without such outcome. In population-based incident case–control studies, cases are obtained from a well-defined population, with controls selected from members of the population who are disease free at the time a case is incident. The advantages of case–control studies are that they require less sample sizes, time and resources compared to prospective studies and often they are the only viable option when studying rare outcomes such as some types of cancer. In case–control studies, past exposure is most often not assessed based on 'direct' measurement but rather through less certain measurements such as a recall captured through interviewer or self-administered



questionnaires or proxies such as job descriptions titles or task histories. Although case–control studies may allow for proper exposure assessment, these studies are prone to recall-bias when estimating exposure. Other challenges include the selection of appropriate controls; as well as the need for appropriate confounder control.

In **cohort studies**, the population under investigation consists of individuals who are at risk of developing a specific disease or health outcome at some point in the future. At baseline and at later follow-ups (prospective cohort studies) relevant exposures, confounding factors and health outcomes are assessed. After an appropriate follow-up period, the frequency of occurrence of the disease is compared among those differently exposed to the previously assessed risk factor of interest. Cohort studies are therefore by design prospective as the assessment of exposure to the risk factor and covariates of interest are measured before the health outcome has occurred. Thus, they can provide better evidence for causal associations compared to the other designs mentioned above. In some cases, cohort studies may be based on estimates of past exposure. Such retrospective exposure assessment is less precise than direct measure and prone to recall bias. As a result, the quality of evidence from cohort studies varies according to the actual method used to assess exposure and the level of detail by which information on covariates were collected. Cohort studies are particularly useful for the study of relatively common outcomes. If sufficiently powered in terms of size, they can also be used to appropriately address relatively rare exposures and health outcomes. Prospective cohort studies are also essential to study different critical exposure windows. An example of this is longitudinal birth cohorts that follow children at regular intervals until adult age. Cohort studies may require a long observation period when outcomes have a long latency prior to onset of disease. Thus, such studies are both complex and expensive to conduct and are prone to loss of follow-up.

## **2.2.** Population and sample size

A key strength of epidemiological studies is that they study diseases in the very population about which conclusions are to be drawn, rather than a proxy species. However, only rarely will it be possible to study the whole population. Instead, a sample will be drawn from the reference population for the purpose of the study. As a result, the observed effect size in the study population may differ from that in the population if the former does not accurately reflect the latter. However, observations made in a non-representative sample may still be valid within that sample but care should then be made when extrapolating findings to the general population.

Having decided how to select individuals for the study, it is also necessary to decide how many participants should minimally be enrolled. The sample size of a study should be large enough to warrant sufficient statistical power. The standard power (also called sensitivity) is 80%, which means the ability of a study to detect an effect of a given magnitude when that effect actually exists in the target population; in other words, there is 80% probability of drawing the right conclusion from the results of the analyses and a corresponding probability of 20% or drawing the wrong conclusion and missing a true effect. Power analysis is often used to calculate the minimum sample size required to likely detect an effect of a given size. Small samples are likely to constitute an unrepresentative sample. The statistical power is also closely related to risk inflation, which needs to be given special attention when interpreting statistically significant results from small or underpowered studies (see Annex D).

Epidemiological studies, like toxicological studies in laboratory animals, are often designed to examine multiple endpoints unlike clinical trials that are designed and conducted to test one single hypothesis, e.g. efficacy of a medical treatment. To put this in context, for laboratory animal toxicology test protocols, OECD guidance for pesticides may prescribe a minimum number of animals to be enrolled in each treatment group. This does not guarantee adequate power for any of the multitude of other endpoints being tested in the same study. It is thus important to appropriately consider the power of a study when conducting both epidemiology and laboratory studies.

## 2.3. Exposure

The quality of the exposure measurements influences the ability of a study to correctly ascertain the causal relationship between the (dose of) exposure and a given adverse health outcome.

In toxicological studies in laboratory animals, the 'treatment regime' i.e. dose, frequency, duration and route are well defined beforehand and its implementation can be verified. This often allows



expression of exposure in terms of external dose administered daily via oral route for example in a 90day study, by multiplying the amount of feed ingested every day by a study animal with the intended (and verified) concentration of the chemical present in the feed. Also, in the future, the internal exposure has to be determined in the pivotal studies.

In the case of pesticides, estimating exposure in a human observational setting is difficult as the dose, its frequency and duration over time and the route of exposure are not controlled and not even well known.

Measuring the intensity, frequency and duration of exposure is often necessary for investigating meaningful associations. Exposure may involve a high dose over a relatively short period of time, or a low-level prolonged dose over a period from weeks to years. While the effects of acute, high-dose pesticide exposure may appear within hours or days, the effects of chronic, low-dose exposures may not appear until years later. Also, a disease may require a minimal level of exposure but increase in probability with longer exposure.

There may be differences in absorption and metabolism via different routes (dermal, inhalation and oral). While dermal or inhalation are often the routes exposure occurs in occupational settings, ingestion (food, water) may be the major route of pesticide exposure for the general population. Pharmacokinetic differences among individuals may result in differing systemic or tissue/organ doses even where the absorbed external doses may appear similar.

## 2.4. Health outcomes

The term health outcome refers to a disease state, event, behaviour or condition associated with health that is under investigation. Health outcomes are those clinical events (usually represented as diagnosis codes, i.e. International Classification of Diseases (ICD) 10) or outcomes (i.e. death) that are the focus of the research. Use of health outcomes requires a well-defined case definition, a system to report and record the cases and a measure to express the frequency of these events.

A well-defined case definition is necessary to ensure that cases are consistently diagnosed, regardless of where, when and by whom they were identified and thus avoid misclassification. A case definition involves a standard set of criteria, which can be a combination of clinical symptoms/signs, sometimes supplemented by confirmatory diagnostic tests with their known sensitivity and specificity. The sensitivity of the whole testing procedure (i.e. the probability that a person with an adverse health condition is truly diagnosed) must be known to estimate the true prevalence or incidence.

The clinical criteria may also involve other characteristics (e.g. age, occupation) that are associated with increased disease risk. At the same time, appropriately measured and defined phenotypes or hard clinical outcomes add validity to the results.

Disease registries contain clinical information of patients on diagnosis, treatment and outcome. These registries periodically update patient information and can thus provide useful data for epidemiological research. Mortality, cancer and other nation-wide health registries generally meet the case-definition requirements and provide (almost) exhaustive data on the incident cases within a population. These health outcomes are recorded and classified in national health statistics databases, which depend on accepted diagnostic criteria that are evolving and differ from one authority to another. This may confound attempts to pool data usefully for societal benefit. Registry data present many opportunities for meaningful analysis, but the degree of data completeness and validity may challenge making appropriate inferences. Also, changes in coding conventions over the lifetime of the database may have an impact on retrospective database research.

Although the disease status is typically expressed as a dichotomous variable, it may also be measured as an ordinal variable (e.g. severe, moderate, mild or no disease) or as a quantitative variable for example by measuring molecular biomarkers of toxic response in target organs or physiological measures such as blood pressure or serum concentration of lipids or specific proteins.

The completeness of the data capture and its consistency are key contributors to the reliability of the study. Harmonisation of diagnostic criteria, data storage and utility would bring benefits to the quality of epidemiological studies.

A surrogate endpoint is used as substitute for a well-defined disease endpoint, an outcome measure, commonly a laboratory measurement (biomarker of response). These measures are considered to be on the causal pathway for the clinical outcome. In contrast to overt clinical disease, such biological markers of health may allow to detect subtle, subclinical toxicodynamic processes. For such outcomes, detailed analytical protocols for quantification should be specified to enable comparison or replication across laboratories. The use of AOPs can highlight differences in case definitions.



Although surrogate outcomes may offer additional information, the suitability of the surrogate outcome examined needs to be carefully assessed. In particular, the validity of surrogate outcomes may represent a major limitation to their use (la Cour et al., 2010). Surrogate endpoints that have not been validated should thus be avoided.

When the health status is captured in other ways, such as from self-completed questionnaires or telephone interviews, from local records (medical or administrative databases) or through clinical examination only, these should be validated to demonstrate that they reflect the underlying case definition.

## 2.5. Statistical analysis and reporting

Reporting in detail materials, methods and results, and conducting appropriate statistical analyses are key steps to ensure quality of epidemiological studies. Regarding statistical analysis, one can distinguish between descriptive statistics and modelling of exposure–health outcome relationship.

## **2.5.1.** Descriptive statistics

Descriptive statistics aim to summarise the important characteristics of the study groups, such as exposure measures, health outcomes, possible confounding factors and other relevant factors. The descriptive statistics often include frequency tables and measures of central tendency (e.g. means and medians) and dispersion (e.g. variance and interquartile range) of the parameters or variables studied.

## 2.5.2. Modelling exposure-health outcome relationship

Modelling of the exposure–health relationship aims to assess the possible relationship between the exposure and the health outcome under consideration. In particular, it can evaluate how this relationship may depend on dose and mode of exposure and other possible intervening factors.

Statistical tests determine the probability that the observations found in scientific studies may have occurred as a result of chance. This is done by summarising the results from individual observations and evaluating whether these summary estimates differ significantly between, e.g. exposed and non-exposed groups, after taking into consideration random errors in the data.

For dichotomous outcomes, the statistical analysis compares study groups by assessing whether there is a difference in disease frequency between the exposed and control populations. This is usually done using a relative measure. The relative risk (RR) in cohort studies estimates the relative magnitude of an association between exposure and disease comparing those that are exposed (or those that have a higher exposure level) with those that are not exposed (or those that have a lower exposure level). It indicates the likelihood of developing the disease in the exposed group relative to those who are not (or less) exposed. An odds ratio (OR), generally an outcome measure in case– control and cross-sectional studies, represents the ratio of the odds of exposure between cases and controls (or diseased and non-diseased individuals in a cross-sectional study) and is often the relative measure used in statistical testing. Different levels or doses of exposure can be compared in order to see if there is a dose–response relationship. For continuous outcome measures, mean or median change in the outcome are often examined across different level of exposure; either through analyses of variance or through other parametric statistics.

While the statistical analysis will show that observed differences are significantly different or not significantly different, both merit careful reflection (Greenland et al., 2016).

**Interpretation of the absence of statistically significant difference.** Failure to reject the null hypothesis does not necessarily mean that no association is present because the study may not have sufficient power to detect it. The power depends on the following factors:

- sample size: with small sample sizes, statistical significance is more difficult to detect, even if true;
- variability in individual response or characteristics, either by chance or by non-random factors: the larger the variability, the more difficult to demonstrate statistical significance;
- effect size or the magnitude of the observed difference between groups: the smaller the size of the effect, the more difficult to demonstrate statistical significance.

**Interpretation of statistically significant difference.** Statistical significance means that the observed difference is not likely due to chance alone. However, such a result still merits careful consideration.



- Biological relevance. Rejection of the null hypothesis does not necessarily mean that the association is biologically meaningful, nor does it mean that the relationship is causal (Skelly, 2011). The key issue is whether the magnitude of the observed difference (or 'effect size') is large enough to be considered biologically relevant. Thus, an association that is statistically significant may be or may be not biologically relevant and vice versa. While epidemiological results that are statistically significant may be dismissed as 'not biologically relevant', non-statistically significant results are seldom determined to be 'biologically relevant'. Increasingly, researchers and regulators are looking beyond statistical significance for evidence of a 'minimal biologically important difference' for commonly used outcomes measures. Factoring biological significance relevance into study design and power calculations, and reporting results in terms of biological as well as statistical significance will become increasingly important for risk assessment (Skelly, 2011). This is the subject of an EFSA Scientific Committee guidance document outlining generic issues and criteria to be taken into account when considering biological relevance (EFSA Scientific Committee, 2017a); also a framework is being developed to consider biological relevance at three main stages related to the process of dealing with evidence (EFSA Scientific Committee, 2017b).
- <u>Random error</u>. Evaluation of statistical precision involves consideration of random error within the study. Random error is the part of the study that cannot be predicted because that part is attributable to chance. Statistical tests determine the probability that the observations found in scientific studies have occurred as a result of chance. In general, as the number of study participants increases, precision (often expressed as standard error) of the estimate of central tendency (e.g. the mean) is increased and the ability to detect a statistically significant difference, if there is a real difference between study groups, i.e. the study's power, is enhanced. However, there is always a possibility, at least in theory, that the results observed are due to chance only and that no true differences exist between the compared groups (Skelly, 2011). Very often this value is set at 5% (significance level).
- <u>Multiple testing</u>. As mentioned previously when discussing sample size, modelling of the exposure-health relationship is in principle hypothesis-driven, i.e. it is to be stated beforehand in the study objectives what will be tested. However, in reality, epidemiological studies (and toxicological studies in laboratory animals) often explore a number of different health outcomes in relation to the same exposure. If many statistical tests are conducted, some 5% of them will be statistically significant by chance. Such testing of multiple endpoints (hypotheses) increases the risk of false positive results and this can be controlled for by use of Bonferroni, Sidak or Benjamini–Hochberg corrections or other suitable methods. But this is often omitted. Thus, when researchers carry out many statistical tests on the same set of data, they can conclude that there are real differences where in fact there are none. Therefore, it is important to consider large number of statistical significance and biological significance notes that the assumptions derived from a statistical analysis should be related to the study design (EFSA, 2011b).
- Effect size magnification. An additional source of bias, albeit one that is lesser known, is that which may result from small sample sizes and the consequent low statistical power. This lesser known type of bias is 'effect size magnification' which can result from low powered studies. While it is generally widely known that small, low-powered studies can result in false negatives since the study power is inadequate to reliably detect a meaningful effect size, it is less well known that these studies can result in inflation of effect sizes if those estimated effects pass a statistical threshold (e.g. the common p < 0.05 threshold used to judge statistical significance). This effect –also known as effect size magnification is a phenomenon by which a 'discovered' association (i.e. one that has passed a given threshold of statistical significance) from a study with suboptimal power to make that discovery will produce an observed effect size that is artificially and systematically inflated. This is because smaller, low-powered studies are more likely to be affected by random variation among individuals than larger ones. Mathematically, conditional on a result passing some predetermined threshold of statistical significance, the estimated effect size is a biased estimate of the true effect size, with the magnitude of this bias inversely related to power of the study.</p>

As an example, if a trial were run thousands of times, there will be a broad distribution of observed effect sizes, with smaller trials systematically producing a wider variation in observed effect sizes than larger trials, but the median of these estimated effect sizes is close to the true



effect size. However, in a small and low powered study, only a small proportion of observed effects will pass any given (high) statistical threshold of significance and these will be only the ones with the greatest of effect sizes. Thus, when these smaller, low powered studies with greater random variation do indeed find a significance-triggered association as a result of passing a given statistical threshold, they are more likely to overestimate the size of that effect. What this means is that research findings of small and significant studies are biased in favour of finding inflated effects. In general, the lower the background (or control or natural) rate, the lower the effect size of interest, and the lower the power of the study, the greater the tendency towards and magnitude of inflated effect sizes.

It is important to note, however, that this phenomenon is only present when a 'pre-screening' for statistical significance is done. The bottom line is that if it is desired to estimate a given quantity such as an OR or RR, 'pre-screening' a series of effect sizes for statistical significance will result in an effect size that is systematically biased away from the null (larger than the true effect size). To the extent that regulators, decision-makers, and others are acting in this way – looking for statistically significant results in what might be considered a sea of comparisons and then using those that cross a given threshold of statistical significance to evaluate and judge the magnitude of the effect – will likely result in an exaggerated sense of the magnitude of the hypothesised association. Additional details and several effect size simulations are provided in Annex D of this document.

**Confounding** occurs when the relationship between the exposure and disease is to some extent attributable to the effect of another risk factor, i.e. the confounder. There are several traditionally recognised requirements for a risk factor to actually act as a confounder as described by McNamee (2003) and illustrated below. The factor must:

- be a cause of the disease, or a surrogate measure of the cause, in unexposed people; factors satisfying this condition are called 'risk factors';
- be correlated, positively or negatively, with exposure in the study populations independently from the presence of the disease. If the study population is classified into exposed and unexposed groups, this means that the factor has a different distribution (prevalence) in the two groups;
- not be an intermediate step in the causal pathway between the exposure and the disease

Confounding can result in an over- or underestimation of the relationship between exposure and disease and occurs because the effects of the two risk factors have not been separated or 'disentangled'. In fact, if strong enough, confounding can also reverse an apparent association. For instance, because agriculture exposures cover many different exposure categories, farmers are likely to be more highly exposed than the general population to a wide array of risk factors, including biological agents (soil organisms, livestock, farm animals), pollen, dust, sunlight and ozone amongst others, which may act as potential confounding factors.

A number of procedures are available for controlling confounding, both in the design phase of the study or in the analytical phase. For large studies, control in the design phase is often preferable. In the design phase, the epidemiological researcher can limit the study population to individuals that share a characteristic which the researcher wishes to control. This is known as 'restriction' and in fact removes the potential effect of confounding caused by the characteristic which is now eliminated. A second method in the design phase through which the researcher can control confounding is by 'matching'. Here, the researcher matches individuals based on the confounding variable which ensures that this is evenly distributed between the two comparison groups.

Beyond the design phase, at the analysis stage, control for confounding can be done by means of either stratification or statistical modelling. One means of control is by stratification in which the association is measured separately, under each of the confounding variables (e.g. males and females, ethnicity or age group). The separate estimates can be 'brought together' statistically – when appropriate – to produce a common OR, RR or other effect size measure by weighting the estimates measured in each stratum (e.g. using Mantel–Haenszel approaches). This can be done at the cost of reducing the sample size for the analysis. Although relatively easy to perform, there can be difficulties associated with the inability of this stratification to deal with multiple confounders simultaneously. For these situations, control can be achieved through statistical modelling (e.g. multiple logistic regression).

Regardless of the approaches available for control of confounding in the design and analysis phases of the study described above, it is important – prior to any epidemiological studies being initiated in the field – that careful consideration be given to confounders because researchers cannot control for a variable which they have not considered in the design or for which they have not collected data.

Epidemiological studies – published or not – are often criticised for ignoring potential confounders that may possibly either falsely implicate or inappropriately negate a given risk factor. Despite these critiques, rarely is an argument presented on the likely size of the impact of the bias from such possible confounding. It should be emphasised that a confounder must be a relatively strong risk factor for the disease to be strongly associated with the exposure of interest to create a substantial distortion in the risk estimate. It is not sufficient to simply raise the possibility of confounding; one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be and how important that impact might be to the interpretation of findings. It is important to consider the magnitude of the association as measured by the RR, OR, risk ratio, regression coefficient, etc. since strong relative risks are unlikely to be due to unmeasured confounding, while weak associations may be due to residual confounding by variables that the investigator did not measure or control in the analysis (US-EPA, 2010b).

Effect modification. Effects of pesticides, and other chemicals, on human health can hardly be expected to be identical across all individuals. For example, the effect that any given active substance might have on adult healthy subjects may not be the same as that it may have on infants, elderly, or pregnant women. Thus, some subsets of the population are more likely to develop a disease when exposed to a chemical because of an increased sensitivity. For this, the term 'vulnerable subpopulation' has been used, which means children, pregnant women, the elderly, individuals with a history of serious illness and other subpopulations identified as being subject to special health risks from exposure to environmental chemicals (i.e. because of genetic polymorphisms of drug-metabolising enzymes, transporters or biological targets). The average effect measures the effect of an exposure averaged over all subpopulations. However, there may be heterogeneity in the strength of an association between various subpopulations. For example, the magnitude of the association between exposure to chemical A and health outcome B may be stronger in children than in healthy adults, and absent in those wearing protective clothing at the time of exposure or in those of different genotype. If heterogeneity is truly present, then any single summary measure of an overall association would be deficient and possibly misleading. The presence of heterogeneity is assessed by testing for the presence of statistically significant interaction between the factor and the effect in the various subpopulations. But, in practice, this requires large sample size.

Investigating the effect in subpopulations defined by relevant factors may advance knowledge on the effect on human health of the risk factor of interest.

## 2.6. Study validity

When either a statistically significant association or no such significant association between, for example, pesticide exposures and a health outcome is observed, there is a need to also evaluate the validity of a research study, assessing factors that might distort the true association and/or influence its interpretation. These imperfections relate to systematic sources of error that result in a (systematically) incorrect estimate of the association between exposure and disease. In addition, the results from a single study takes on increased validity when it is replicated in independent investigations conducted on other populations of individuals at risk of developing the disease.

**Temporal sequence.** Any claim of causation must involve the cause preceding in time the presumed effect. Rothman (2002) considered temporality as the only criterion that is truly causal, such that lack of temporality rules out causality. While the temporal sequence of an epidemiological association implies the necessity for the exposure to precede the outcome (effect) in time, measurement of the exposure is not required to precede measurement of the outcome. This requirement is easier met in prospective study designs (i.e. cohort studies), than when exposure is assessed retrospectively (case–control studies) or assessed at the same time than the outcome (cross-sectional studies). However, also in prospective studies, the time sequence for cause and effect and the temporal direction might be difficult to ascertain if a disease developed slowly and initial forms of disease were difficult to measure (Höfler, 2005).

The generalisability of the result from the population under study to a broader population should also be considered for study validity. While the random error discussed previously is considered a precision problem and is affected by sampling variability, **bias** is considered a validity issue. More



specifically, bias issues generally involve methodological imperfections in study design or study analysis that affect whether the correct population parameter is being estimated. The main types of bias include selection bias, information bias (including recall bias and interviewer/observer bias) and confounding. An additional potential source of bias is effect size magnification, which has already been mentioned.

**Selection bias** concerns a systematic error relating to validity that occurs as a result of the procedures and methods used to select subjects into the study, the way that subjects are lost from the study or otherwise influence continuing study participation.

Typically, such a bias occurs in a case–control study when inclusion (or exclusion) of study subjects on the basis of disease is somehow related to the prior exposure status being studied. One example might be the tendency for initial publicity or media attention to a suspected association between an exposure and a health outcome to result in preferential diagnosis of those that had been exposed compared to those that had not. Selection bias can also occur in cohort studies if the exposed and unexposed groups are not truly comparable as when, for example, those that are lost from the study (loss to follow-up, withdrawn or non-response) are different in status to those who remain. Selection bias can also occur in cross-sectional studies due to selective survival: only those that have survived are included in the study. These types of bias can generally be dealt with by careful design and conduct of a study (see also Sections 4, 6 and 8).

The 'healthy worker effect' (HWE) is a commonly recognised selection bias that illustrates a specific bias that can occur in occupational epidemiology studies: workers tend to be healthier than individuals from the general population overall since they need to be employable in a workforce and can thus often have a more favourable outcome status than a population-based sample obtained from the general population. Such a HWE bias can result in observed associations that are masked or lessened compared to the true effect and thus can lead to the appearance of lower mortality or morbidity rates for workers exposed to chemicals or other deleterious substances.

**Information bias** concerns a systematic error when there are systematic differences in the way information regarding exposure or the health outcome are obtained from the different study groups that result in incorrect or otherwise erroneous information being obtained or measured with respect to one or more covariates being measured in the study. Information bias results in misclassification which in turn leads to incorrect categorisation with respect to either exposure or disease status and thus the potential for bias in any resulting epidemiological effect size measure such as an OR or RR.

Misclassification of exposure status can result from imprecise, inadequate or incorrect measurements; from a subject's incorrect self-report; or from incorrect coding of exposure data.

Misclassification of disease status can, for example, arise from laboratory error, from detection bias, from incorrect or inconsistent coding of the disease status in the database, or from incorrect recall. Recall bias is a type of information bias that concerns a systematic error when the reporting of disease status is different, depending on the exposure status (or vice versa). Interviewer bias is another kind of information bias that occurs where interviewers are aware of the exposure status of individuals and may probe for answers on disease status differentially – whether intended or not – between exposure groups. This can be a particularly pernicious form of misclassification – at least for case–control studies – since a diseased subject may be more likely to recall an exposure that occurred at an earlier time period than a non-diseased subject. This will lead to a bias away from null value (of no relation between exposure and disease) in any effect measure.

Importantly, such misclassifications as described above can be 'differential' or 'non-differential' and these relate to (i) the degree to which a person that is truly exposed (or diseased) is correctly classified as being truly exposed or diseased and (ii) the degree to which an individual who is truly not exposed (or diseased) is correctly classified in that way. The former is known as 'sensitivity' while the latter is referred to as 'specificity' and both of these play a role in determining the existence and possible direction of bias. Differential misclassification means that misclassification has occurred in a way that depends on the values of other variables, while non-differential misclassification refers to misclassifications that do not depend on the value of other variables.

What is important from an epidemiological perspective is that misclassification biases – either differential or non-differential – depend on the sensitivity and specificity of the study's methods used to categorise such exposures and can have a predictable effect on the direction of bias under certain (limited) conditions: this ability to characterise the direction of the bias based on knowledge of the study methods and analyses can be useful to the regulatory decision-maker since it allows the decision maker to determine whether the epidemiological effect sizes being considered (e.g. OR, RR) are likely underestimates or overestimates of the true effect size. While it is commonly assumed by some that



non-differential misclassification bias produces predictable biases towards the null (and thus systematically under-predicts the effect size), this is not necessarily the case. Also, the sometimes common assumption in epidemiology studies that misclassification is non-differential (which is sometimes also paired with the assumption that non-differential misclassification bias is always towards the null) is not always justified (e.g. see Jurek et al., 2005).

When unmeasured confounders are thought to affect the results, researchers should conduct sensitivity analyses to estimate the range of impacts and the resulting range of adjusted effect measures (US-EPA, 2010b). Quantitative sensitivity (or bias) analyses are, however, not typically conducted in many epidemiological studies, with most researchers instead describing various potential biases qualitatively in the form of a narrative in the discussion section of a paper.

It is often advisable that the epidemiological investigator performs sensitivity analysis to estimate the impact of biases, such as exposure misclassification or selection bias, by known but unmeasured risk factors or to demonstrate the potential effects that a missing or unaccounted for confounder may have on the observed effect sizes (see Lash et al., 2009; Gustafson and McCandless, 2010). Sensitivity analyses should be incorporated in the list of criteria for reviewing epidemiological data for risk assessment purposes.

# 3. Key limitations of the available epidemiological studies on pesticides

3.1. Limitations identified by the authors of the EFSA external scientific report

The EFSA External scientific report (Ntzani et al., 2013; summarised in Annex A) identified a plethora of epidemiological studies which investigate diverse health outcomes. In an effort to systematically appraise the epidemiological evidence, a number of methodological limitations were highlighted. In the presence of these limitations, robust conclusions could not be drawn, but outcomes for which supportive evidence from epidemiology existed were highlighted for future investigation. The main limitations identified included (Ntzani et al., 2013):

- Lack of prospective studies and frequent use of study designs that are prone to bias (casecontrol and cross-sectional studies). In addition, many of the studies assessed appeared to be insufficiently powered.
- Lack of detailed exposure assessment, at least compared to many other fields within epidemiology. The information on specific pesticide exposure and co-exposures was often lacking, and appropriate biomarkers were seldom used. Instead, many studies relied on broad definition of exposure assessed through questionnaires (often not validated).
- Deficiencies in outcome assessment (broad outcome definitions and use of self-reported outcomes or surrogate outcomes).
- Deficiencies in reporting and analysis (interpretation of effect estimates, confounder control and multiple testing).
- Selective reporting, publication bias and other biases (e.g. conflict of interest).

The observed heterogeneity in the results within each studied outcome was often large. However, heterogeneity is not always a result of biases and may be genuine and consideration of *a priori* defined subgroup analysis and meta-regression should be part of evidence synthesis efforts. Occupational studies, which are of particular importance to pesticide exposure, are also vulnerable to the healthy worker effect, a bias resulting in lower morbidity and mortality rates within the workforce than in the general population. The healthy worker effect tends to decline with increasing duration of employment and length of follow-up.

Studies with sufficient statistical power, detailed definition of pesticide exposure, data for many health outcomes and transparent reporting are rare, apart from the Agricultural Health Study (AHS) and other similarly designed studies. It is important to note that several of these methodological limitations have not been limited to pesticide exposure studies and, most importantly, are not specific in epidemiology and have been observed in other specific fields including in animal studies (Tsilidis et al., 2013).

Given the wide range of pesticides with various definitions found in the EFSA External scientific report, it is difficult to harmonise this information across studies. Although heterogeneity of findings across studies can be as informative as homogeneity, information needs to be harmonised such that replication can be assessed and summary effect sizes be calculated. This does not mean that if there is



genuine heterogeneity the different studies cannot be pooled. Limited conclusions can be made from a single study. Nonetheless, the report highlighted a number of associations between pesticides and health effects that merit further consideration and investigation. Of interest is the fact that a considerable proportion of the published literature focused on pesticides no longer approved for use in the EU and in most developed countries e.g. studies focusing solely on DDT and its metabolites constituted almost 10% of the eligible studies (Ntzani et al., 2013). These may still be appropriate since they may persist as pesticide residues or because they continue to be used in developing countries. Also, the report focused on epidemiological evidence in relation to any health outcome across an approximately 5-year window. Although the report is valuable in describing the field of epidemiological assessment of pesticide–health associations, it is not able to answer specific disease-pesticide guestions thoroughly. A more in-depth analysis of specific disease endpoints associated with pesticides exposure is needed, where this information is available, and studies published earlier than the time window covered by the EFSA External scientific report should be also included.

#### 3.2. Limitations in study designs

For ethical reasons, randomised controlled trials are not allowed to test the safety of low dose pesticide exposure in the EU. Therefore, information on potential adverse health consequences in humans has to be extracted using observational studies.

For diseases with long-latency periods, measurement of exposure at one time point may not accurately reflect the long-term exposure which is needed to develop such diseases. This is particularly important for non-persistent pesticides, whose levels in biological samples are not constant but vary quite often. Thus, those studies that claim an association between a single measurement in urine samples and a long latency outcome should be carefully interpreted.

Among the 795 studies reviewed in the Ntzani report, 38% were case-control studies and 32% cross-sectional studies. As a result, evidence on potential adverse health consequences of pesticide exposure is largely based on studies that lack prospective design at least for outcomes that have long latency periods. For the cross-sectional studies, directionality cannot be assessed and observed associations may often reflect reverse causation (is the disease caused by the exposure, or does the disease influence the exposure?). Although reverse causation is a potential problem of cross-sectional studies in many fields of epidemiology, in pesticide epidemiology, it is less of an issue, because in most situations it is unlikely that a disease will cause exposure to pesticides.

Although case–control studies are frequently used for rare outcomes, such as several cancers, their main limitation is that they are prone to recall bias and they have to rely on retrospective assessment of exposure. However, they can still provide useful information, especially for rare outcomes. It is important to examine whether results from case–control and prospective studies converge. This was, for example, the case amongst studies that were conducted to examine associations between intake of *trans*-fatty acids and cardiovascular disease (EFSA, 2004), where both case–control and prospective studies consistently reported positive associations. The effect estimates between the two study designs were systematically different with prospective studies reporting more modest effect sizes but both study designs reached similar conclusions. As for pesticides, similar values have been observed for the magnitude of association between Parkinson's disease and pesticide exposure irrespective of the study design (reviewed in Hernández et al., 2016).

#### **3.3.** Relevance of study populations

Because the environmentally relevant doses of pesticides to which individuals are exposed are lower than those required to induce observed toxicity in animal models, the associated toxic effects need to be understood in the context of differences of susceptibility of subpopulations. Potentially vulnerable groups are at an increased risk against exposure to low levels of pesticides than healthy individuals, sometimes during sensitive windows of exposure. This is the case of genetic susceptibility, which represents a critical factor for risk assessment that should be accounted for (Gómez-Martín et al., 2015). Genetic susceptibility largely depends on functional genetic polymorphisms affecting toxicokinetics (e.g. genes encoding xenobiotic metabolising enzymes and membrane transporters) and/or toxicodynamics (e.g. different receptor gene polymorphisms). This genetic variability should be considered on the basis of a plausible scientific hypothesis.

While different disorders, particularly neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis) have been linked to exposures to environmental factors (e.g.



pesticides), in many instances the genetic architecture of the disorder has not been taken into account. The prevalence of specific gene mutations may reach 5–10% and sometimes over 20% of cases in certain populations (Gibson et al., 2017), so that the links of these diseases to pesticide exposure may be heavily influenced by genetic structure within populations under study. Given the small effect sizes for many of these disorders, the underlying effects of specific genes not accounted for in the study design may modify the disease risk estimates. Hence, associations with pesticide exposure may need to be evaluated in the light of common genetic influences known to be associated with a spectrum of neurodegenerative diseases. However, genetic variation by itself does not predispose people for an increased pesticide exposure.

A subgroup of population of special interest is represented by children, because their metabolism, physiology, diet and exposure patterns to environmental chemicals differ from those of adults and can make them more susceptible to their harmful effects. The window(s) of biologic susceptibility remain unknown for the most part, and would be expected to vary by mechanism. Gender-based susceptibility also merits consideration in case of pesticide-related reproductive toxicity and endocrine disruption. Those subgroups are currently considered during the risk assessment process but may deserve more attention to provide additional protection.

#### **3.4.** Challenges in exposure assessment

The main limitations of epidemiological studies conducted on pesticides derive from uncertainty in exposure assessment. Limitations include the fact that most currently approved pesticides tend to have short elimination half-lives and that their use involves application of various formulations depending on the crop and season. As a result, accurate assessment needs to capture intermittent long-term exposure of these non-persistent chemicals as well as being able to quantify exposure to individual pesticides.

Numerous studies have assessed internal exposure by measuring urinary non-active metabolites common for a large group of pesticides (for example, dialkyl phosphates for organophosphates, 3-phenoxybenzoic acid for pyrethroids or 6-chloronicotinic acid for neonicotinoids). These data should not be utilised to infer any risk because: (a) a fraction of these metabolites might reflect direct exposure through ingestion of preformed metabolites from food and other sources, rather than ingestion of the parent compound and (b) the potency of the different parent pesticides can vary by orders of magnitude. Thereby, HBM data based on those urine metabolites can be unhelpful unless they are paired with other data indicating the actual pesticide exposure.

Ideally exposure should be quantified on an individual level using biomarkers of internal dose. As most available biomarkers reflect short term (few hours or days) exposure and given the cost and difficulty of collecting multiple samples over time, many studies quantify exposure in terms of external dose. Quantitative estimation of external dose needs to account for both frequency and duration of exposure and should preferably be done on an individual but not group level. Often external exposure is quantified using proxy measures such as:

- subject- or relative-reported jobs, job titles, tasks or other lifestyle habits which are being associated with the potential exposure to or actual use of pesticides in general;
- handling of a specific product or set of products and potential exposure to these as documented through existing pesticide records or diaries or estimated from crops grown;
- environmental data: environmental pesticide monitoring, e.g. in water, distance from and/or duration of residence in a particular geographical area considered to be a site of exposure.

In many cases, these proxy measures are recorded with use of questionnaires, which can be either interviewer-administered or based on self-report. However, questionnaire data often rely on individual recall and knowledge and are thus potentially subject to both recall bias and bias introduced by the interviewer or study subjects. These sources of bias can to some extent be quantified if the questionnaires are validated against biomarkers (that is, to what extent do individual questions predict biomarker concentrations in a sub-sample of participants). If the exposure is assessed retrospectively the accuracy of the recall is for obvious reasons more likely to be compromised and impossible to validate. When exposure is based on records, similar difficulties may occur due to, e.g. incomplete or inaccurate records.

In many previous studies, duration of exposure is often used as a surrogate of cumulative exposure, assuming that exposure is uniform and continuous over time (e.g. the employment period) but this assumption must be challenged for pesticides. Although for some chemicals the exposure patterns may be fairly constant, exposures for the large number of pesticides available in the market



will vary with season, by personal protective equipment (PPE) and by work practices, and in many cases, uses are not highly repetitive. At an individual level, exposures can vary on a daily and even hourly basis, and often involve several pesticides. This temporal variability can result in particularly high variation in systemic exposures for pesticides with short biological half-lives and considerable uncertainty in extrapolating single or few measurements to individual exposures over a longer term. Hence, many repeated measurements over time may be required to improve exposure estimates.

### **3.5.** Inappropriate or non-validated surrogates of health outcomes

Self-reported health outcomes are frequently used in epidemiological research because of the difficulty of verifying responses in studies with large samples and limited funds, among other reasons. Although a number of studies have examined agreement between self-reported outcomes and medical records, the lack of verification of such metrics can lead to misclassification, particularly in large population-based studies, which may detract from reliability of the associations found.

Reliance on clinically manifested outcomes can increase the likelihood that individuals who have progressed along the toxicodynamic continuum from exposure to disease but have not yet reached an overt clinical disease state will be misclassified as not having the disease (Nachman et al., 2011). Thereby, delay in onset of clinical symptoms following exposure may cause underreporting where clinical assessment alone is used at an inappropriate point in time.

In the case of carcinogenesis, there are some examples where subclinical outcomes have been assessed as preneoplastic lesions with potential to progress to neoplastic conditions. This is the case of monoclonal gammopathy of undetermined significance (MGUS), which has been associated with pesticide exposure in the AHS (Landgren et al., 2009), as this condition has a 1% average annual risk of progression to malignant multiple myeloma (Zingone and Kuehl, 2011). However, it is difficult to predict if and when an MGUS will progress to multiple myeloma. Since there are studies indicating that pesticide exposure may be associated with the risk of precancerous lesions in animal research, a combined epidemiological analysis of both preneoplastic and neoplastic outcomes may increase the power of such an analysis.

Surrogate outcomes may seem an attractive alternative to clinically relevant outcomes since there may be various surrogates for the same disease and they may occur sooner and/or be easier to assess, thereby shortening the time to diagnosis. A valid surrogate endpoint must, however, be predictive of the causal relationship and accurately predict the outcome of interest. In addition, these surrogates should be relevant to the mode of action of a pesticide such that they should be anchored to established toxicological endpoints to support their predictivity. Although surrogate markers may correlate with an outcome, they may not capture the effect of a factor on the outcome. This may be because the surrogate may not be causally or strongly related to the clinical outcome, but only a concomitant factor, and thus may not be predictive of the clinical outcome. The validity of surrogate outcomes may thus represent a major limitation to their use (la Cour et al., 2010).

However, concerns arise as to whether critical regulatory decisions can be made based on epidemiological studies that did not directly measure the adverse health outcome but valid surrogates instead. The use of surrogates as replacement endpoints should be considered only when there is substantial evidence to establish their reliability in predicting clinical meaningful effects.

# **3.6.** Statistical analyses and interpretation of results

The statistical analyses and the interpretation of scientific findings that appear in the epidemiological literature on the relationship between pesticides and health outcomes do not substantially deviate from those reported in other fields of epidemiological research. Therefore, the advantages and limitations of epidemiological studies presented in Section 2.5 also apply to the epidemiological studies on pesticides.

The few distinctive features of the epidemiological studies on pesticides include the following: (a) sparse use of appropriate statistical analyses in the presence of measurement errors when assessing exposure to pesticides and (b) paucity of information on other important factors that may affect the exposure–health outcome relationship. These features are expanded on in the following paragraphs.

a) Statistical analyses in the presence of measurement errors

The difficulties inherent in correctly measuring exposure are frequent in many areas of epidemiological research, such as nutritional epidemiology and environmental epidemiology. It is not



easy to gauge the short- and long-term exposure outside controlled laboratory experimental settings. In large populations, individuals are exposed to a variety of different agents in a variety of different forms for varying durations and with varying intensities.

Unlike nutritional or environmental epidemiology, however, pesticide epidemiology has so far made little use of statistical analyses that would appropriately incorporate measurement errors, despite their wide availability and sizable literature on the topic. A direct consequence of this is that the inferential conclusions may not have been as accurate and as precise as they could have been if these statistical methods were utilised (Bengtson et al., 2016; Dionisio et al., 2016; Spiegelman, 2016).

#### b) Information on other important factors of interest

Identifying and measuring the other relevant factors that might affect an outcome of interest is a recurrent and crucial issue in all fields of science. For example, knowing that a drug effectively cures a disease on average may not suffice if such drug is indeed harmful to children or pregnant women. Whether or not age, pregnancy and other characteristics affect the efficacy of a drug is an essential piece of information to doctors, patients, drug manufacturers and drug-approval agencies alike.

Pesticide epidemiology provides an opportunity for careful identification, accurate measuring and thorough assessment of possible relevant factors and their role in the exposure-health outcome relationship. Most often, relevant factors have been screened as potential confounders. When confounding effects were detected, these needed to be adjusted for in the statistical analyses. This has left room for further investigations that would shed light on this important issue by reconsidering data that have already been collected and that may be collected in future studies. The statistical methods in the pesticide literature have been mainly restricted to standard applications of basic regression analyses, such as binary probability and hazard regression models. Potentially useful analytical approaches, such as propensity score matching, mediation analyses, and causal inference, would be helpful for pesticide epidemiology (Imbens and Rubin, 2015).

# 4. Proposals for refinement to future epidemiological studies for pesticide risk assessment

This section is aimed at addressing methods for assessment of available pesticide epidemiological studies and proposals for improvement of such studies to be useful for regulatory purposes.

When considering the potential regulatory use of epidemiological data, many of the existing epidemiological studies on pesticides exposure and health effects suffer from a range of methodological limitations or deficiencies which limit their value in the assessment of individual active substances. Epidemiological studies on pesticides exposure and health effects would ideally generate semi-quantitative data or be able to have greater relevance to quantitative risk assessment with respect to the output from prediction models. This would allow epidemiological results to be expressed in terms more comparable to the quantitative risk assessments, which are more typically used in evaluating the risks of pesticides. The question arises how such epidemiological data could be considered for risk assessment when judged in comparison to the predictive models. A precisely measured quantitative dose–response relationship is presently rarely attainable as a result of current pesticide epidemiological studies.

The quality, reliability and relevance of the epidemiological evidence in relation to pesticide exposure and health effects can be enhanced by improving (a) the quality of each individual study and (b) the assessment of the combined evidence accrued from all available studies.

# 4.1. Assessing and reporting the quality of epidemiological studies

The quality and relevance of epidemiological research should be considered when selecting epidemiological studies from the literature for use in risk assessment. The quality of this research can be enhanced by (US-EPA, 2012; Hernández et al., 2016):

- a) an adequate assessment of exposure, preferentially biomarker concentrations at individual level reported in a way which will allow for a dose–response assessment;
- b) a reasonably valid and reliable outcome assessment (well-defined clinical entities or validated surrogates);
- c) an adequate accounting for potentially confounding variables (including exposure to multiple chemicals);
- d) the conduct and reporting of subgroup analysis (e.g. stratification by gender, age, ethnicity).



It is widely accepted that biomedical research is subject to and suffers from diverse limitations. An assessment of weaknesses in the design, conduct and analysis of epidemiology research studies on pesticides is essential to identify potentially misleading results and identify reliable data.

Guidelines and checklists help individuals meet certain standards by providing sets of rules or principles that guide towards the best behaviour in a particular area. Several tools and guidelines have been developed to aid the assessment of epidemiological evidence; however, there is no specific tool for assessing studies on pesticides. Although these studies have special considerations around exposure assessment that require specific attention, standard epidemiological instruments for critical appraisal of existing studies may apply. Existing reporting guidelines usually specify a minimum set of information needed for a complete and clear account of what was done and what was found during a research study focusing on aspects that might have introduced bias into the research (Simera et al., 2010).

A number of tools were specifically designed for quality appraisal of observational epidemiological studies, such as the Newcastle–Ottawa scale (NOS) and the Research Triangle Institute (RTI) item bank. The latter is a practical and validated tool which consists of a checklist of 29 questions for evaluating the risk of bias and precision of epidemiological studies of chemical exposures. In addition, the Biomonitoring, Environmental Epidemiological research that use biomonitoring to assess short-lived chemicals (LaKind et al., 2015), but it can also be used for persistent chemicals and environmental measures as its main elements are cross-cutting and are more broadly applicable. Two earlier efforts to develop evaluative schemes focused on epidemiology research on environmental chemical exposures and neurodevelopment (Amler et al., 2006; Youngstrom et al., 2011).

Regarding guality of reporting, the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) Network, officially launched in June 2008, is an international initiative that promotes transparent and accurate reporting of health research studies. It currently lists over 90 reporting guidelines with some of them being specific for observational epidemiological studies (e.g. Strengthening the Reporting of OBservational studies in Epidemiology (STROBE)). The STROBE statement includes recommendations on what should be included in an accurate and complete report of an observational study including cross-sectional, case-control and cohort studies using a checklist of 22 items that relate to the title, abstract, introduction, methods, results and discussion sections of articles (von Elm et al., 2007). The STROBE statement has been endorsed by a growing number of biomedical journals which refer to it in their instructions for authors. Table 1 presents a summary of the main features that STROBE proposes to be taking into account when assessing the quality of reporting epidemiological studies. Extensions to STROBE are available including the STROBE Extension to Genetic Association studies (STREGA) initiative and the STROBE-ME statement for assessment of molecular epidemiology studies. Since the STROBE checklist mentions only in a general way exposure and health outcomes, the PPR Panel recommends that an extension of the STROBE statement be developed, for inclusion in the EQUATOR network library, specifically relevant to the area of pesticide exposure and health outcomes. This would greatly assist researchers and regulatory bodies in the critical evaluation of study guality.

Factor	Item	Recommendation	
Title and Abstract			
	1	<ul><li>a) Indicate the study's design with a commonly used term in the title of the abstract</li><li>b) Provide in the abstract an informative and balanced summary of what was done and what was found</li></ul>	
Introduction			
Background/rationale	2	2 Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including prespecified hypotheses	
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up and data collection	

Table 1:	Main features of the STROBE tool to	assess quality of reporting of epidemiological studies
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STROBE Statement	Items		
Factor	Item	Recommendation	
Participants	6	<ul> <li>a) Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study – Give eligibility criteria, and the sources and methods of selection of participants</li> <li>b) Cohort study – For matched studies, give matching criteria and the number of exposed and unexposed Case-control study – For matched studies, giving matching criteria and the number of controls per case</li> </ul>	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurements	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	<ul> <li>a) Describe all statistical methods, including those used to control for confounding</li> <li>b) Describe any methods used to examine subgroups and interactions</li> <li>c) Explain how missing data were addressed</li> <li>d) Cohort study – If applicable, explain how loss to follow-up was addressed Case-control study – If applicable, explain how matching of cases and controls was addressed</li> <li>Cross-sectional study – If applicable, describe analytical methods taking account of sampling strategy</li> <li>e) Describe any sensitivity analyses</li> </ul>	
Results			
Participants	13*	<ul> <li>a) Report numbers of individuals at each stage of study – e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up and analysed</li> <li>b) Give reasons for non-participation at each stage</li> <li>c) Consider use of a flow diagram</li> </ul>	
Descriptive data	14*	<ul> <li>a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders</li> <li>b) Indicate number of participants with missing data for each variable of interest</li> <li>c) <i>Cohort study</i> – Summarise follow-up time (e.g. average and total amount)</li> </ul>	
Outcome data	15*	Cohort study – Report numbers of outcome events or summary measures over time Case–control study – Report numbers in each exposure category, or summary measures of exposure Cross-sectional study – Report numbers of outcome events or summary measures	
Main results	16	<ul> <li>a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included</li> <li>b) Report category boundaries when continuous variables were categorised</li> <li>c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</li> </ul>	



STROBE Statement Items			
Factor Item Recommendation		Recommendation	
Other analyses	17	Report other analyses done – e.g. analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

\*: Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Selective reporting can occur because non-significant results or unappealing significant results may not be published. Investigators should avoid the selective reporting of significant results and high-risk estimates. In this regard, standardisation of reporting of epidemiological studies could help to reduce or avoid selective reporting. The STROBE statement and similar efforts are useful tools for this purpose. Although some epidemiological research will remain exploratory and *post hoc* in nature, this should be clarified in the publications and selective reporting minimised, so that epidemiological findings could be interpreted in the most appropriate perspective (Kavvoura et al., 2007).

Preregistration of studies and prepublication of protocols are the measures taken by some Journal editors and Ethics Committees to reduce reporting bias and publication bias in clinical trials on pharmaceuticals. Although a similar proposal has been suggested for observational epidemiological studies in order to be conducted as transparently as possible to reduce reporting bias and publication bias, there is no consensus among epidemiologists (Pearce, 2011; Rushton, 2011). In contrast, a number of initiatives have been undertaken by professional societies to foster good epidemiological practice. This is the case, for example, of the International Epidemiological Association (IEA, 2007) or the Dutch Society for Epidemiology on responsible epidemiologic Research Practice (DSE, 2017).

Data quality assessment of formal epidemiological studies is based solely on the methodological features of each individual study rather than on the results, regardless of whether they provide evidence for or against an exposure/outcome association. However, for risk assessment, it is important to assess not only the quality of study methods but also the quality of the information they provide. Indeed, good studies may be dismissed during the formal quality assessment by the poor reporting of the information.

# 4.2. Study design

Well conducted prospective studies with appropriate exposure assessment provide the most reliable information and are less prone to biases. When prospective studies are available, results from studies of less robust design can give additional support. In the absence of prospective studies the results from cross-sectional and case-control studies should be considered but interpreted with caution. However, it is acknowledged that a well-designed case-control study may be superior to a less well designed cohort study. Analytical approaches should be congruent with the study design, and assumptions that the statistical methods required should be carefully evaluated.

Ideally observational studies for long-term diseases should be prospective and designed such that the temporal separation between the exposure and the health outcome is appropriate with respect to the time it takes to develop the disease. For outcomes such as cancer or cardiovascular diseases, which often have a long latency period (> 10 years), exposure should be assessed more than once prior to the outcome assessment. For other outcomes with a shorter latency period, such as immune function disturbances, the appropriate temporal separation may be in the range of days or weeks and a single exposure assessment may be adequate. In short, the ideal design of a study depends on the latency period for the outcome under consideration. The expected latency period then determines both the length of follow-up and the frequency for which the exposure has to be quantified.



# 4.3. Study populations

The EU population, which exceeds 500 million people, can be assumed to be fairly heterogeneous and so expected to include a number of more sensitive individuals that may be affected at lower doses of pesticide exposure. To address this, in stratified sampling, the target population is divided into subgroups following some key population characteristics (e.g. sex, age, geographic distribution, ethnicity or genetic variation) and a random sample is taken within each subgroup. This allows subpopulations to be represented in a balanced manner in the study population.

Vulnerable populations should then be examined in epidemiological studies either through subgroup or sensitivity analysis. However, such analyses need to be defined *a priori*. In case of ad hoc subgroup sensitivity analysis, the statistical thresholds should be adjusted accordingly and the replication of results should follow. Evidence of vulnerable subpopulations would ideally involve prospective studies that include assessment of biomarkers of exposure, subclinical endpoints and disease incidence over time.

It may be impossible to find a threshold of a toxic-induced increase in disease in the population because a large number of people are in a preclinical state and would be sensitive to the low end of the dose-response curve. For that to be evident, the epidemiology data would need to characterise the relationship between chemical exposure and risk of disease in a broad cross-section of the population (or look at precursor lesions or key events) and allow a robust examination of a low-dose slope.

On the basis of the degree of evidence relevant to a vulnerable subpopulation, consideration should be given to whether dose-response assessment will focus on the population as a whole or will involve separate assessments for the general population and susceptible subgroups. If it is the population as a whole, the traditional approach is to address variability with uncertainty factors; it may also be possible to analyse the effect of variability on risk by evaluating how the risk distribution of the disease shifts in response to the toxicant. In essence, the risk distribution based on a subclinical biomarker is an expression of toxicodynamic variability that can be captured in dose-response assessment.

The alternative approach is to address vulnerable subpopulations as separate from the general population and assign them unique potencies via dose–response modelling specific to the groups that might be based on actual dose–response data for the groups, on adjustments for specific toxicokinetic or toxicodynamic factors, or on more generic adjustment or uncertainty factors. For a pesticide, if it is known that a particular age group, disease (or disease-related end-point), genetic variant or co-exposure creates unique vulnerability, efforts should be made to estimate the potency differences relative to the general population and on that basis to consider developing separate potency values or basing a single value on the most sensitive group or on the overall population with adjustments for vulnerable groups.

#### 4.4. Improvement of exposure assessment

The difficulties often associated with pesticide exposure assessment in epidemiological studies have been highlighted above. The description of pesticide exposure (in particular quantitative information on exposure to individual pesticides) is generally reported in insufficient detail for regulatory purposes and this limitation is difficult to overcome, especially for diseases with a long latency period (e.g. many cancers and neurodegenerative disorders).

It is noteworthy that the methods necessary to conduct exposure monitoring are to be submitted by the applicant in the dossier. The regulation requirements do ask for validated methods that can be used for determining exposure. The Commission Regulation (EU) No 283/2013, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of PPP on the market, addresses information on methods of analysis required to support both pre-approval studies and post-approval monitoring. In this context, the post-approval requirements are the most relevant and the regulation literally states:

'4.2. Methods for post-approval control and monitoring purposes – Methods, with a full description, shall be submitted for:

 a) the determination of all components included in the monitoring residue definition as submitted in accordance with the provisions of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin;



- b) the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2;
- c) the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible;
- d) the analysis in body fluids and tissues for active substances and relevant metabolites.

As far as practicable these methods shall employ the simplest approach, involve the minimum cost, and require commonly available equipment. The specificity of the methods shall be determined and reported. It shall enable all components included in the monitoring residue definition to be determined. Validated confirmatory methods shall be submitted if appropriate. The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. The LOQ shall be determined and reported for each component included in the monitoring residue definition. For residues in or on food and feed of plant and animal origin and residues in drinking water, the reproducibility of the method shall be determined by means of an independent laboratory validation (ILV) and reported'.

From this, it can be concluded that the requirements exist, but are somewhat less stringent for human biomonitoring than for monitoring of residues in food and feed.

Failure to use these existing methods restricts the potential for the use of epidemiological evidence in the regulation of specific pesticides. It is therefore important that those contemplating future studies carefully consider approaches to be used to avoid misclassification of exposure, and to conduct appropriate detailed exposure assessments for specific pesticides, which allow for sound dose– response analyses, and demonstrate the validity of the methods used.

A given exposure may have a different health impact depending on the period in the lifespan when exposure takes place. Greater attention needs to be paid to exposures occurring during periods of potential susceptibility for disease development by ensuring that the exposure assessment adequately addresses such critical times. This may be particularly relevant for studies involving neurodevelopment, obesity or allergic responses, which are complex multistage developmental processes that occur either prenatally or in the early post-natal life. For this reason, measurement of the exposure at one single time period may not properly characterise relevant exposures for all health effects of the environmental factors, and thus, the possibility arises of needing to measure the exposure at several critical periods of biological vulnerability to environmental factors. It is particularly challenging to construct an assessment of historical exposure swhich may deviate from current exposures, in both the range of chemicals and intensity of exposure and also co-exposure to other substances which are not included in the scope of study.

There are advantages and disadvantages to all methods of measuring pesticide exposure, and specific study designs and aims should be carefully considered to inform a specific optimal approach.

Exposure assessment can be improved at the *individual* level in observational research by using:

a) Personal exposure monitoring: This can be used to document exposures as readings measure pesticide concentration at the point of contact. Personal exposure monitors have been costly and burdensome for study participants. However, technological advances have recently driven personal exposure monitoring for airborne exposures to inexpensive, easy to use devices and these are suitable for population research. Personal exposure monitors that are specific to pesticide exposure could involve sensors to measure airborne concentrations, 'skin' patches to measure dermal concentrations, indoor home monitors that capture dust to measure other means of exposure. These mobile technology advances can be employed to provide observational studies with detailed and robust exposure assessments. Such equipment is now increasingly being adapted to serve large-scale population research and to capture data from large cohort studies. These coupled with other technological advances, such as real time data transfers via mobile phones and mobile phone applications to capture lifestyle and other habits, could bring next generation observational studies far more detailed and robust exposure assessments compared to current evidence. However, the generation of huge volumes of data can pose organisational, statistical and technical challenges, particularly with extended follow-up times. Ethics and personal data protection issue should be taken into account, and local regulations may prevent extensive use of such technologies. However, use of such personal monitors only provides information for one of the different potential routes of exposure.

b) **Biomarkers of exposure** (human biomonitoring (HBM)). An alternative and/or complementary approach is to ascertain the internal dose, which is the result of exposure via different routes (dermal,



inhalation and dietary exposure). These biomarkers have the potential to play an important role in assessing aggregate exposure to pesticides and informing cumulative risk assessment. Biomonitoring requires measurements in biological samples of concentrations of chemical under consideration (parent or metabolites) or markers of pathophysiologic effects thereof (such as adducts). However, challenges may include uncertainties relating to extrapolation of measured concentrations in biological samples to relevant doses.

Although biomonitoring has the potential to provide robust estimates of absorbed doses of xenobiotics, modern pesticides and their metabolites are eliminated from the body relatively quickly, with excretion half-lives typically measured in a few days (Oulhote and Bouchard, 2013). Consequently, use of biomarkers is both resource intensive and intrusive. The process is even more intrusive when it has to be conducted repeatedly on large numbers of individuals to monitor exposures over long durations.

Nevertheless, because of the potential to provide accurate integrated estimates of absorbed doses, biological monitoring of pesticides and their metabolites can be usefully employed to calibrate other approaches of exposure assessment. A good example of such an approach is that used by the Agricultural Health Study (Thomas et al., 2010; Coble et al., 2011; Hines et al., 2011). Also, HBM methods can be used with other forms of exposure assessment for the construction of long exposure histories.

Biomonitoring improves the precision in characterisation of exposure and allows the investigation of changes in exposure that occur at environmentally relevant exposure concentrations. Data collected in large-scale biomonitoring studies can be useful in setting reference ranges to assist in exposure classification in further epidemiological studies. Biomonitoring data also provide critical information for conducting improved risk assessment and help to identify subpopulations at special risk for adverse outcomes.

Biobanks, as repositories of biological samples, can be exploited to assess biomarkers of exposure with the aim of investigating early exposure–late effect relationships. That is, whether exposures occurring during early life are critical for disease development later in life (e.g. neurobehavioral impairment, children tumours, immunotoxic disorders, etc.) and to retrospectively assess health risks according to current health guidelines.

The results of measurements of metabolite levels in human matrices, e.g. urine, blood or hair do not provide the complete story with respect to the actual received dose. Additional assessment, possibly employing physiological-based toxicokinetic (PBTK) approaches, may be required to estimate the total systemic or tissue/organ doses. A PBTK model is a physiologically based compartmental model used to characterise toxicokinetic behaviour of a chemical, in particular for predicting the fate of chemicals in humans. Data on blood flow rates, metabolic and other processes that the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBTK model. PBTK models cannot be used only to translate external exposures into an internal (target) dose in the body, but also to infer external exposures from biomonitoring data. Furthermore, PBTK models need to be validated.

Toxicokinetic processes (ADME) determine the 'internal concentration' of an active substance reaching the target and help to relate this concentration/dose to the observed toxicity effect. Studies have been prescribed by the current regulations, but it would be beneficial to survey all the evidence, be it from *in vitro*, animal or human studies, about toxicokinetic behaviour of an active substance. Further discussion on quality assurance issues and factors to consider in relation to HBM studies is present in the report of the EFSA outsourced project (Bevan et al., 2017).

Exposure assessment can also be improved at the *population* level in observational research by using:

a) Larger epidemiological studies that make use of novel technologies and big data availability, such as **registry data** or data derived from large databases (including administrative databases) on health effects and pesticide usage, could provide more robust findings that might eventually be used for informed decision-making and regulation. Much effort needs to concentrate around the use of registered data which may contain records of pesticide use by different populations, such as farmers or other professional users that are required to maintain.<sup>9</sup> Such data could be further linked to

<sup>&</sup>lt;sup>9</sup> Regulation 1107/2009 Article 67 states: Record-keeping 1. Producers, suppliers, distributors, importers, and exporters of plant protection products shall keep records of the plant protection products they produce, import, export, store or place on the market for at least 5 years. Professional users of plant protection products shall, for at least 3 years, keep records of the plant protection products they use, containing the name of the plant protection product, the time and the dose of application, the area and the crop where the plant protection product was used. They shall make the relevant information contained in these records available to the competent authority on request. Third parties such as the drinking water industry, retailers or residents, may request access to this information by addressing the competent authority. The competent authorities shall provide access to such information in accordance with applicable national or Community law.



electronic health records (*vide supra*) and provide studies with unprecedented sample size and information on exposure and subsequent disease and will eventually be able to answer robustly previously unanswered questions. At the same time, information on active substances needs to be better captured in these registries and large databases. Dietary pesticide residue exposure can be estimated more accurately by using spraying journal data in combination with supervised residue trials. This method has the advantage of including more comprehensive and robust source data, more complete coverage of used pesticides and more reliable and precise estimates of residues below standard limit of quantification (LOQ) (Larsson et al., 2017).

b) Novel sophisticated approaches to **geographical information systems** (GIS) and small area studies might also serve as an additional way to provide estimates of residential exposures. Exposure indices based on GIS (i.e. residential proximity to agricultural fields and crop surface with influence around houses), when validated, may represent a useful complementary tool to biomonitoring and have been used to assess exposure to pesticides with short biological half-lives (Cornelis et al., 2009). As some such exposures maybe influenced by wind direction, amongst other factors, this should be taken into account through a special analysis of outcomes to make best of use of the approach. Also, these indices could be more representative, albeit non-specific, measures of cumulative exposure to non-persistent pesticides for long periods of time than biomonitoring data (González-Alzaga et al., 2015).

As already discussed, to be useful for the regulatory risk assessments of individual compounds epidemiological exposure assessments should provide information on specific pesticides. However, epidemiological studies which include more generic exposure assessments also have the potential to identify general risk factors and suggest inferences of causal associations in relevant human populations. Such observations may be important both informing overall regulatory policies, and for identification of matters for further epidemiological research.

Recent advances in modern technologies make it possible to estimate pesticide exposures to an unprecedented extent using novel analytical strategies:

a) The development of the so called **-omic techniques**, such as metabolomics and adductomics, also presents intriguing possibilities for improving exposure assessment through measurement of a wide range of molecules, from xenobiotics and metabolites recorded over time in biological matrices (blood, saliva, urine, hair, nails, etc.), to covalent complexes with DNA and proteins (adductomics) and understanding biological pathways. These methodologies could be used in conjunction with other tools. There is also both interest and the recognition that further work is required before such techniques can be applied in regulatory toxicology. The use of the exposome (the totality of exposures received by an individual during life) might be better defined by using 'omics' technologies and biomarkers appropriate for human biomonitoring. Nevertheless, important limitations have to be acknowledged because of the lack of validation of these methodologies and their cost, which limits their use at large scale.

b) Environmental exposures are traditionally assessed following 'one-exposure-one-health-effect' approach. In contrast, the **exposome** encompass the totality of human environmental exposures from conception onward complementing the genetics knowledge to characterise better the environmental components in disease aetiology. As such, the exposome includes not only any lifetime chemical exposures but also other external and or internal environmental factors, such as infections, physical activity, diet, stress and internal biological factors (metabolic factors, gut microflora, inflammation and oxidative stress). A complete exposome would have to integrate many external and internal exposures from different sources continuously over the life course. However, a truly complete exposome will likely never be measured. Although all these domains of the exposome need to be captured by using different approaches than the traditional ones, it is envisaged that no single tool will be enough to this end.

The more holistic approach of exposure is not intended to replace the traditional 'one-exposureone-health-effect' approach of current epidemiological studies. However, it would improve our understanding of the predictors, risk factors and protective factors of complex, multifactorial chronic diseases. The exposome offers a framework that describes and integrates, holistically, the environmental influences or exposures over a lifetime (Nieuwenhuijsen, 2015).

Collaborative research and integration of epidemiological or exploratory studies forming large consortia are needed to validate these potential biomarkers and eventually lead to improved exposure assessment. The incorporation of the exposome paradigm into traditional biomonitoring approaches offers a means to improve exposure assessment. Exposome-wide association studies (EWAS) allow to measurement of thousands of chemicals in blood from healthy and diseased people, test for disease



associations and identify useful biomarkers of exposure that can be targeted in subsequent investigations to locate exposure sources, establish mechanisms of action and confirm causality (Rappaport, 2012). After identifying these key chemicals and verifying their disease associations in independent samples of cases and controls, the chemicals can be used as biomarkers of exposures or disease progression in targeted analyses of blood from large populations.

In relation to the exposome concept, the -omics technologies have the potential to measure profiles or signatures of the biological response to the cumulative exposure to complex chemical mixtures. An important advance would be to identify a unique biological matrix where the exposome could be characterised without assessing each individual exposure separately in a given biological sample. The untargeted nature of omics data will capture biological responses to exposure in a more holistic way and will provide mechanistic information supporting exposure-related health effects. Importantly, omics tools could shed light on how diverse exposures act on common pathways to cause the same health outcomes.

While improved exposure assessment increases the power to detect associations, in any individual study it is necessary to maximise the overall power of the study by optimising the balance between the resource used for conducting an exposure assessment for each subject and the total number of subjects.

#### 4.5. Health outcomes

For pesticides, the health outcomes are broad as these chemicals have not shown a particular effect in relation to just one single disease area. For each health outcome, multiple definitions may exist in the literature with a varying degree of validation and unknown reproducibility across different databases, which are limited by the lack of generalisability. A proper definition of a health outcome is critical to the validity and reproducibility of observational epidemiological studies, and the consistency and clarity of these definitions need to be considered across studies. While prospective observational studies have explicit outcome definitions, inclusion and exclusion criteria and standardised data collection, retrospective studies usually rely on identification of health outcomes based largely on coded data, and classification and coding of diseases may change over time. Detailed description of the actual codes used to define key health outcomes and the results of any validation efforts are valuable to future research efforts (Stang et al., 2012; Reich et al., 2013). An example of coded diseases is the ICD-10, which for instance can be used as a tool to standardise the broad spectrum of malignant diseases.

In some surveillance studies, it is preferable to use broader definitions with a higher sensitivity to identify all potential cases and then apply a narrower and more precise definition with a high positive predictive value to reduce the number of false positives and resulting in more accurate cases. In contrast, in formal epidemiological studies, a specific event definition is used and validated to determine its precision; however, the 'validation' does not test alternative definitions, so it is not possible to determine sensitivity or specificity.

Surrogate endpoints should be avoided unless they have been validated. Some criteria to assess the validity of a surrogate outcome include:

- The surrogate has been shown to be in the causal pathway of the disease. This can be supported by the following evidence: correlation of biomarker response to pathology and improved performance relative to other biomarkers; biological understanding and relevance to toxicity (mechanism of response); consistent response across mechanistically different compounds and similar response across sex, strain and species; the presence of dose– response and temporal relationship to the magnitude of response; specificity of response to toxicity; that is, the biomarker should not reflect the response to toxicities in other tissues, or to physiological effects without toxicity in the target organ.
- At least one well conducted trial using both the surrogate and true outcome (Grimes and Schulz, 2005; la Cour et al., 2010). Several statistical methods are used to assess these criteria and if they are fulfilled the validity of the surrogate is increased. However, many times some uncertainty remains, making it difficult to apply surrogates in epidemiological studies (la Cour et al., 2010).

The data on health outcomes over the whole EU is potentially very extensive. If it can be managed effectively, it will open the prospect of greater statistical power for epidemiological studies assessing deleterious effects using very large sample sizes. Necessary prerequisites for these studies which may



detect new subtle effects, chronic effects or effects on subpopulations when stratified are beyond the remit of risk assessment. They include trans-national approaches to health informatics where harmonised diagnostics, data storage and informatics coupled with legally approved access to anonymised personal data for societal benefit are established. Health records should include adequate toxidrome classification. The latter may in turn require improvements in medical and paramedical training to ensure the quality of the input data.

Another opportunity for biological monitoring to be employed is where the investigation involves the so-called biomarkers of effect. That is a quantifiable biochemical, physiological, or other change that, depending on the magnitude, is associated with an established or possible health impairment or disease. Biomarkers of effect should reflect early biochemical modifications that precede functional or structural damage. Thus, knowledge of the mechanism ultimately leading to toxicity is necessary to develop specific and useful biomarkers, and vice versa, an effect biomarker may help to explain a mechanistic pathway of the development of a disease. Such biomarkers should identify early and reversible events in biological systems that may be predictive of later responses, so that they are considered to be preclinical in nature. Advances in experimental -omics technologies will show promise and provide sound information for risk assessment strategies, i.e. on mode of action, response biomarkers, estimation of internal dose and dose–response relationships (DeBord et al., 2015). These technologies must be validated to assess their relevance and reliability. Once validated, they can be made available for regulatory purposes.

# 5. Contribution of vigilance data to pesticides risk assessment

In addition to the formal epidemiological studies discussed in Sections 2–4, other human health data can be generated from ad hoc reports or as a planned process, i.e. through monitoring systems that have been implemented at the national level by public health authorities or authorisation holders. Consistent with Sections 2–4, this section first reviews how such a monitoring system should operate, what the current situation is regarding the monitoring of pesticides and what recommendations for improvement can be made.

# 5.1. General framework of case incident studies

A continuous process of collection, reporting and evaluation of adverse incidents has the potential to improve the protection of health and safety of users and others by reducing the likelihood of the occurrence of the same adverse incident in different places at later times, and also to alleviate consequences of such incidents. This obviously also requires timely dissemination of the information collected on such incidents. Such a process is referred to as vigilance.<sup>10</sup>

For example in the EU, the safety monitoring of medicines is known as pharmacovigilance; the pharmacovigilance system operates between the regulatory authorities in Member States, the European Commission and the European Medicines Agency (EMA). In some Member States, regional centres are in place under the coordination of the national Competent Authorities. Manufacturers and health care professionals report incidents to the Competent Authority at the national level, which ensures that any information regarding adverse reactions is recorded and evaluated centrally and also notifies other authorities for subsequent actions. The records are then centralised by the EMA which supports the coordination of the European pharmacovigilance system and provides advice on the safe and effective use of medicines.

# 5.2. Key limitations of current framework of case incident reporting

Several EU regulations require the notification and/or collection and/or reporting of adverse events caused by pesticides in humans (occurring after acute or chronic exposure in the occupational setting, accidental or deliberate poisoning, etc.). These include:

• Article 56 of EC Regulation 1107/2009 requires that 'The holder of an authorisation for a plant protection product shall immediately notify the Member States [...] In particular, potentially

<sup>&</sup>lt;sup>10</sup> The concept of <u>survey</u> refers to a single effort to measure and record something, and <u>surveillance</u> refers to repeated standardized surveys to detect trends in populations in order to demonstrate the absence of disease or to identify its presence or distribution to allow for timely dissemination of information. <u>Monitoring</u> implies the intermittent analysis of routine measurements and observations to detect changes in the environment or health status of a population, but without eliciting a response. <u>Vigilance</u> is distinct from surveillance and mere monitoring as it implies a process of paying close and continuous attention, and in this context addresses specifically post marketing events related to the use of a chemical.



harmful effects of that plant protection product, or of residues of an active substance, its metabolites, a safener, synergist or co-formulant contained in it on human health [...] shall be notified. To this end the authorisation holder shall record and report all suspected adverse reactions in humans, in animals and the environment related to the use of the plant protection product. The obligation to notify shall include relevant information on decisions or assessments by international organisations or by public bodies which authorise plant protection products or active substances in third countries'.

Article 7 of EC Directive 128/2009 establishing a framework for Community action to achieve
the sustainable use of pesticides requires that: '2. <u>Member States</u> shall put in place systems for
gathering information on pesticide acute poisoning incidents, as well as chronic poisoning
developments where available, among groups that may be exposed regularly to pesticides such
as operators, agricultural workers or persons living close to pesticide application areas. 3. To
enhance the comparability of information, <u>the Commission</u>, in cooperation with the Member
States, shall develop by 14 December 2012 a strategic guidance document on monitoring and
surveying of impacts of pesticide use on human health and the environment'. However, at the
time of publishing this scientific opinion, this document has still not been released.

There are three additional regulations that apply, although indirectly, to pesticides and reporting:

- EC Regulation 1185/2009 concerning statistics on pesticides requires that Member States shall collect data on pesticide sales and uses according to a harmonised format. The statistics on the placing on the market shall be transmitted yearly to the Commission and the statistics on agricultural use shall be transmitted every 5 year.
- Article 50 of Regulation (EC) 178/2002, laying down the general principles and requirements of food law, set up an improved and broadened rapid alert system covering food and feed (RASFF). The system is managed by the <u>Commission</u> and includes as members of the network Member States, the Commission and the Authority. It reports on non-authorised occurrences of pesticides residues and food poisoning cases.
- Article 45 (4) of EC Regulation 1272/2008 (CLP Regulation): importers and downstream users placing hazardous chemical mixtures on the market of an EU Member State will have to submit a notification to the Appointed Body/Poison Centre of that Member State. The notification needs to contain certain information on the chemical mixture, such as the chemical composition and toxicological information, as well as the product category to which the mixture belongs. The inclusion of information on the product category in a notification allows Appointed Bodies/Poison Centres to carry out comparable statistical analysis (e.g. to define risk management measures), to fulfil reporting obligations and to exchange information among MS. The product category is therefore not used for the actual emergency health response as such, but allows the identification of exposure or poisoning trends and of possible measures to prevent future poisoning cases. When formally adopted, the new Regulation will apply as of 1 January 2020.

While there are substantial legislative provisions, to this date a single unified EU 'phytopharmacovigilance'<sup>11</sup> system akin to the pharmacovigilance system does not exist for PPP. Rather, a number of alerting systems have been developed within the EU to alert, notify, report and share information on chemical hazards that may pose a risk to public health in Member States. These systems cover different sectors including medicines, food stuffs, consumer products, industrial accidents, notifications under International Health Regulations (IHR) and events detected by EU Poisons Centres and Public Health Authorities. Each of these systems notify and distribute timely warnings to competent authorities, public organisations, governments, regulatory authorities and public health officials to enable them to take effective action to minimise and manage the risk to public health (Orford et al., 2014).

In the EU, information on acute pesticide exposure/incident originates mainly from data collected and reported by Poison Control Centres (PCC's). PCC's collect both cases of acute and chronic exposure/poisoning they are aware of, in the general population and in occupational settings. Cases are usually well-documented and information includes circumstances of exposure/incident, description of the suspected causal agent, level and duration of exposure, the clinical course and treatment and an assessment of the causal relationship. In severe cases, the toxin and/or the metabolites are usually

<sup>&</sup>lt;sup>11</sup> 'phytovigilance' would refer to a vigilance system for plants; as pesticides are intended to be 'medicines' for crops, the term 'phytopharmacovigilance' is considered to be the more appropriate one here. Furthermore, it is a broad term used in France covering soil, water, air, environment, animal data, etc.



measured in blood or urine. However, follow-up of cases reported to the centres merits further attention to identify potential long-term protracted effects.

There are two key obstacles to using Poison Centres data: official reports from national Poisons Centres are not always publicly available and when they are, there is a large heterogeneity in the format of data collections and coding, and assessment of the causal relationship. Indeed, each Member State has developed its own tools for collection activities resulting in difficulties for comparing and exchanging exposure data. In 2012, the European Commission funded a collaborative research and development project to support the European response to emerging chemical events: the Alerting and Reporting System for Chemical Health Threats, Phase III (ASHTIII) project. Among the various tools and methodologies that were considered, methods to exchange and compare exposure data from European PCC's were developed. As a feasibility study, work-package 5 included the development of a harmonised and robust coding system to enable Member States to compare pesticide exposure data. However, results of a consultation with the PCC community showed that further coordination of data coding and collection activities is supported. It was concluded that more support and coordination is required at the EU and Member States level so that exposures data can be compared between Member States (Orford et al., 2015).

In addition to data collected by PCC's, several Member States have set up programmes dedicated to occupational health surveillance.<sup>12</sup> The purpose of these programmes is to identify the kinds of jobs, types of circumstances and pesticides that cause health problems in workers in order to learn more about occupational pesticide illnesses and injuries and how to prevent them. They are based on voluntary event notification by physicians (sometimes self-reporting by users) of any case of suspected work-related pesticide injury or illness or poisoning. In addition to medical data, information gathered includes data regarding type of crop, mode of application, temperature, wind speed, wearing of personal protection equipment, etc. Once collected, these data are examined and a report is released periodically; they provide a useful support to evaluate the safety of the products under re-registration. These data also highlight emerging problems and allow definition of evidence-based preventive measures for policy-makers. At EU level, the European Agency for Safety and Health at Work (EU-OSHA)<sup>13</sup> has very little in the way of monitoring of occupational pesticide-related illnesses data. In the USA, a programme specifically dedicated to pesticides funded and administered by the National Institute for Occupational Safety and Health (NIOSH) is in operation in a number of States.<sup>14</sup>

In summary, currently human data may be collected in the form of case reports or case series, poison centres information, coroner's court findings, occupational health surveillance programmes or post-marketing surveillance programmes. However, not all this information is present in the medical data submitted by applicants mainly because the different sources of information are diverse and heterogeneous by nature, which makes some of them sometimes not accessible.

- Data collected through occupational health surveillance of the plant production workers or if they do so, the medical data are quite limited being typically basic clinical blood measurements, physical examinations, potentially with simple indications of how and where exposed took place, and there usually is no long-term follow up. Furthermore, worker exposures in modern plants (especially in the EU) are commonly very low, and often their potential exposure is to a variety of pesticides (unless it is a facility dedicated to a specific chemical).
- Moreover, the reporting of data from occupational exposure to the active substances during manufacture is often combined with results from observations arising from contact with the formulated plant protection product as the latter information results from case reports on poisoning incidents and epidemiological studies of those exposed as a result of PPP use. Indeed, the presence of co-formulants in a plant protection product can modify the acute toxicological profile. Thus, to facilitate proper assessment, when reporting findings collected in humans it should be clearly specified whether it refers to the active substance per se or a PPP.

With regard to the requirements of specific data on diagnoses of poisoning by the active substance or formulated plant protection products and proposed treatments, which are also part of chapter 5.9 of the EC Regulation 283/2013, information is often missing or limited to those cases where the toxic mode of action is known to occur in humans and a specific antidote has been identified.

<sup>&</sup>lt;sup>12</sup> For example: Phyt'attitude in France is a vigilance programme developed by the Mutualité Sociale Agricole: http://www.msa. fr/lfr/sst/phyt-attitude

<sup>&</sup>lt;sup>13</sup> https://osha.europa.eu/en/about-eu-osha

<sup>&</sup>lt;sup>14</sup> SENSOR programme: https://www.cdc.gov/niosh/topics/pesticides/overview.html



# 5.3. Proposals for improvement of current framework of case incident reporting

In order to avoid duplication and waste of effort, a logical next step would be to now develop, with all concerned public and private sector actors, an EU 'phytopharmacovigilance' system for chemicals similar to the ones that have been put in place for medicines. This network could be based on committed and specifically trained occupational health physicians and general practitioners in rural areas, and resources should be allocated by Member States to establish and to successfully maintain the system. Indeed such a network would be useful in detecting acute effects; it would also act as a sentinel surveillance network for specific health effects (such as asthma, sensitisation, etc.) or for the detection of emerging work-related disease. In fact, while much experience has already been gained on how to gradually build such a system, it is nevertheless envisioned that this will take a number of years to be put in place. Several difficulties will arise because of the nature of the data collected (the sources of information are potentially diverse), the quality and completeness of the collected information for every case (especially the circumstances), the grading of severity and accountability of the observed effects (the link between the observed effect and the product). Rules should be defined so that they are identical from one 'evaluator' to another. The network should be stable over time (e.g. continuity in national organisations involved, consistent methodology employed, etc.), to ensure that the phytopharmacovigilance system fully complies with the objectives, i.e. monitoring changes over time. The use of phytopharmacovigilance data is unlikely to be limited to risk assessment purposes and may have an impact on risk management decisions (e.g. revisions in the terms and conditions of product authorisations or ultimately product withdrawal); this should be clear to all stakeholders from the outset.

Such a system may not merit being established solely for chemicals that are (predominantly) used as pesticides. However, given the legislative provisions already in place for pesticides, its development may need to be prioritised for pesticides.

In conclusion, the European Commission together with the Member States should initiate the development of an EU-wide vigilance framework for pesticides. These should include:

- harmonisation of human incident data collection activities at the EU level;
- coordination of the compilation of EU-wide databases;
- improving the collaboration between Poison Centres and regulatory authorities at national level in order to collect all the PPP poisonings produced in each Member State;
- guidance document on monitoring the impact of pesticide use on human health with harmonisation of data assessment for causal relationships;
- regular EU-wide reports.

# 6. Proposed use of epidemiological studies and vigilance data in support of the risk assessment of pesticides

This section briefly reviews the risk assessment process (Section 6.1) based on experimental studies and discusses what information epidemiological studies could add to that process. Next, the assessment of the reliability of epidemiological studies is addressed in Section 6.2. In Section 6.3, the relevance of one or more studies found to be reliable is assessed.

# 6.1. The risk assessment process

Risk assessment is the process of evaluating risks to humans and the environment from chemicals or other contaminants and agents that can adversely affect health. For regulatory purposes, the process used to inform risk managers consists of four steps (EFSA, 2012a). On the one hand, information is gathered on the nature of toxic effects (hazard identification) and the possible dose– response relationships between the pesticide and the toxic effects (hazard characterisation). On the other hand, information is sought about the potential exposure of humans (consumers, applicators, workers, bystanders and residents) and of the environment (exposure assessment). These two elements are weighed in the risk characterisation to estimate that populations be potentially exposed to quantities exceeding the reference dose values, that is, to estimate the extra risk of impaired health in the exposed populations. Classically, this is used to inform risk managers for regulatory purposes.

a) Step 1. Hazard identification.



Epidemiological studies and vigilance data are relevant for hazard identification as they can point to potential link between pesticide exposure and health. In this context, epidemiological data can provide invaluable information in 'scanning the horizon' for effects not picked up in experimental models. Importantly, these studies also provide information about potentially enhanced risks for vulnerable population subgroups, sensitive parts of the lifespan, and gender selective effects.

b) Step 2. *Hazard characterisation* (dose–response assessment). As previously discussed a classic dose–response framework is not normally considered when using epidemiological data as the exposure dose is rarely assigned. The challenge presented when high quality epidemiological studies are available is to see whether these can best be integrated into the scheme as numerical input. A dose–response framework is rarely considered when using epidemiological data for risk assessment of pesticides. However, previous scientific opinions of the EFSA CONTAM Panel have used epidemiology as basis for setting reference values, particularly in the case of cadmium, lead, arsenic and mercury, which are the most well-known and data rich (EFSA, 2009a,b, 2010b, 2012b). Even when they may not form the basis of a dose–response assessment, vigilance and epidemiological data may provide supportive evidence to validate or invalidate a dose–response study carried out in laboratory animals. Characterisation of the relationships between varying doses of a chemical and incidences of adverse effects in exposed populations requires characterisation of exposure or dose, assessment of response and selection of a dose–response model to fit the observed data in order to find a no-effect level. This raises two questions: can a dose–response be derived from epidemiological data to identify a no-effect level. If not, can epidemiological information otherwise contribute to the hazard characterisation?

Understanding dose–response relationships could also be relevant where adverse health outcomes are demonstrated to be associated with uses with higher exposures than EU good plant protection practice would give rise to, but where no association is observed from uses with lower exposures. It is clear that in this context the statistical summary of an epidemiological study defining RR or OR is potentially useful quantitative information to feed into the hazard characterisation process, when the study design meets the necessary standards.

c) Step 3. *Exposure assessment*. Data concerning the assessment of exposure are often hard to estimate in complex situations where a variety of uncontrolled 'real-world' factors confound the analysis. As discussed previously, contemporary biological monitoring is rarely carried out in the general human population for practical reasons including high cost, test availability and logistics. However, it is anticipated that in the near future biomonitoring studies and data on quantitative exposure to pesticides will increase.

Step 4. *Risk characterisation*. In this final step, data on exposure are compared with health-based reference values to estimate the extra risk of impaired health in the exposed populations. Human data can indeed help verify the validity of estimations made based on extrapolation from the full toxicological database regarding target organs, dose–response relationships and the reversibility of toxic effects, and to provide reassurance on the extrapolation process without direct effects on the definition of reference values (London et al., 2010).

Epidemiological data might also be considered in the context of uncertainty factors (UFs). An UF of 10 is generally used on animal data to account for interspecies variability of effects and this is combined with a further factor of 10 to account for variation in susceptibility of different parts of the human population. However, there are cases where only human data are considered (when this is more critical than animals data) and a single factor of 10 for intraspecies variability will apply. It is noted that at this moment Regulation (EC) No 1107/2009 Article 4(6) stipulates that: 'In relation to human health, no data collected on humans shall be used to lower the safety margins resulting from tests on animals'. The implication of this is that for risk assessment epidemiological data may only be used to increase the level of precaution used in the risk assessment, and not to decrease UFs even where relevant human data are available.

# 6.2. Assessment of the reliability of individual epidemiological studies

Factors to be considered in determining how epidemiology should be considered for a WoE assessment are described below and have been extensively outlined by available risk of bias tools for observational epidemiological studies.<sup>15</sup> The following examples represent factors to look for not an exhaustive list:

• *Study design and conduct*. Was the study design appropriate to account for the expected distributions of the exposure and outcome, and population at risk? Was the study conducted primarily in a hypothesis generating or a hypothesis-testing mode?

<sup>&</sup>lt;sup>15</sup> Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the RTI Item Bank (https://www.ncbi.nlm.nih.gov/books/NBK154464/) and Cochrane handbook.



- *Population*. Did the study sample the individuals of interest from a well-defined population? Did the study have adequate statistical power and precision to detect meaningful differences for outcomes between exposed and unexposed groups?
- *Exposure assessment*. Were the methods used for assessing exposure valid, reliable and adequate? Was a wide range of exposures examined? Was exposure assessed at quantitative level or in a categorical or dichotomous (e.g. ever vs never) manner? Was exposure assessed prospectively or retrospectively?
- *Outcome assessment*. Were the methods used for assessing outcomes valid, reliable and adequate? Was a standardised procedure used for collecting data on health outcomes? Were health outcomes ascertained independently from exposure status to avoid information bias?
- *Confounder control*: were potential confounding factors appropriately identified and considered? How were they controlled for? Were the methods used to document these factors valid, reliable and adequate?
- *Statistical analysis*. Did the study estimate quantitatively the independent effect of an exposure on a health outcome of interest? Were confounding factors appropriately controlled in the analyses of the data?
- Is the *reporting* of the study adequate and following the principles of transparency and the guidelines of the STROBE statement (or similar tools)?

Study evaluation should provide an indication on the nature of the potential limitations each specific study may have and an assessment of overall confidence in the epidemiological database.

Furthermore, the nature and the specificity of the outcome with regards to other known risk factors can influence the evaluation of human data for risk assessment purposes, particularly in case of complex health endpoints such as chronic effects with long induction and latency periods.

Table 2 shows the main parameters to be evaluated in single epidemiological studies and the associated weight (low, medium and high) for each parameter. Specific scientific considerations should be applied on a case-by-case basis, but it would be unrealistic to implement these criteria in a rigid and unambiguous manner.

Parameter	High	Moderate	Low
Study design and conduct	Prospective studies. Prespecified hypothesis (compound and outcome specific)	Case–control studies. Prospective studies not adequately covering exposure or outcome assessment	Cross-sectional, ecological studies Case–control studies not adequately covering exposure or outcome assessment
Population	Random sampling. Sample size large enough to warrant sufficient power Population characteristics well defined (including vulnerable subgroups)	Questionable study power, not justified in detail Non-representative sample of the target population Population characteristics not sufficiently defined	No detailed information on how the study population was selected Population characteristics poorly defined
Exposure assessment	Accurate and precise quantitative exposure assessment (human biomonitoring or external exposure) using validated methods Validated questionnaire and/or interview for chemical-specific	Non-valid surrogate or biomarker in a specified matrix and external exposure Questionnaire and/or interview for chemical-specific exposure	Poor surrogate Low-quality questionnaire and/or interview; information collected for groups of chemicals No chemical-specific exposure information collected;
	exposure answered by subjects	answered by subjects or proxy individuals	
Outcome Assessment	Valid and reliable outcome assessment. Standardised and validated in study population Medical record or diagnosis confirmed	Standardised outcome, not validated in population, or screening tool; or, medical record non-confirmed	Non-standardised and non-validated health outcome Inappropriate or self-reported outcomes.

Table 2:	Study quality	considerations for	weighting	epidemiological	observational studies <sup>(a),(</sup>	(b)
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Parameter	High	Moderate	Low
Confounder control	Adequate control for important confounders relevant to scientific question, and standard confounders Careful consideration is given to clearly indicated confounders	Confounders are partially controlled for Moderately control of confounders and standard variables Not all variables relevant for scientific question are considered	No control of potential confounders and effect modifiers in the design and analysis phases of the study
Statistical Analysis	Appropriate to study design, supported by adequate sample size, maximising use of data, reported well (not selective) Statistical methods to control for confounding are used and adjusted and unadjusted estimates are presented. Subgroups and interaction analysis are conducted	Acceptable methods, analytic choices that lose information, not reported clearly Post hoc analysis conducted but clearly indicated	Only descriptive statistics or questionable bivariate analysis is made Comparisons not performed or described clearly Deficiencies in analysis (e.g. multiple testing)
Reporting	Key elements of the Material and Methods, and results are reported with sufficient detail Numbers of individuals at each stage of study is reported A plausible mechanism for the association under investigation is provided	Some elements of the Material and Methods or results are not reported with sufficient detail Interpretation of results moderately addressed	Deficiencies in reporting (interpretation of effect estimates, confounder control) Selective reporting Paucity of information on relevant factors that may affect the exposure-health relationship. Misplaced focus of the inferential objectives Not justified conclusions

(a): Overall study quality ranking based on comprehensive assessment across the parameters.

(b): Adapted from US-EPA (2016), based in turn on Muñoz-Quezada et al. (2013) and LaKind et al. (2014).

If the above assessment is part of the evidence synthesis exercise, where epidemiological research is being assessed and quantitatively summarised, it permits more accurate estimation of absolute risk related to pesticide exposure and further quantitative risk assessment.

In the particular case of pesticide epidemiology data, three basic categories are proposed as a first tier to organise human data with respect to risk of bias and reliability<sup>16</sup>: (a) low risk of bias and high reliability (all or most of the above quality factors have been addressed with minor methodological limitations); (b) medium risk of bias and medium reliability (many of the above quality factors have been addressed with moderate methodological limitations); (c) high risk of bias and low reliability, because of serious methodological limitations or flaws that reduce the validity of results or make them largely uninterpretable for a potential causal association. The latter studies are considered unacceptable for risk assessment mainly because of poor exposure assessment, misclassification of exposure and/or health outcome, or lack of statistical adjustment for relevant confounders. Risk assessment should not be based on results of epidemiological studies that do not meet well-defined data quality standards. Furthermore, results of exploratory research will need to be confirmed in future research before they can be used for risk assessment.

# 6.3. Assessment of strength of evidence of epidemiological studies

This section briefly discusses some important issues specifically related to combining and summarising results from different epidemiological studies on the association between pesticides and human health.

The approach for weighting epidemiological studies is mainly based on the modified Bradford Hill criteria, which are a group of conditions that provide evidence bearing on a potentially causal relationship between an incidence and a possible consequence (strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy) (Table 3). Clearly, the

<sup>&</sup>lt;sup>16</sup> These categories are in accordance with those currently used by EFSA for the peer review of pesticide active substances: acceptable, supplementary and unacceptable.



more of these criteria that are met the stronger the basis for invoking the association as evidence for a meaningful association. However, Bradford Hill was unwilling to define what causality was and never saw the criteria as sufficient or even absolutely necessary but simply of importance to consider in a common-sense evaluation.

Table 3:	Considerations for WoE analysis based on the modified Bradford Hill criteria for evidence	
	integration	

Category	Considerations
Strength of Association	The assessment of the strength of association (not only the magnitude of association but also statistical significance) requires examination of underlying methods, comparison to the WoE in the literature and consideration of other contextual factors including the other criteria discussed herein
Consistency of Association	Associations should be consistent across multiple independent studies, particularly those conducted with different designs and in different populations under different circumstances. This criterion also applies to findings consistent across all lines of evidence (epidemiology, animal testing, <i>in vitro</i> systems, etc.) in light of modern data integration
Specificity	The original criteria of evidence linking a specific outcome to an exposure can provide a strong argument for causation has evolved and may have new and interesting implications within the context of data integration. Data integration may elucidate some mechanistic specificity among the varied outcomes associated with complex exposures. The lack of specificity can help to narrow down specific agents associated with disease
Temporality	Evidence of a temporal sequence between exposure to an agent and appearance of the effect within an appropriate time frame constitutes one of the best arguments in favour of causality. Thus, study designs that ensure a temporal progression between the two measures are more persuasive in causal inference
Biological Gradient (Dose–response)	Increased effects associated with greater exposures, or duration of exposures, strongly suggest a causal relationship. However, its absence does not preclude a causal association
Biological Plausibility	Data explained and supported by biologically plausible mechanisms based on experimental evidence strengthen the likelihood that an association is causal. However, lack of mechanistic data should not be taken as evidence against causality
Coherence	The interpretation of evidence should make sense and not to conflict with what is known about the biology of the outcome in question under the exposure-to-disease paradigm. If it does, the species closest to humans should be considered to have more relevance to humans
Experimental Evidence	Results from randomised experiments provide stronger evidence for a causal association than results based on other study designs. Alternatively, an association from a non- experimental study may be considered as causal if a randomised prevention derived from the association confirms the finding
Sequence of Key events	Provide a clear description of each of the key events (i.e. measurable parameters from a combination of <i>in vitro</i> , <i>in vivo</i> or human data sources) that underlie the established MoA/AOP for a particular health outcome. A fully elucidated MoA/AOP is a not requirement for using epidemiology studies in human health risk assessment

Adapted from Höfler (2005), Fedak et al. (2015) and US-EPA (2016).

For predictive causality, care must be taken to avoid the logical fallacy *post hoc ergo propter hoc* that states 'Since event Y followed event X, event Y must have been caused by event X'. Höfler (2005) quotes a more accurate 'counterfactual' definition as follows 'but for E, D will not occur or would not have occurred, but given E it will/would have occurred'. Yet, more detailed descriptions using symbolic logic are also available (Maldonado and Greenland, 2002). Rothman and Greenland (2008) stated that 'the only *sine qua non* for a counterfactual effect is the condition that the cause must precede the effect. If the event proposed as a result or "effect" precedes its cause, there may be an association between the events but certainly no causal relationship'.

#### 6.3.1. Synthesis of epidemiological evidence

Systematic reviews and meta-analysis of observational studies can provide information that strengthens the understanding of the potential hazards of pesticides, exposure–response characterisation, exposure scenarios and methods for assessing exposure, and ultimately risk characterisation (van den Brandt, 2002). Systematic reviews entail a detailed and comprehensive plan



and search strategy defined *a priori* aimed at reducing bias by identifying, appraising and synthesising all relevant studies on a particular topic. The major steps of a systematic review are as follows: formulation of the research question; definition of inclusion and exclusion criteria; search strategy for studies across different databases; selection of studies according to predefined strategy; data extraction and creation of evidence tables; assessment of methodological quality of the selected studies; including the risk of bias; synthesis of data (a meta-analysis can be performed if studies allow); and interpretation of results and drawing of conclusions (EFSA, 2010a). Evidence synthesis is, however, challenging in the field of pesticide epidemiology as standardisation and harmonisation is difficult. Nonetheless, evidence synthesis should play a pivotal role in assessing the robustness and relevance of epidemiological studies.

Statistical tools have been developed that can help assess this evidence. When multiple studies on nearly identical sets of exposures and outcomes are available, these can provide important scientific evidence. Where exposure and outcomes are quantified and harmonised across studies, data from individual epidemiological studies with similar designs can be combined to gain enough power to obtain more precise risk estimates and to facilitate assessment of heterogeneity. Appropriate systematic reviews and quantitative synthesis of the evidence needs to be performed regularly (e.g. see World Cancer Research Fund approach to continuous update of meta-analysis for cancer risk factor<sup>17</sup>). Studies should be evaluated according to previously published criteria for observational research and carefully examine possible selection bias, measurement error, sampling error, heterogeneity, study design, and reporting and presentation of results.

Meta-analysis is the term generally used to indicate the collection of statistical methods for combining and contrasting the results reported by different studies (Greenland and O'Rourke, 2008). Meta-analysis techniques could be used to examine the presence of diverse biases in the field such as small study effects and excess significance bias. Meta-analyses, however, do not overcome the underlying biases that may be associated with each study design (i.e. confounding, recall bias or other sources of bias are not eliminated). The extent to which a systematic review or meta-analysis can draw conclusions about the effects of a pesticide depends strongly on whether the data and results from the included studies are valid, that is, on the quality of the studies considered. In particular, consistent findings among original studies resulting from a consistent bias will produce a biased conclusion in the systematic review. Likewise, a meta-analysis of invalid studies may produce a misleading result, yielding a narrow confidence interval around the wrong effect estimate.

In addition to summarising the basic study characteristics of the literature reviewed, a typical metaanalysis should include the following components: (a) the average effect size and effect size distribution for each outcome of interest and an examination of the heterogeneity in the effect size distributions; (b) subgroup analysis in which the variability present in the effect size distribution is systematically analysed to identify study characteristics that are associated with larger or smaller effect sizes; (c) publication bias analysis and other sensitivity analyses to assess the validity of conclusions drawn (Wilson and Tanner-Smith, 2014).

In a meta-analysis, it is important to specify a model that adequately describes the effect size distribution of the underlying population of studies. Meta-analysis using meaningful effect size distributions will help to integrate quantitative risk into risk assessment models. The conventional normal fixed- and random-effects models assume a normal effect size population distribution, conditionally on parameters and covariates. Such models may be adequate for estimating the overall effect size, but surely not for prediction if the effect size distribution exhibits a non-normal shape (Karabatsos et al., 2015).

#### 6.3.2. Meta-analysis as a tool to explore heterogeneity across studies

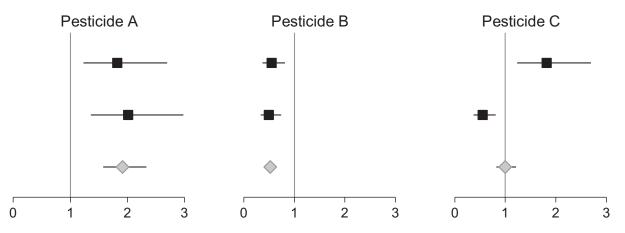
When evaluating the findings of different studies, many aspects should be carefully evaluated. Researchers conducting meta-analyses may tend to limit the scope of their investigation to the determination of the size of association averaged over the considered studies. The motivation often is that aggregating the results yields greater statistical power and precision for the effect of interest. Because individual estimates of effect vary by chance, some variation is expected. However, estimates must be summarised only when meaningful. An important aspect that is often overlooked is heterogeneity of the strength of associations across subgroups of individuals. Heterogeneity between

<sup>&</sup>lt;sup>17</sup> World Cancer Research Fund International. Continuous Update Project (CUP) http://www.wcrf.org/int/research-we-fund/ continuous-update-project-cup



studies needs to be assessed and quantified when present (Higgins, 2008). In meta-analysis, heterogeneity among results from different studies may indeed be as informative as homogeneity. Exploring the reasons underlying any observed inconsistencies of findings is generally conducive of great understanding.

Figure 1 shows three forest plots from a fictitious example in which each of three pesticides (A, B and C) is evaluated in meta-analysis of two studies. It is assumed that both studies for each pesticide are of the highest quality and scientific rigor. No biases are suspected.



**Figure 1:** Forest plots from a fictitious example in which each of three pesticides (A, B and C) is evaluated in a meta-analysis of two studies. The x-axis in each plot represents the estimated risk ratio of the disease of interest comparing exposed and unexposed individuals. The squares denote the estimated risk ratio in each study and the grey diamonds the summarised risk ratio. The horizontal lines indicate 95% confidence intervals

The following text contains short comments on the interpretation of the results in Figure 1, one pesticide at a time.

- Exposure to pesticide A seems to double the risk of the disease. The results are consistent between the two studies and the confidence intervals do not contain the null value, one. These results, however, do not imply that (a) the risk ratio would be about 2 in any other study that was conducted on the same exposure and disease; or that (b) the risk ratio is two in any group of individuals (e.g. males or females, young or old).
- Exposure to pesticide B seems to halve the risk of the disease. The results are consistent between the two studies and the confidence intervals do not contain the null value, one. These results, however, do not imply that (a) the risk ratio would be about a half in any other study that was conducted on the same exposure and disease; or that (b) the risk ratio is about a half in any group of individuals (e.g. males or females, young or old).
- Exposure to pesticide C seems to double the risk of the disease in one study and to halve the risk in the other. The results are inconsistent between the two studies and the confidence intervals do not contain the null value, one. These results, however, do not imply that (a) the risk ratio would be about one in any other study that was conducted on the same exposure and disease; or that (b) the risk ratio is about one in any group of individuals (e.g. males or females, young or old).

What evidence can the results shown in Figure 1 provide?

The risk ratio reported by any study can be generalised to other populations only if all the relevant factors have been controlled for (Bottai, 2014; Santacatterina and Bottai, 2015). In this context, relevant factors are variables that are stochastically dependent with the health outcome of interest. For example, cardiovascular diseases are more prevalent among older subjects than among younger individuals. Age is therefore a relevant factor for cardiovascular diseases. The evidence provided by the results shown in Figure 1 are potentially valid only if this step was taken in each of the studies considered. If that was the case for the studies, then, there is evidence that exposure to pesticide A doubles the risk in the specific group of individuals considered by each of the two studies. If the risk ratios are summary measures over the respective study populations, then none of the findings should be generalised. However, if the risk ratios for pesticide A were not adjusted for any factor, and the underlying populations were very different



across the two studies, then there would still be evidence that there may be no relevant factors and pesticide A doubles the risk in any subgroup of individuals. Pesticide B appears to halve the risk, and the estimated confidence intervals are narrower for pesticide B than for pesticide A. Generalisability of the findings, however, holds for pesticide B under the conditions stated above for pesticide A. As for pesticide C, the forest plot provides evidence that exposure to this pesticide raises the risk of the disease in the group of individuals in one of the studies and decreases it in the group considered in the other study. Again, if the risk ratios are summary measures over the respective study populations, then none of the findings should be generalised. Investigating the reasons behind the inconsistency between the two studies on pesticide C can provide as much scientific insight as investigating the reasons behind the similarity between the studies on pesticide A or pesticide B.

In general, the overall summary measures provided by forest plots, such as the silver diamonds in each of the three panels of Figure 1, are of little scientific interest. When evaluating the findings of different studies, many aspects should be carefully evaluated. An important aspect that is often overlooked is heterogeneity of the strength of associations across subgroups of individuals. When information about subgroup analysis is provided in the publications that describe a study, this should be carefully evaluated. Sensitivity analyses should complement the results provided by different studies. These should aim to evaluate heterogeneity and the possible impact of uncontrolled for relevant factors along with information and sampling error. A synoptic diagram is displayed in Figure 2.

#### Bias

• Information error, such as measurement error, effect size magnification

#### Relevant Factors

- Which were considered and which were not considered
- How were they distributed in each study
- What population is the resulting inference on

#### Sampling Error

• Standard errors, not p-values, of the estimates of the parameters of interest

Sensitivity Analyses

• Range of the parameters of interest that are consistent with observed data

#### Figure 2: Items to consider when evaluating and comparing multiple studies

#### 6.3.3. Usefulness of meta-analysis for hazard identification

Human data can be used for many stages of risk assessment. Single epidemiological studies, if further studies on the same pesticide are not available, should not be used as a sole source for hazard identification, unless they are high quality studies (according to criteria shown in Table 2). Evidence synthesis techniques which bring together many studies, such as systematic reviews and meta-analysis (where appropriate) should be utilised instead. Although many meta-analyses have been carried out for the quantitative synthesis of data related to chronic diseases, their application for risk assessment modelling is still limited.

Importantly, evidence synthesis will provide a methodological assessment and a risk of bias assessment of the current evidence highlighting areas of uncertainties and identifying associations with robust and credible evidence.

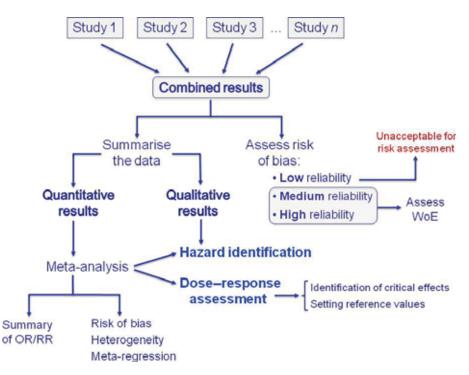
Figure 3 shows a simple methodology proposed for the application of epidemiological studies into risk assessment. The first consideration is the need of combining different epidemiological studies



addressing the same outcome. This can be made following criteria proposed by EFSA guidance for systematic reviews (EFSA, 2010a). Then, the risk of bias is assessed based on the factors described in Section 6.2 for a WoE assessment, namely: study design and conduct, population, exposure assessment, outcome assessment, confounder control, statistical analysis and reporting of results. Those studies categorised as of low reliability will be considered unacceptable for risk assessment. The remaining studies will be weighted and used for hazard identification.

If quantitative data are available, a meta-analysis can be conducted to create summary data and to improve the statistical power and precision of risk estimates (OR, RR) by combining the results of all individual studies available or meeting the selection criteria. As meta-analyses determine the size of association averaged over the considered studies, they provide a stronger basis for hazard identification. Moreover, under certain circumstances, there is the possibility to move towards risk characterisation metrics because these measured differences in health outcomes (OR, RR) can be converted to dose–response relationships (Nachman et al., 2011). Although quite unusual in practice, this would allow for the identification of critical effects in humans and/or setting reference values without the need of using animal extrapolation.

Since heterogeneity is common in meta-analyses, there is a need to assess which studies could be combined quantitatively. Heterogeneity can be genuine, representing diverse effects in different subgroups, or might represent the presence of bias. If heterogeneity is high (I<sup>2</sup> greater than 50%), individual studies should not be combined to obtain a summary measure because of the high risk of aggregating bias from different sources. Sources of heterogeneity should be explored through sensitivity analysis and/or meta-regression. Furthermore, the presence of diverse biases in the meta-analysis should be examined, such as small study effects, publication bias and excess significance bias. It is important to find models that adequately describe the effect size distribution of the underlying studied populations.





6.3.4. Pooling data from similar epidemiological studies for potential dose-response modelling

As in other fields of research, findings from a single epidemiological study merit verification through replication. When the number of replications is abundant, it may be worthwhile to assess the entire set of replicate epidemiological studies through a meta-analysis and ascertain whether, for key outcomes, findings are consistent across studies. Such an approach will provide more robust conclusions about the existence of cause-effect relationships.



Once a hazard has been identified, the next step in risk assessment is to conduct a dose-response assessment to estimate the risk of the adverse effect at different levels of exposure and/or the concentration level below which no appreciable adverse health effect can be assumed for a given population. However, this step requires fully quantitative (or at least semi-quantitative) exposure data at an individual level. Summary estimates resulting from quantitative synthesis would be more informative for risk assessment if they present an OR for a given change in the continuous variable of exposure (or per a given percentile change in exposure) as this allows for relative comparisons across studies and could be of help to derive health-based reference values. Only within such a framework can data from human studies with similar designs be merged to gain enough power to model proper dose-response curves (Greenland and Longnecker, 1992; Orsini et al., 2012).

Conversely, meta-analytical approaches may be of limited value if a combined OR is calculated based on meta-analyses interpreting exposure as a 'yes' or a 'no' (ever vs never) because exposures are not necessarily to active ingredients in the same proportion in all studies included. Even though in these cases, meta-analyses may consistently find an increased risk associated with pesticide exposure, for risk assessment the exposure needs to characterise the effect of specific pesticide classes or even better individual pesticides as their potency may differ within the same class (Hernández et al., 2016).

This approach would allow points of departure to be identified (e.g. benchmark doses (BMD)) and would be relevant for the integration of epidemiological studies into quantitative risk assessment. Although BMD modelling is currently used for analysing dose–response data from experimental studies, it is possible to apply the same approach to data from observational epidemiological studies (Budtz-Jørgenson et al., 2004). The EFSA Scientific Committee confirmed that the BMD approach is a scientifically more advanced method compared to the no observed-adverse-effect level (NOAEL) approach for deriving a Reference Point, since it makes extended use of the dose–response data from experimental and epidemiological studies to better characterise and quantify potential risks. This approach, in principle, can be applicable to human data (EFSA Scientific Committee, 2017b), although the corresponding guidelines are yet to be developed.

Dose–response data from observational epidemiological studies may differ from typical animal toxicity data in several respects and these differences are relevant to BMD calculations. Exposure data often do not fall into a small number of well-defined dosage groups. Unlike most experimental studies, observational studies may not include a fully unexposed control group, because all individuals may be exposed to some extent to a chemical contaminant. In this case, the BMD approach still applies since fitting a dose–response curve does not necessarily require observations at zero exposure. However, the response at zero exposure would then need to be estimated by low-dose extrapolation. Hence, the BMD derived from epidemiological data can be strongly model-dependent (Budtz-Jørgensen et al., 2001).

Epidemiology data need to be of sufficient quality to allow the application of the BMD approach, especially in terms of assigning an effect to a specific pesticide and its exposure. Clear rules and guidance, and definition of model parameters need to be considered for such a BMD approach, which might differ from BMD approaches from controlled experimental environments. Although the BMD modelling approach has been applied to epidemiological data on heavy metals and alcohol (Lachenmeier et al., 2011), currently, few individual studies on pesticides are suitable for use in dose–response modelling, much less in combination with other studies. However, future studies should be conducted and similarly reported so that they could be pooled together for a more robust assessment.

# 7. Integrating the diverse streams of evidence: human (epidemiology and vigilance data) and experimental information

This section first considers in Section 7.1 the different nature of the main streams of evidence, i.e. originating either from experimental studies or from epidemiological studies. The approach used is that recommended by the EFSA Scientific Committee Guidance on WoE (EFSA Scientific Committee, 2017b), which distinguishes three successive phases to assess and integrate these different streams of information: reliability, relevance and consistency. The first step, consists in the assessment of the reliability of individual studies be they epidemiological (addressed in Section 6) or experimental (beyond the scope of this Scientific Opinion). Then, the relevance (strength of evidence) of one or more studies found to be reliable is assessed using principles of epidemiology (addressed in Section 6) and toxicology. Next, Section 7.2 considers how to bring together different streams of relevant information from epidemiological and experimental studies, which is considered in a WoE approach, to assess consistency and biological plausibility for humans.



# 7.1. Sources and nature of the different streams of evidence Comparison of experimental and epidemiological approaches

In the regulatory risk assessment of pesticides, the information on the toxic effects is based on the results of a full set of experiments as required by Regulation (EC) 283/2013 and 284/2013, and conducted according to OECD guidelines. They are carried out *in vivo* or *in vitro*, so there will always be some high-quality experimental data available for pesticides as required to be provided by applicants under Regulation (EC) 1107/2009. A number of categories are established for rating the reliability of each stream of evidence according to the EFSA peer review of active substances: acceptable, supplementary and unacceptable. The data quality and reliability of *in vivo* or *in vitro* toxicity studies should be assessed using evaluation methods that better provide more structured support for determining a study's adequacy for hazard and risk assessments. Criteria have been proposed for conducting and reporting experimental studies to enable their use in health risk assessment for pesticides (Kaltenhäuser et al., 2017).

Animal (*in vivo*) studies on pesticide active substances conducted according to standardised test guidelines and good laboratory practices (GLP, e.g. OECD test guidelines) are usually attributed higher reliability than other research studies. Notwithstanding, since there is no evidence that studies conducted under such framework have a lower risk of bias (Vandenberg et al., 2016), evidence from all relevant studies, both GLP and non-GLP, should also be considered and weighted. Thus, data from peer-reviewed scientific literature should be taken into account for regulatory risk assessment of pesticide active substances, provide they are of sufficient quality after being assessed for methodological reliability. Their contribution to the overall WoE is influenced by factors including test organism, study design and statistical methods, as well as test item identification, documentation and reporting of results (Kaltenhäuser et al., 2017).

The internal validity of *in vitro* toxicity studies should be evaluated as well to provide a better support for determining a study's adequacy for hazard and risk assessments. *In silico* modelling can be used to derive structure–activity relationships (SAR) and to complement current toxicity tests for the identification and characterisation of the mode or mechanisms of action of the active substance in humans. These alternative toxicity testing (and non-testing) approaches could be helpful in the absence of animal data, e.g. to screen for potential neurodevelopmental or endocrine disruption effects of pesticides, and to increase confidence in animal testing. Considering the demand for minimising the number of animal studies for regulatory purposes, non-animal testing information can provide relevant stand-alone evidence that can be used in the WoE assessment.

A number of toxicological issues are amenable for systematic review, from the impact of chemicals on human health to risks associated with a specific exposure, the toxicity of chemical mixtures, the relevance of biomarkers of toxic response or the assessment of new toxicological test methods (Hoffmann et al., 2017). For instance, in a previous Scientific Opinion EFSA used a systematic review for the determination of toxicological mechanisms in the frame of AOP approach (Choi et al., 2016; EFSA Scientific Committee, 2017c).

Besides toxicity data on the active substance, such data may also be required on metabolites or residues if human exposure occur through the diet or drinking water. Results from these studies are then considered in relation to expected human exposures estimated through food consumption and other sources of exposure. The strength of this approach is that *in vivo* studies account for potential toxic metabolites, though not always animal metabolic pathways parallels the ones of humans.

Experimental studies in laboratory animals are controlled studies where confounding is eliminated by design, which is not always the case with epidemiological studies. Animals used in regulatory studies are, however, typically inbred, genetically homogeneous and due to the controlled environment they lack the full range of quantitative and qualitative chemical susceptibility profiles. Nevertheless, animal surrogates of human diseases are being challenged by their scientific validity and translatability to humans, and the lack of correlation often found between animal data and human outcomes can be attributed to the substantial interspecies differences in disease pathways and disease-induced changes in gene expression profiles (Esch et al., 2015). Thereby, many experimental models do not capture complex multifactorial diseases making animal-to-human extrapolation subject to considerable uncertainty. Current risk assessment is therefore by its nature predictive and may be insufficient because it is chemical-specific and humans are exposed to a large number of chemicals from environmental, dietary and occupational sources or because of different toxicokinetic differences. In recognition of the uncertain nature of animal-to-human extrapolation, the regulatory risk assessment advice does not just consider the relevant point(s) of departure (NOAEL, LOAEL or BMDL) that have



been identified as safe but lowers these values using uncertainty factors (UFs) to propose safe reference dose values, either for acute or chronic toxicity.

Given the limitations of studies in laboratory animals, epidemiological studies in the 'real world' are needed, even if they have limitations of their own. Epidemiological studies incorporate the true (or estimated) range of population exposures, which usually are intermittent and at inconsistent doses instead of occurring at a consistent rate and dose magnitude (Nachman et al., 2011). Since epidemiological studies are based on real-world exposures, they provide insight into actual human exposures that can then be linked to diseases, avoiding the uncertainty associated with extrapolation across species. Hence, it can be said that they address the requirements of Regulation 1107/2009 Article 4, which stipulates that the risk assessment should be based on good plant protection practice and realistic use conditions. Thus, epidemiological studies assist problem formulation and hazard/risk characterisation whilst avoiding the need for high dose extrapolation (US-EPA, 2010).

Epidemiological studies therefore provide the opportunity to (a) identify links with specific human health outcomes that are difficult to detect in animal models; (b) affirmation of the human relevance of effects identified in animal models; (c) ability to evaluate health effects for which animal models are unavailable or limited (Raffaele et al., 2011). Epidemiological evidence will be considered over experimental animal evidence only when sufficiently robust pesticide epidemiological studies are available. However, in epidemiological studies, there are always a variety of factors that may affect the health outcome and confound the results. For example, when epidemiological data suggest that exposures to pesticide formulations are harmful they usually cannot identify what component may be responsible due to the complexity of accurately assessing human exposures to pesticides. While some co-formulants are not intrinsically toxic, they can be toxicologically relevant if they change the toxicokinetics of the active substance. In addition, confounding by unmeasured factor(s) associated with the exposure can never be fully excluded; however, a hypothetical confounder (yet unrecognised) may not be an actual confounder and has to be strongly associated with disease and exposure in order to have a meaningful effect on the risk (or effect size) estimate, which is not always the case.

Many diseases are known to be associated with multiple risk factors; however, a hazard-by-hazard approach is usually considered for evaluating the consequences of individual pesticide hazards on vulnerable systems (Figure 4A). Specifically, single-risk analysis allows a determination of the individual risk arising from one particular hazard and process occurring under specific conditions, while it does not provide an integrated assessment of multiple risks triggered by different environmental stressors (either natural or anthropogenic) (Figure 4B). Risk assessment would benefit by developing procedures for evaluating evidence for co-occurrence of multiple adverse outcomes (Nachman et al., 2011), which is more in line with what happens in human setting. For these reasons, if appropriately conducted, epidemiological studies can be highly relevant for the risk assessment process.



# A Classical <u>single hazard</u> approach: driven by regulatory frameworks

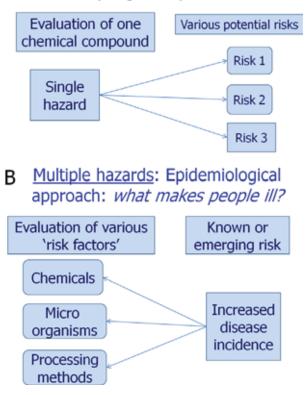


Figure 4: Role of epidemiological studies when compared to classical toxicological studies

In parallel with epidemiological data, vigilance data can provide an additional stream of evidence, especially for acute toxicity. Cases are usually well-documented and information can be used at different steps of the risk assessment; these include: level and duration of exposure, clinical course and assessment of the causal relationship. In severe cases, the toxin and/or the metabolites are usually measured in blood or urine which allows for comparison with animal data and in some cases for setting toxicological values.

In summary, experimental studies or epidemiological studies and vigilance data represent two different approaches to collect and assess evidence i.e. one emanating from controlled exposures (usually to a single substance) using experimental study design and a relatively homogeneous surrogate population, the other reflecting the changes observed in a heterogeneous target population from mixed (and varying) exposure conditions using non-experimental study design (ECETOC, 2009). Epidemiology and toxicology each bring important and different contributions to the identification of human hazards. This makes both streams of evidence complementary, and their combination represents a powerful approach. Animal studies should always inform the interpretation of epidemiological studies and vice versa; hence, they should not be studied and interpreted independently.

# 7.2. Principles for weighting of human observational and laboratory animal experimental data

Following the identification of reliable human (epidemiological or vigilance) studies and the assessment of the relevance of the pooled human studies, the separate lines of evidence that were found to be relevant need to be integrated with other lines of evidence that were equally found to be relevant.

The first consideration is thus how well the health outcome under consideration is covered by toxicological and epidemiological studies. When both animal and human studies are considered to be available for a given outcome/endpoint, this means that individual studies will first have been assessed for reliability and strength of evidence (Sections 6.2 and 6.3, respectively, for epidemiological studies)

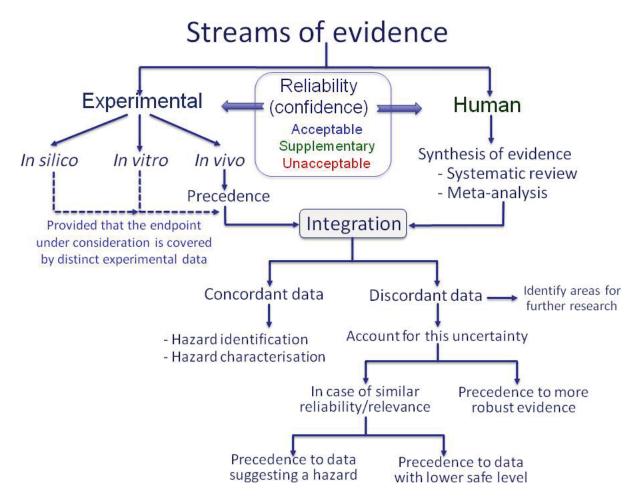


prior to the weighting of the various sources of evidence. Although the different sets of data can be complementary and confirmatory, individually they may be insufficient and pose challenges for characterising properly human health risks. Where good observational data are lacking, experimental data have to be used. Conversely, when no experimental data is available, or the existing experimental data were found not to be relevant to humans, the risk assessment may have to rely on the available and adequate observational studies.

A framework is proposed for a systematic integration of data from multiple lines of evidence (in particular, human and experimental studies) for risk assessment (Figure 5). Such integration is based on a WoE analysis accounting for relevance, consistency and biological plausibility using modified Bradford Hill criteria (Table 3). For a comparative interpretation of human and animal data, this framework should rely on the following principles (adapted from ECETOC, 2009; Lavelle et al., 2012):

- Although the totality of evidence should be assessed, only the studies that are found to be reliable (those categorised as acceptable or supplementary evidence) are considered further. If the data from the human or the experimental studies is considered to be of low reliability (categorised as unacceptable), no risk assessment can be conducted.
- A WoE approach should be followed where several lines of evidence are found to be relevant. For pesticide active substances, experimental studies following OECD test guidelines are deemed high reliability unless there is evidence to the contrary. The strength of evidence from animal studies can be upgraded if there is high confidence in alternative pesticide toxicity testing or non-testing methods (e.g. *in vitro* and *in silico* studies, respectively). As for epidemiological evidence, the conduct of meta-analysis provides a more precise estimate of the magnitude of the effect than individual studies and also allows for examining variability across studies (see Section 6.3).
- Next, the studies that are found to be more relevant for the stage being assessed are to be given more weight, regardless of whether the data comes from human or animal studies. Where human data are of highest relevance, and supported by a mechanistic scientific foundation, they should take precedence for each stage of the risk assessment. When human and experimental data are of equal or similar relevance, it is important to assess their concordance (consistency across the lines of evidence) in order to determine whether and which data set may be given precedence.
  - In case of concordance between human and experimental data, the risk assessment should use all the data as both yield similar results in either hazard identification (e.g. both indicate the same hazard) or hazard characterisation (e.g. both suggest similar safe dose levels). Thus, both can reinforce each other and similar mechanisms may be assumed in both cases.
  - In case of non-concordance, the framework needs to account for this uncertainty. For hazard identification, the data suggesting the presence of a hazard should generally take precedence. For dose-response, the data resulting in the lower acceptable level should take precedence. In every situation of discordance, the reasons for this difference should be considered. If the reason is related to the underlying biological mechanisms, or toxicokinetic differences between humans and animal models, then confidence in the risk assessment will increase. Conversely, if the reason cannot be understood or explained, then the risk assessment may be less certain. In such cases, efforts should be made to develop a better understanding of the biological basis for the contradiction.





**Figure 5:** Methodology for the integration of human and animal data for risk assessment

Epidemiological studies provide complementary data to analyse risk and should be contextualised in conjunction with well-designed toxicological *in vivo* studies and mechanistic studies. The overall strength of the evidence achieved from integrating multiple lines of evidence will be at least as high as the highest evidence obtained for any single line. This integrated approach provides explicit guidance on how to weight and integrate toxicological and epidemiological evidence. This is a complex task that becomes even more difficult when epidemiological data deal with multifactorial, multihit, chronic diseases for which toxicological models, or disease-specific animal models, are limited.

# 7.3. Weighting all the different sources of evidence

The WHO/IPCS defines the WoE approach as a process in which all of the evidence considered relevant for risk assessment is evaluated and weighted (WHO/IPCS, 2009). The WoE approach, taking the risk assessment of chemical substances as an example, requires the evaluation of distinct lines of evidence (*in vivo, in vitro, in silico,* population studies, modelled and measured exposure data, etc.). The challenge is to weight these types of evidence in a systematic, consistent and transparent way (SCENIHR, 2012). The weighting may be formally quantitative or rely on categorisation according to criterion referencing of risk.

An EFSA Working Group was established to provide transparent criteria for the use of the WoE approach for the evaluation of scientific data by EFSA's Panels and Scientific Committee (EFSA, 2015b). The aim of this Working Group was to provide support to stakeholders on how individual studies should be selected and weighted, how the findings integrated to reach the final conclusions and to identify uncertainties regarding the conclusions.

The WoE approach is not consistently considered in the risk assessment of pesticides in the peer review process of DAR or RAR. Expert judgement alone, without a structured WoE approach, has been more commonly used. A few examples can be found, such as the peer review of glyphosate (EFSA, 2015c), where the rapporteur Member State (RMS) considered all the data either from industry or



from public literature, including epidemiological data, and took a specific WoE approach with established *ad hoc* criteria and considering all data available for proposing an 'overall' NOAEL for each endpoint of toxicity explored.

The US-EPA has recently applied specific criteria for the WoE approach to the peer review of the pesticide chlorpyrifos by following the 'Framework for incorporating human epidemiologic & incident data in health risk assessment'. In this specific case, a WoE analysis has been conducted to integrate quantitative and qualitative findings across many lines of evidence including experimental toxicology studies, epidemiological studies and physiologically based pharmacokinetic and pharmacodynamic (PBPK-PD) modelling. Chlorpyrifos was also used as an example for the EFSA Guidance on literature search under Regulation (EC) No 1107/2009. In addition, an EFSA conclusion (EFSA, 2014a) took into consideration the US-EPA review (2011) to revise its first conclusion produced in 2011.

In sum, a broader WoE approach can be applied to evaluate the available scientific data using modified Bradford Hill criteria as an organisational tool to increase the likelihood of an underlying causal relationship (Table 3). Although epidemiology increasingly contributes to establishing causation, an important step to this end is the establishment of biological plausibility (US-EPA, 2010; Adami et al., 2011; Buonsante et al., 2014).

# 7.4. Biological mechanisms underlying the outcomes

A biological mechanism describes the major steps leading to a health effect following interaction of a pesticide with its biological targets. The mechanism of toxicity is described as the major steps leading to an adverse health effect. An understanding of all steps leading to an effect is not necessary, but identification of the key events following chemical interaction is required to describe a mechanism (of toxicity in the case of an adverse health effect). While many epidemiological studies have shown associations between pesticide exposures and chronic diseases, complementary experimental research is needed to provide mechanistic support and biological plausibility to the human epidemiological observations. Experimental exposures should be relevant to the human population provided that the biologic mechanisms in laboratory animals occur in humans.

Establishing biological plausibility as part of the interpretation of epidemiological studies is relevant and should take advantage of modern technologies and approaches (Section 7.6). In this context, the AOP framework can be used as a tool for systematically organising and integrating complex information from different sources to investigate the biological mechanisms underlying toxic outcomes and to inform the causal nature of links observed in both experimental and observational studies (Section 7.5).

The use of data to inform specific underlying biological mechanisms or pathways of the potential toxic action of pesticides is limited since only selected pesticide chemicals have been investigated for biological function in relation to a specific health outcome. It may be possible to formulate a mode of action (MoA) hypothesis, particularly where there is concordance between results of comparable animal studies or when different chemicals show the same pattern of toxicity. It is essential to identify the toxicant and the target organ as well as the dose–response curve of the considered effect and its temporal relationship. If the different key events leading to toxicity and a MoA hypothesis can be identified, it is sometimes possible to evaluate the plausibility of these events to humans (ECETOC, 2009).

Sulfoxaflor is an example where MoA has been extensively studied and has been also widely used as an example during the ECHA/EFSA MOA/HRF workshop held in November 2014. Sulfoxaflor induced hepatic carcinogenicity in both rats and mice. Studies to determine the MoA for these liver tumours were performed in an integrated and prospective manner as part of the standard battery of toxicology studies such that the MoA data were available prior to, or by the time of, the completion of the carcinogenicity studies. The MoA data evaluated in a WoE approach indicated that the identified rodent liver tumour MoA for sulfoxaflor would not occur in humans. For this reason, sulfoxaflor is considered not to be a potential human liver carcinogen.

Furthermore, sometimes MoA data may indicate a lack of possible effects. If there are biological data that indicate an adverse effect is not likely to occur in humans, this should inform the interpretation of epidemiological studies. Nevertheless, while primary target site selectivity between pests and humans plays an important role in pesticides safety, secondary targets in mammals must also be considered.

In the case of exposure to multiple pesticides, the decision to combine risks can be taken if the pesticides share a common mechanism of toxicity (act on the same molecular target at the same target tissue, act by the same biochemical mechanism of action, and share a common toxic intermediate) which may cause the same critical effect or just based on the observation that they share the same target organ (EFSA 2013a,b). However, cumulative risk assessment is beyond the scope of this Opinion.

# 7.5. Adverse Outcome Pathways (AOPs)

The AOP methodology provides a framework to collect and evaluate relevant chemical, biological and toxicological information in such a way that is useful for risk assessment (OECD, 2013). An AOP may be defined as the sequence of key events following the interaction of a chemical with a biological target (molecular initiating event (MIE)) to the *in vivo* adverse outcome relevant to human health. All these key events are necessary elements of the MoA and should be empirically observable or constitute biologically based markers for such an event. An AOP is therefore a linear pathway from one MIE to one adverse outcome at a level of biological organisation relevant to risk assessment. The goal of an AOP is to provide a flexible framework to describe the cascade of key events that lead from a MIE to an adverse outcome in a causal linkage (EFSA PPR Panel, 2017). The 'key events' must be experimentally measurable and the final adverse effect is usually associated with an *in vivo* OECD Test Guideline. However, in some cases the adverse outcome may be at a level of biological organisation below that of the apical endpoint described in a test guideline (OECD, 2013).

A particular MIE may lead to several final adverse effects and, conversely, several MIEs may converge in the same final adverse effect. However, each AOP will have only one MIE and one final adverse effect, but may involve an unlimited number of intermediate steps (Vinken, 2013). It should be noted that key events at different levels of biological organisation provide a greater WoE than multiple events at the same level of organisation (OECD, 2013).

The essential biochemical steps involved in a toxic response are identified and retrieved from an indepth survey of relevant scientific literature or from experimental studies. Any type of information can be incorporated into an AOP, including structural data, 'omics-based' data and *in vitro*, *in vivo* or *in silico* data. However, *in vivo* data are preferred over *in vitro* data and endpoints of interest are preferred to surrogate endpoints (Vinken, 2013). The AOPs identified must not be incompatible with normal biological processes, since they need to be biologically plausible.

Qualitative AOPs (intended as an AOP including the assembly and evaluation of the supporting WoE following the OECD guidance for AOP development) should be the starting and standard approach in the process of integration of epidemiology studies into risk assessment by supporting (or identifying the lack of support for) the biological plausibility of the link between exposure to pesticides affecting the pathway and the adverse outcome. Accordingly, qualitative AOPs may be developed solely for the purpose of hazard identification, to support biological plausibility of epidemiological studies based on mechanistic knowledge (EFSA PPR Panel, 2017).

The AOP framework is a flexible and transparent tool for the review, organisation and interpretation of complex information gathered from different sources. This approach has the additional advantage of qualitatively characterising the uncertainty associated with any inference of causality and identifying whether additional mechanistic studies or epidemiological research would be more effective in reducing uncertainty. The AOP framework is therefore a useful tool for risk assessment to explore whether an adverse outcome is biologically plausible or not. For the purpose of analysing the biological plausibility, AOPs can serve as an important tool, particularly when the regulatory animal toxicological studies are negative but the evaluation of the apical endpoint (or relevant biomarkers) observed in epidemiological studies is considered inadequate based on the AOP. By means of mechanistically describing apical endpoints, the AOP framework is chemically agnostic, if complemented by the MoA and/or Integrated Approach on Testing and Assessment (IATA) framework, it will support the chemical specific risk assessment (EFSA PPR Panel, 2017).

AOP and MoA data can be used to assess the findings of epidemiological studies to weight their conclusions. Whether those findings are inconsistent with deep understanding of biological mechanisms, or simply empirical, they should be given less weight than other findings that are consistent with AOP or MoA frameworks once established. However, there are relatively few examples of well-documented AOPs and a full AOP/MoA framework is not a requirement for using epidemiological studies in risk assessment.

AOPs are thus a critical element to facilitate moving towards a mechanistic-based risk assessment instead of the current testing paradigm relying heavily on apical effects observed in animal studies. Shifting the risk assessment paradigm towards mechanistic understanding would reduce limitations of the animal data in predicting human health effects for a single pesticide, and also support the current efforts being made on cumulative risk assessment of pesticide exposure (EFSA PPR Panel, 2017).



# **7.6.** Novel tools for identifying biological pathways and mechanisms underlying toxicity

The elucidation of toxicity pathways brings the opportunity of identifying novel biomarkers of early biological perturbations in the toxicodynamic progression towards overt disease, particularly from advances in biomonitoring, in -omics technologies and systems biology (toxicology). The revolution of omics in epidemiology holds the promise of novel biomarkers of early effect and offers an opportunity to investigate mechanisms, biochemical pathways and causality of associations.

The growing recognition of the value of biomonitoring data in epidemiological investigations may help to reduce misclassification by providing objective measures of exposure and outcome. As long as biomarker data for exposure, outcome and susceptibility are increasingly generated, epidemiology will have a greater impact in the understanding of toxicodynamic progression as a function of pesticide exposure and eventually in risk assessment. A challenge for risk assessors will be to acknowledge where subtle and early changes along the toxicodynamic pathway are indicative of increased potential for downstream effects (Nachman et al., 2011). Omics data can be used for gaining insight to the MoA by identifying pathways affected by pesticides and as such can assist hazard identification, the first step in risk assessment.

Transcriptomic, metabolomic, epigenomic and proteomic profiles of biological samples provide a detailed picture, sometimes at individual molecule resolution, of the evolving state of cells under the influence of environmental chemicals, thus revealing early mechanistic links with potential health effects. Nowadays, the challenges and benefits that advances in -omics techniques can bring to regulatory toxicology are still being explored (Marx-Stoelting et al., 2015). Clear rules for assessing the specificity of these biomarkers are necessary.

Those -omic applications most relevant and advanced in the context of toxicology are analysis of MoA and the derivations of AOP, and biomarker identification, all of which potentially assist epidemiology too. For example, (a) transcriptomics: comparing gene expression (mRNA) profiles can be used for biomarker discovery, grouping expressed genes into functional groups (Gene Ontology categories) or for Gene Set Analysis. Such techniques may provide varying information regarding biological mechanisms. (b) Proteomics: studying the protein profile of samples, with sophisticated analysis of protein quantity and post-translational modifications which may be associated with changes in biological pathways following exposure and possible disease development, utilising informatics and protein databases for identification and quantification. (c) Metabolomics uses nuclear magnetic resonance spectroscopy or mass-spectrometry based techniques to produce data which are analysed via software, and databases, to identify markers (molecular signatures and pathways) that correlate with exposure or disease. (d) The use of the exposome (the totality of exposures received by an individual during life) might be better defined by using -omics technologies and biomarkers appropriate for human biomonitoring. Nevertheless, important limitations stemming from the lack of validation of these methodologies and their cost limit their use at large scale.

The application of -omics technologies to environmental health research requires special consideration to study design, validation, replications, temporal variance and meta-data analysis (Vlaanderen et al., 2010). For larger studies, intra-individual variability in the molecular profiles measured in biological samples should show less variability than the interindividual variation in profiles of gene expression, protein levels or metabolites, which are highly variable over time. It is important that these inter-individual variations should not be larger than variation related to exposure changes, but it is not certain if this will be true.

The biologically meaningful omics signatures identified by performing omics-exposure and omicshealth association studies provide useful data for advanced risk assessment. This approach supports moving away from apical toxicity endpoints towards earlier key events in the toxicity pathway resulting from chemical-induced perturbation of molecular/cellular responses (NRC, 2007).

# 7.7. New data opportunities in epidemiology

The current technological landscape permits the digitisation and storage of unprecedented amount of data from many sources, including smart phones, text messages, credit card purchases, online activity, electronic medical records, global positioning system (GPS) and supermarket purchasing data. While some of these data sources may provide valuable information for risk assessment, many of them contain personal information that can outpace legal frameworks and arise questions about the ethics of its use for scientific or regulatory purposes. A specific example is constituted by data containing



personal information related to health, which are considered sensitive or especially protected, such as electronic medical records, information from occupational or environmental questionnaires, geographic location, health or social security number, etc. These various forms of health information are being easily created, stored and accessed. Big data provide researchers with the ability to match or link records across a number of data sources. Linking of big data sources of health and heritable information offers great promise for understanding disease predictors (Salerno et al., 2017); however, there are challenges in using current methods to process, analyse and interpret the data systematically and efficiently or to find relevant signals in potential oceans of noise, as noted by the Board on Environmental Studies and Toxicology of the National Academies of Sciences, Engineering, and Medicine in its 2017 report.<sup>18</sup>

In addition, medico-administrative data, such as drug reimbursements drawn from National Health Insurance or hospital discharge databases, can be cross-linked with data on agricultural activities drawn from agricultural census or geographical mapping. It is acknowledged that in several instances this information can be obtained at group level only, and an important challenge will be to obtain data at individual level and/or on individual habits.

Biobanks also constitute new data sources from healthy or diseased populations. They consist of an organised collection of human biological specimens and associated information stored for diverse research purposes. These biosamples are available for application of novel technologies with potential for generating data valuable for exposure assessment or exposure reconstruction. If studies' design and conduct are harmonised, data and samples can be shared between biobanks to promote powerful pooled analyses and replications studies (Burton et al., 2010).

Large scale epidemiological studies with deep phenotyping provide also unprecedented opportunities to link well phenotyped study participants with the aforementioned data. For example, UK Biobank, has recruited over 500,000 individuals with questionnaire, medical history and physical measurements data as well as stored blood and urine samples with available genome wide association data for all 500,000 participants, and linkage to Hospital Episode Statistics, national registry data and primary care records. To gain information on air pollution and noise levels, the postcode of participants has been linked to air pollution or noise estimates. In addition, piloting of personal exposure monitoring will take place in order to collect individual level data on these exposures. These approaches could be extended to gain information on pesticide exposure, either through geographical linkage, linkage with purchasing and occupational registries, and personal exposure monitoring. Similar biobanks exist in many other EU countries (http://www.bbmri-eric.eu/BBMRI-ERIC has collected most EU studies).

# 8. Overall recommendations

# 8.1. Recommendations for single epidemiological studies:

The following recommendations for improving epidemiological studies are aimed to conform to the 'recognised standards' mentioned in Regulation (EU) No 1107/2009 to make them of particular value to risk assessment of pesticides ('where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards, epidemiological studies are of particular value and must be submitted'). Accordingly, these recommendations can indeed not be considered as a practical guidance for researchers on how to conduct such studies, but for those who are planning to conduct a study for further use in pesticide risk assessment.

#### a) Study design (including confounding)

- 1) Since prospective epidemiological designs provide stronger evidence for causal inference, these studies are encouraged over the other designs for pesticide risk assessment.
- 2) Future epidemiological studies should be conducted using the appropriate sample size in order to properly answer the question under investigation. A power analysis should thus be performed at the study design stage.
- Future studies should take into consideration heterogeneity, subpopulations, exposure windows and susceptibility periods and conditions (pregnancy, development, diseases, etc.).

<sup>&</sup>lt;sup>18</sup> National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Environmental Studies and Toxicology; Committee on Incorporating 21st Century Science into Risk-Based Evaluations. Washington (DC): National Academies Press (US); 2017 Jan.



- 4) A wide range of potential confounding variables (including co-exposure to other chemicals, lifestyle, socioeconomic factors, etc.) should be measured or accounted for during the design stage (e.g. matching) of the study.
- 5) Consideration of host factors that may influence toxicity and act as effect modifiers. These will include genetic polymorphisms data (e.g. paraoxonase-1 genotype) or nutritional factors (e.g. iodine status) among others.
- 6) Collaboration between researchers is encouraged to build-up consortia that enhance the effectiveness of individual cohorts.

Collection and appropriately storage of relevant biological material should be undertaken for future exposure assessment, including the use of novel technologies.

- **b) Exposure** (measurement, data transformation for reporting and statistical analysis):
  - 1) Collection of specific information on exposure should avoid as far as possible broad definitions of exposure, non-specific pesticide descriptions and broad exposures classifications such as 'never' *vs.* 'ever' categories. Nevertheless, these categories may be valuable under certain circumstances, e.g. to anticipate a class effect.
  - 2) Studies which only look at broad classes of pesticides (generic groups of unrelated substances), or 'insecticides', 'herbicides', etc. or even just 'pesticides' in general are of much less use (if any) for risk assessment. Studies that investigate specific named pesticides and co-formulants are more useful for risk assessment.
  - 3) Pesticides belonging to the same chemical class or eliciting the same mode of toxic action or toxicological effects might be grouped in the same category. Further refinement with information on frequency, duration and intensity of exposure might help in estimating exposure patterns.
  - 4) In occupational epidemiology studies, operator and worker behaviour and proper use of PPE should be adequately reported as these exposure modifiers may significantly change exposures and thereby potential associations.
  - 5) Improving the accuracy of exposure measurement is increasingly important, particularly for cohort studies. Long-term cohort studies which cover the etiologically relevant time period should improve the accuracy of measures of exposures by use of repeated biologic measures or repeated updates of self-reported exposures.
  - 6) Indirect measures of environmental exposure for wider populations, including records on pesticide use, registry data, GIS, geographical mapping, etc., as well as data derived from large databases (including administrative databases) may be valuable for exploratory studies. If these data are not available, records/registries should be initiated. Likewise, estimation of dietary exposure to pesticide from food consumption databases and levels of pesticide residues from monitoring programmes can be used as well. As with direct exposure assessment, each method of indirect measurement should be reviewed for risk of bias and misclassification and weighted appropriately.
  - 7) Whenever possible, exposure assessment should use direct measurements of exposure to named pesticides in order to establish different levels of exposure (e.g. personal exposure metering/biological monitoring), possibly in conjunction with other methods of exposure assessment which are more practicable or even necessary for large studies and historical exposures. New studies should explore novel ways of personal exposure monitoring. Results should be expressed using standardised units to normalise exposure across populations
  - 8) The characterisation of exposure assessment over time can benefit by undertaken a more comprehensive exposure monitoring strategy coupled with information on exposure determinants over a longer time period collected from questionnaires or job-exposure matrices supported by biomonitoring data. Exposure assessment models can be comprehensively supported by HBM studies, which would allow identification of the critical exposure parameters. If such case, adjustments can then be made to the parameter assumptions within the models, leading to more realistic evaluations of exposure.
  - 9) The use of the exposome concept and metabolomics in particular hold great promise for next-generation epidemiological studies both for better exposure measurement (biomarkers of exposure), for identification of vulnerable subpopulations and for biological interpretation of toxicity pathways (biomarkers of disease).



10) Improved knowledge on exposure (and toxicity) to pesticide mixtures will be beneficial for comprehensive risk assessment. Consideration of the joint action of combined exposures to multiple pesticides acting on common targets, or eliciting similar adverse effects, is relevant for cumulative risk assessment. This requires all the components of the mixture to be known as well as an understanding of the MoA, dose–response characteristics and potential interactions between components. Characterisation of the exposure is a key element for combined exposure to multiple pesticides where the pattern and magnitude of exposure changes over time.

#### c) Adverse Outcomes (measurement, data transformation for reporting and statistical analysis):

- 1) Self-reported health outcomes should be avoided or confirmed by independent, blinded assessment of disease status by a medical expert assigned to the study.
- 2) Outcomes under study should be well defined and surrogate endpoints should be avoided unless they have been validated. Care must be taken when definitions of diseases and subclasses of diseases change over time (cancer, neurodegenerative disorders, etc.).
- 3) Use should be made of biological markers of early biological effect to improve the understanding of the pathogenesis of diseases. These quantitative biological parameters from mechanistic toxicology will enhance the usefulness of epidemiology because they improve the study sensitivity, reduce misclassification and enhance human relevance as compared to findings from studies in experimental animals. Since these refined endpoints are early events in the toxicodynamic pathway and often measured on a continuous scale, they might be preferable to more overt and traditional outcomes.
- 4) The use of biomarkers of effect may be helpful in assessing aggregate exposure to pesticides and informing cumulative risk assessment.
- 5) Developing read across methods allowing health outcomes to be identified using epidemiological studies and to link acute and chronic incidents records with experimental findings.
- **d) Statistical** (descriptive statistics, modelling of exposure–effect relationship):
  - 1) Statistical analysis should be based on *a priori* defined analytical (statistical) protocols, to avoid post hoc analyses for exploratory studies and report all the results, regardless of whether they are statistically significant or not.
  - 2) Data should be reported in such a way that permit, where appropriate, mathematical modelling to estimate individual/population exposures and dose–response assessment irrespective of whether direct or indirect measures are used.
  - 3) Reports should include both unadjusted and adjusted proportions and rates of outcome of interest across studies that are based on underlying populations with different structure of relevant factors and exposures.
  - 4) Possible relevant factors, and their role in the exposure-health outcome relationship, should be carefully identified, accurately measured and thoroughly assessed. Most often, relevant factors have been screened as potential confounders. When confounding effects were detected, these needed to be adjusted for using appropriate statistical methods that include sensitivity analysis.
  - 5) Potentially useful analytical approaches, such as propensity score matching, mediation analyses, and causal inference are encouraged to be applied in pesticide epidemiology.
  - 6) When the association between a given pesticide exposure and a disease is found to be statistically significant, particularly in (presumed) low powered studies, it would be general good practice to perform a power analysis/design calculation to determine the degree to which the statistically significant effect size estimate (e.g. OR or RR) may be artificially inflated or magnified.<sup>19</sup>

<sup>&</sup>lt;sup>19</sup> Additional information on power and sample size recommendations and related issues including effect size magnification and design calculations are provided in Annex D to this report. Specifically, a power calculation requires 3 values to be clearly reported by epidemiological studies: (i) the number of subjects in the non-exposed group (including individuals with and without the disease of interest); (ii) the number of subjects in the exposed group (also including individuals with and without the disease of interest); (iii) the number of diseased subjects in the non-exposed group.



#### e) Reporting of results:

- 1) These should follow practices of good reporting of epidemiological research outlined in the STROBE statement and in the EFSA guideline on statistical reporting (EFSA, 2014b) and include the further suggestions identified in this Opinion including effect size inflation estimates.
- 2) Although some epidemiological research will remain exploratory and post hoc in nature, this should be acknowledged and supported by appropriate statistical analysis.
- Epidemiological studies are encouraged to provide access to raw data for further investigations and to deposit their full results and scripts or software packages used for analyses.
- 4) Report, or deposit using online sources, all results along with scripts and statistical tools used to allow the reproducibility of results to be tested.
- 5) Report all sources of funding and adequately report financial and other potential conflicts of interest.

As a general recommendation, the PPR Panel encourages development of guidance for epidemiological research in order to increase its value, transparency and accountability for risk assessment.<sup>20</sup> An increased quality of epidemiological studies, together with responsible research conduct and scientific integrity, will benefit the incorporation of these studies into risk assessment.

#### 8.2. Surveillance

- 1) Increase the reporting of acute and chronic incidents by setting up post-marketing surveillance programmes (occupational and general population) as required by article 7 of EU directive 2009/128; this should be fulfilled by developing surveillance networks with occupational health physicians and by boosting the collaboration between national authorities dealing with PPP and poison control information centres.
- 2) Develop a valid method for assessing the weight/strength of the causal relationship ('imputability') for acute and chronic incidents, and develop glossaries and a thesaurus to support harmonised reporting between EU member states.
- Harmonised data from member states should be gathered at the EU level and examined periodically by the Commission/EFSA and a report should be released focussing on the most relevant findings.
- 4) Develop an EU-wide vigilance framework for pesticides.
- 5) There is scope for training improvements regarding pesticide toxidromes in toxicology courses for medical and paramedical staff responsible for diagnostic decisions, data entry and management.

#### 8.3. Meta-analysis of multiple epidemiological studies

- 1) Evidence from epidemiological studies might be pooled by taking into account a thorough evaluation of the methods and biases of individual studies, an assessment of the degree of heterogeneity among studies, development of explanations underlying any heterogeneity and a quantitative summary of the evidence (provided that it is consistent).
- 2) For every evidence synthesis effort, studies should be reviewed using relevant risk of bias tools. Studies with different designs, or with different design features, may require (some) different questions for risk of bias assessments.
- 3) Evidence syntheses should not be restricted to specific time frames; they should include the totality of evidence. These efforts are more relevant if focused on specific health outcome or disease categories.
- 4) In evidence synthesis efforts, beyond the quantitative synthesis of the effect sizes, there should be consideration on the calculated predictive intervals, small study effects and asymmetry bias, conflicts of interest, confounding, excess significance bias,<sup>21</sup> and heterogeneity estimates.

<sup>&</sup>lt;sup>20</sup> An example is the guideline developed by the Dutch Society for Epidemiology on responsible epidemiologic Research Practice (2017).

<sup>&</sup>lt;sup>21</sup> Excess significance bias refers to the situation in which there are too many studies with statistically significant results in the published literature on a particular outcome. This pattern suggests strong biases in the literature, with publication bias, selective outcome reporting, selective analyses reporting, or fabricated data being possible explanations (Ioannidis and Trikalinos, 2007).



- 5) In the presence of heterogeneity, studies with highly selected populations, albeit unrepresentative of their respective populations, may prove valuable and deserve consideration as they may represent genuine and not statistical heterogeneity.
- 6) A more consistent reporting such as for age, race and gender across studies would enhance the meta-analyses.
- 7) Where quantitative data of individual pesticides are available from epidemiological studies, they can be combined or pooled for dose-response modelling, which could enable development of quantitative risk estimates and points of departure (BMDL, NOAEL).
- 8) International consortium of cohort studies should be encouraged to support data pooling to study disease–exposure associations that individual cohorts do not have sufficient statistical power to study (e.g. AGRICOH).

# 8.4. Integration of epidemiological evidence with other sources of information

- 1) All lines of evidence (epidemiology, animal, *in vitro* data) should be equally scrutinised for biases.
- 2) Validated and harmonised methods should be developed to combine observational studies, animal/basic science studies and other sources of evidence for risk assessment.
- 3) Experimental and human data should both contribute to hazard identification and to dose–response assessment.
- 4) A systematic integration of data from multiple lines of evidence should be based on a WoE analysis accounting for relevance, consistency and biological plausibility using modified Bradford Hill criteria. The principles underlying this framework are described in Section 7.2 and summarised in Figure 5.
- 5) Epidemiological findings should be integrated with other sources of information (data from experimental toxicology, mechanism of action/AOP) by using a WoE approach. An integrated and harmonised approach should be developed by bringing together animal, mechanistic and human data in an overall WoE framework in a systematic and consistent manner.
- 6) The AOP framework offers a structured platform for the integration of various kinds of research results.
- 7) Animal, *in vitro* data and human data should be assessed as a whole for each endpoint. A conclusion can be drawn as to whether the results from the experiments are confirmed by human data for each endpoint and this could be included in the RARs.

#### 9. Conclusions

This Scientific Opinion is intended to help the peer review process during the renewal of pesticides authorisation (and, where possible, during the approval process) under Regulation 1107/2009 which requires a search of the scientific peer-reviewed open literature, including existing epidemiological studies. These are more suitable for the renewal process of active substances, also in compliance with Regulation 1141/2010, which indicates that the dossiers submitted for renewal should include new data relevant to the active substance.

The four key elements of the terms of reference are repeated below and the parts of the text addressing the individual terms are identified in order. As they follow from the text passages grouped with each of the ToRs the recommendations relevant to each of the ToRs are also indicated as follows.

'The PPR Panel will discuss the associations between pesticide exposure and human health effects observed in the External scientific report (Ntzani et al., 2013) and how these findings could be interpreted in a regulatory pesticide risk assessment context. Hence, the PPR Panel will systematically assess the epidemiological studies collected in the report by addressing major data gaps and limitations of the studies and provide recommendations thereof'.

'The PPR Panel will specifically':

- 1) Collect and review all sources of gaps and limitations, based on (but not necessarily limited to) those identified in the External Scientific report in regard to the quality and relevance of the available epidemiological studies. Responses in Section 3 pp. 20–24, Section 5.2 pp. 33–35: no Recommendations appropriate.
- 2) Based on the gaps and limitations identified in point 1, propose potential refinements for future epidemiological studies to increase the quality, relevance and reliability of the findings



and how they may impact pesticide risk assessment. This may include study design, exposure assessment, data quality and access, diagnostic classification of health outcomes, and statistical analysis. Responses in Section 4 pp 24–33: recommendations in Sections 8.1, 8.2 and 8.3 pp. 54–58.

- 3) Identify areas in which information and/or criteria are insufficient or lacking and propose recommendations for how to conduct pesticide epidemiological studies in order to improve and optimise the application in risk assessment. These recommendations should include harmonisation of exposure assessment (including use of biomonitoring data), vulnerable population sub-groups and/or health outcomes of interest (at biochemical, functional, morphological and clinical level) based on the gaps and limitations identified in point 1. Responses in Sections 4.2–4.5 pp. 27–33, Section 5.3 pp. 36: recommendations in Section 8.1 c) 1–4, pp. 56.
- 4) Discuss how to make appropriate use of epidemiological findings in risk assessment of pesticides during the peer review process of draft assessment reports, e.g. WoE as well as integrating the epidemiological information with data from experimental toxicology, AOPs, mechanism of actions, etc. Responses in Sections 6.2 and 6.3 pp. 37–45 and 7 pp. 45–54: Responses in Section 8.4 pp. 58.

As explained above, appropriate epidemiological data and post-approval surveillance may usefully contribute to the risk assessment framework by hazard identification, and – with methodological improvements – hazard characterisation. It can be improved by contributions from WoE analysis, Uncertainty analysis, and identification and estimation of biases. It is the responsibility of applicants to collect the available relevant literature, to consider its relevance and quality using relevant EFSA criteria including those for systematic review and to introduce discussion of the outcomes within the DAR, RAR and post-approval frameworks that are prescribed under EU law.

The definition of appropriate quality will require analysis of sample size, statistical procedures, estimates of effect size inflation, assessment of biases and their contribution to the conclusions drawn.

The nature of the studies will require consideration at all relevant points in the risk assessment process so that for example epidemiological data on reproductive topics will be considered alongside laboratory animal studies designed to reveal reproductive effects and in the context of recommendation for labelling for reproductive toxicity (for ECHA).

Unless there is history of use in countries outside the EU, the relevant epidemiological studies will be restricted in their effect on the DAR but the RAR and Surveillance framework is potentially able to benefit from epidemiology progressively as time after first approval passes and from prior use of Active Ingredients in other jurisdictions. It is recommended that RAR and surveillance protocols should reflect this difference.

The specific recommendations listed above follow from detailed arguments based on an analysis of present and foreseen **s**trengths **w**eaknesses **o**pportunities and **t**hreats related to the use of epidemiological data in risk assessment. Broadly these are as follows:

#### Strengths. Include:

- The fact that the evidence concerns human specific risks.
- That health outcomes are integrated measures of the effects of all exposure to toxins.
- The ability to elicit subjective experience from potentially affected people.

#### Weaknesses. Include:

- The exposures to pesticides are usually complex; contribution of a specific active ingredient is not easily deciphered.
- The exposures occur in various settings where precisely controlled conditions are lacking.
- Most data reflect the responses of mixed populations.
- Many data show low level associations that are inconsistently repeatable and require sophisticated analysis.

**Opportunities**. Despite the range of limitations described in this Opinion, which apply to many available published epidemiological studies, there are opportunities to benefit risk assessment of pesticides. These include:

• The access to very large numbers of potentially exposed individuals for studies that may reveal subtle health effects and reveal the experience of sensitive sub-groups.



- The prospect of improving exposure estimation using biomonitoring and new molecular approaches to establish tissue burdens of potential toxins and their residues.
- The possibility of fully integrating human data into the conventional risk assessment based on responses in laboratory animals.
- Utilising WoE, AOP, Expert judgement, Expert Knowledge Elicitation (EKE) and Uncertainty Analysis to evaluate differences in the quality of potentially relevant data.
- The opportunity to engage professional epidemiologists and statisticians to refine interpretation of epidemiological findings and to recommend improved designs to tackle difficult areas such as chronic and combined exposure risks and dose–response data.
- A major information technology opportunity exists in pooling data from a variety of national sources. Once the relevant legal, methodological and ethical issues are overcome much more valuable data can be collected. When this data is made available, in a form that can be used in a 'big data' setting for societal benefit there will be potential for significant improvements in epidemiological studies. First, however, it will be necessary to preserve individual privacy and essential commercial confidentiality. Once these obstacles are overcome the statistical power of epidemiological studies can be improved and applied to identify and possibly characterise hazards better. These aims can be realised effectively by agreed actions at a high EU level. Interstate approval for providing data and interactive platforms will need to be backed by harmonisation of population health information, food consumption data, active substance and co-formulant spatial and temporal application data. Such rich data can be expected to assist in increasing consistency, a criterion that strengthens evidence of causality and reliability. It promises larger sample sizes for epidemiological studies that will be better able to identify vulnerable groups that may require special protection from pesticide toxicity.

#### Threats. Include:

- Widespread perception of risk levels to the human population or to wildlife and the environment that are unrealistic and that cause negative consequences in societies.
- Poor experimental design yielding false positive or false negative conclusions that undermine data from other valid sources.
- Failure to respond to emerging risks as a result of ineffective surveillance or unwillingness to make appropriate anonymised data available for societal benefit.
- Waste of data through failure to collect appropriate information regarding exposure (specifically occupational exposure) by registries (cancer or congenital anomalies) or surveillance programmes which hinders linking health outcomes to exposure.
- Waste of data through failure to harmonise diagnostic criteria, failure to record data in a sufficiently detailed combinable form for integrated analysis, poor training of medical and paramedical staff in relevant toxidromes that will allow optimum quality of data entered into Health Statistics Databases.

#### References

- Adami HO, Berry SC, Breckenridge CB, Smith LL, Swenberg JA, Trichopoulos D, Weiss NS and Pastoor TP, 2011. Toxicology and epidemiology: improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. Toxicology Sciences, 122, 223–234.
- Amler RW, Barone Jr S, Belger A, Berlin Jr CM, Cox C, Frank H, Goodman M, Harry J, Hooper SR, Ladda R, LaKind JS, Lipkin PH, Lipsitt LP, Lorber MN, Myers G, Mason AM, Needham LL, Sonawane B, Wachs TD and Yager JW, 2006. Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical exposure. Neurotoxicology, 27, 861–874.
- Bengtson AM, Westreich D, Musonda P, Pettifor A, Chibwesha C, Chi BH, Vwalika B, Pence BW, Stringer JS and Miller WC, 2016. Multiple overimputation to address missing data and measurement error: application to HIV treatment during pregnancy and pregnancy outcomes. Epidemiology, 27, 642–650.
- Bevan R, Brown T, Matthies F, Sams C, Jones K, Hanlon J and La Vedrine M, 2017. Human Biomonitoring data collection from occupational exposure to pesticides. EFSA supporting publication 2017:EN-1185, 207 pp.
- Bottai M, 2014. Lessons in biostatistics: inferences and conjectures about average and conditional treatment effects in randomized trials and observational studies. Journal of Internal Medicine, 276, 229–237.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2001. Benchmark dose calculation from epidemiological data. Biometrics, 57, 698–706.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2004. Effects of exposure imprecision on estimation of the benchmark dose. Risk Analysis, 24, 1689–1696.



- Buonsante VA, Muilerman H, Santos T, Robinson C and Tweedale AC, 2014. Risk assessment's insensitive toxicity testing may cause it to fail. Environmental Research, 135, 139–147.
- Burton PR, Fortier I and Knoppers BM, 2010. The global emergence of epidemiological biobanks: opportunities and challenges. In: Khoury M, Bedrosian S, Gwinn M, Higgins J, Ioannidis J and Little J (eds.). Human Genome Epidemiology. Building the evidence for using genetic information to improve health and prevent disease. 2nd Edition, Oxford University Press, Oxford. pp. 77–99.
- Choi J, Polcher A and Joas A, 2016. Systematic literature review on Parkinson's disease and Childhood Leukaemia and mode of actions for pesticides. EFSA supporting publication 2016:EN-955, 256 pp. Available online: http://www.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-955/pdf
- Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Beane Freeman LE, Blair A, Sandler DP and Alavanja MC, 2011. An updated algorithm for estimation of pesticide exposure intensity in the agricultural health study. International Journal of Environmental Research and Public Health, 8, 4608–4622.
- Coggon D, 1995. Questionnaire based exposure assessment methods. Science of the Total Environment, 168, 175–178.
- Cornelis C, Schoeters G, Kellen E, Buntinx F and Zeegers M, 2009. Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case-control study on bladder cancer. International Journal of Hygiene and Environmental Health, 212, 172–185.
- la Cour JL, Brok J and Gøtzsche PC, 2010. Inconsistent reporting of surrogate outcomes in randomised clinical trials: cohort study. BMJ, 341, c3653.
- DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS and Yucesoy B, 2015. Systems biology and biomarkers of early effects for occupational exposure limit setting. The Journal of Occupational and Environmental Hygiene, 12(Suppl 1), S41–S54.
- Dionisio KL, Chang HH and Baxter LK, 2016. A simulation study to quantify the impacts of exposure measurement error on air pollution health risk estimates in copollutant time-series models. Environmental Health, 15, 114.
- DSE (Dutch Society for Epidemiology), 2017. Responsible Epidemiologic Research Practice (RERP). A guideline developed by the RERP working group of the Dutch Society for Epidemiology, 2017 (available at https://www.epidemiologie.nl/home.html, https://epidemiologie.nl/fileadmin/Media/docs/Onderzoek/Responsible\_Epide miologic\_Research\_Practice.2017.pdf)
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2009. Framework for the Integration of Human and Animal Data in Chemical Risk Assessment. Technical Report No. 104. Brussels. Available online: http://www.ecetoc.org/uploads/Publications/documents/TR%20104.pdf
- ECHA/EFSA, 2014. Workshop on Mode of action and Human relevance framework in the context of classification and labelling (CLH) and regulatory assessment of biocides and pesticides. November 2014. Available online: https://echa.europa.eu/documents/10162/22816050/moaws\_workshop\_proceedings\_en.pdf/a656803e-4d97-438f-87ff-fc984cfe4836
- EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids. EFSA Journal 2004;81, 1–49 pp. https://doi.org/10.2903/j.efsa.2004.81
- EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;980, 1–139 pp. https:// doi.org/10.2903/j.efsa.2009.980
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2009b. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
- EFSA (European Food Safety Authority), 2010a. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637
- EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM), 2010b. Scientific Opinion on Lead in Food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
- EFSA (European Food Safety Authority), 2011a. Submission of scientific-peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092
- EFSA (European Food Safety Authority), 2011b. Statistical significance and biological relevance. EFSA Journal 2011;9(9):2372, 17 pp. https://doi.org/10.2903/j.efsa.2011.2372
- EFSA (European Food Safety Authority), 2012a. Scientific Opinion on risk assessment terminology. EFSA Journal 2012;10(5):2664, 43 pp. https://www.efsa.europa.eu/en/efsajournal/pub/2664
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2012b. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985
- EFSA (European Food Safety Authority), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293



- EFSA (European Food Safety Authority), 2013b. Scientific Opinion on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12):3472, 40 pp. https://doi.org/10.2903/j.efsa.2013.3472
- EFSA (European Food Safety Authority), 2014a. Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos. EFSA Journal 2014;12(4):3640, 34 pp. https://doi.org/ 10.2903/j.efsa.2014.3640
- EFSA (European Food Safety Authority), 2014b. Guidance on statistical reporting. EFSA Journal 2014;12(12): 3908, 18 pp. https://doi.org/10.2903/j.efsa.2014.3908
- EFSA (European Food Safety Authority), 2015a. Stakeholder Workshop on the use of epidemiological data in pesticide risk assessment. EFSA supporting publication 2015:EN-798, 8 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/798e
- EFSA (European Food Safety Authority), 2015b. Increasing robustness, transparency and openness of scientific assessments Report of the Workshop held on 29–30 June 2015 in Brussels. EFSA supporting publication 2015:EN-913. 29 pp. Available online: http://www.efsa.europa.eu/sites/default/files/corporate\_publications/ files/913e.pdf
- EFSA (European Food Safety Authority), 2015c. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302, 107 pp. https://doi.org/10.2903/j.efsa.2015.4302
- EFSA PPR Panel (European Food Safety Authority Panel on Plant Protection Products and their Residues), 2017. Scientific Opinion on the investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 2017;15(3):4691, 325 pp. https://doi.org/10.2903/j.efsa.2017.4691
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971.
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017c. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1): 4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658
- von Elm E, Altman DG, Egger M, Pocock SJ and Gøtzsche PC, Vandenbroucke JP and STROBE Initiative, 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ, 335, 806–808.
- Esch EW, Bahinski A and Huh D, 2015. Organs-on-chips at the frontiers of drug discovery. Nature Reviews. Drug Discovery, 14, 248–260.
- Fedak KM, Bernal A, Capshaw ZA and Gross S, 2015. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerging Themes in Epidemiology, 30, 14.
- Gibson SB, Downie JM, Tsetsou S, Feusier JE, Figueroa KP, Bromberg MB, Jorde LB and Pulst SM, 2017. The evolving genetic risk for sporadic ALS. Neurology, 89, 226–233.
- Gómez-Martín A, Hernández AF, Martínez-González LJ, González-Alzaga B, Rodríguez-Barranco M, López-Flores I, Aguilar-Garduno C and Lacasana M, 2015. Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities. Chemosphere, 139, 534–540.
- González-Alzaga B, Hernández AF, Rodríguez-Barranco M, Gómez I, Aguilar-Garduño C, López-Flores I, Parrón T and Lacasaña M, 2015. Pre- and postnatal exposures to pesticides and neurodevelopmental effects in children living in agricultural communities from South-Eastern Spain. Environment International, 85, 229–237.
- Greenland S and Longnecker MP, 1992. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. American Journal of Epidemiology, 135, 1301–1309.
- Greenland S and O'Rourke K, 2008. Meta-analysis. In: Rothman K, Greenland S and Lash T (eds). *Modern Epidemiology. 3*. Lippincott Williams & and Wilkins, Philadelphia. pp. 652–682.
- Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN and Altman DG, 2016. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. European Journal of Epidemiology, 31, 337–350.
- Grimes DA and Schulz KF, 2005. Surrogate end points in clinical research: hazardous to your health. Obstetrics and Gynecology, 105, 1114–1118.
- Gustafson P and McCandless LC, 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. International Journal of Environmental Research and Public Health, 7, 1520–1539.
- Hernández AF, González-Alzaga B, López-Flores I and Lacasaña M, 2016. Systematic reviews on neurodevelopmental and neurodegenerative disorders linked to pesticide exposure: methodological features and impact on risk assessment. Environment International, 92–93, 657–679.
- Higgins JP, 2008. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. International Journal of Epidemiology, 37, 1158–1160.



Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295–300.

Hines CJ, Deddens JA, Coble J, Kamel F and Alavanja MC, 2011. Determinants of captan air and dermal exposures among orchard pesticide applicators in the Agricultural Health Study. Annals of Occupational Hygiene, 55, 620–633.

Hoffmann S, de Vries RBM, Stephens ML, Beck NB, Dirven HAAM, Fowle JR 3rd, Goodman JE, Hartung T, Kimber I, Lalu MM, Thayer K, Whaley P, Wikoff D and Tsaioun K, 2017. A primer on systematic reviews in toxicology. Archives of Toxicology, 91, 2551–2575.

Höfler M, 2005. The Bradford Hill considerations on causality: a counterfactual perspective. Emerging Themes in Epidemiology, 2, 11.

IEA (International Epidemiological Association), 2007. Good Epidemiological Practice (GEP) 2007. Available online: http://ieaweb.org/good-epidemiological-practice-gep/

Imbens G and Rubin D, 2015. *Causal Inference for Statistics, Social, and Biomedical Sciences: An Introduction*. Cambridge University Press, New York, NY.

INSERM, 2013. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.

Ioannidis JP and Trikalinos TA, 2007. An exploratory test for an excess of significant findings. Clinical Trials, 4, 245–253.

Jurek AM, Greenland S, Maldonado G and Church TR, 2005. Proper interpretation of non-differential misclassification effects: expectations vs observations. International Journal of Epidemiology, 34, 680–687.

Kaltenhäuser J, Kneuer C, Marx-Stoelting P, Niemann L, Schubert J, Stein B and Solecki R, 2017. Relevance and reliability of experimental data in human health risk assessment of pesticides. Regulatory Toxicology and Pharmacology, 88, 227–237.

Karabatsos G, Talbott E and Walker SG, 2015. A Bayesian nonparametric meta-analysis model. Research Synthesis Methods, 6, 28–44.

Kavvoura FK, Liberopoulos G and Ioannidis JP, 2007. Selection in reported epidemiological risks: an empirical assessment. PLoS Medicine, 4, e79.

Lachenmeier DW, Kanteres F and Rehm J, 2011. Epidemiology-based risk assessment using the benchmark dose/ margin of exposure approach: the example of ethanol and liver cirrhosis. International Journal of Epidemiology, 40, 210–218.

LaKind JS, Sobus JR, Goodman M, Barr DB, Furst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teequarden J, Tornero-Velez R and Weisel CP, 2014. A proposal for assessing study quality: biomonitoring, environmental epidemiology, and short-lived chemicals (BEES-C) instrument. Environmental International, 73, 195–207.

LaKind JS, Goodman M, Barr DB, Weisel CP and Schoeters G, 2015. Lessons learned from the application of BEES-C: systematic assessment of study quality of epidemiologic research on BPA, neurodevelopment, and respiratory health. Environment International, 80, 41–71.

Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV and Alavanja MC, 2009. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. Blood, 113, 6386–6391.

Larsson MO, Nielsen VS, Brandt CØ, Bjerre N, Laporte F and Cedergreen N, 2017. Quantifying dietary exposure to pesticide residues using spraying journal data. Food and Chemical Toxicology, 105, 407–428.

Lash TL, Fox MP and Fink AK, 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lavelle KS, Robert Schnatter A, Travis KZ, Swaen GM, Pallapies D, Money C, Priem P and Vrijhof H, 2012. Framework for integrating human and animal data in chemical risk assessment. Regulatory Toxicology and Pharmacology, 62, 302–312.

London L, Coggon D, Moretto A, Westerholm P, Wilks MF and Colosio C, 2010. The ethics of human volunteer studies involving experimental exposure to pesticides: unanswered dilemmas. Environmental Health, 18, 50.

Maldonado G and Greenland S, 2002. Estimating causal effects. International Journal of Epidemiology, 31, 422–429.

Marx-Stoelting P, Braeuning A, Buhrke T, Lampen A, Niemann L, Oelgeschlaeger M, Rieke S, Schmidt F, Heise T, Pfeil R and Solecki R, 2015. Application of omics data in regulatory toxicology: report of an international BfR expert workshop. Archives of Toxicology, 89, 2177–2184.

McNamee R, 2003. Confounding and confounders. Occupational and Environmental Medicine, 60, 227–234.

Monson R, 1990. Occupational Epidemiology, 2nd Edition. CRC Press, Boca Ration, FL.

Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E and Vega C, 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology, 39, 158–168.

Nachman KE, Fox MA, Sheehan MC, Burke TA, Rodricks JV and Woodruff TJ, 2011. Leveraging epidemiology to improve risk assessment. Open Epidemiology Journal, 4, 3–29.

Nieuwenhuijsen MJ, 2015. Exposure assessment in environmental epidemiology. In: Vrijheid M (ed.). The Exposome-Concept and Implementation in Birth Cohorts Chapter 14. Oxford University Press.

NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press.

NRC (National Research Council), 2009. *Science and Decisions: Advancing Risk Assessment*. The National Academies Press, Washington, DC.



Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E and Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013:EN-497, 159 pp.

OECD (Organisation for Economic Co-operation and Development), 2013. Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment, No. 184. Paris. Avilable online: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282013%296&doclanguage=en

Orford R, Crabbe H, Hague C, Schaper A and Duarte-Davidson R, 2014. EU alerting and reporting systems for potential chemical public health threats and hazards. Environment International, 72, 15–25.

Orford R, Hague C, Duarte-Davidson R, Settimi L, Davanzo F, Desel H, Pelclova D, Dragelyte G, Mathieu-Nolf M, Jackson G and Adams R, 2015. Detecting, alerting and monitoring emerging chemical health threats: ASHTIII. European Journal of Public Health, 25(supp 3), 218.

Orsini N, Li R, Wolk A, Khudyakov P and Spiegelman D, 2012. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. American Journal of Epidemiology, 175, 66–73.

Oulhote Y and Bouchard MF, 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environmental Health Perspectives, 121, 1378–1384.

Pearce N, 2011. Registration of protocols for observational research is unnecessary and would do more harm than good. Occupational and Environmental Medicine, 68, 86–88.

Pearce N, 2012. Classification of epidemiological study designs. International Journal of Epidemiology, 41, 393–397.

- Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA, Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Hemminki K, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, Lebailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, 't Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Sanjose S, Schernhammer E, Costantini AS, Seixas N, Shy C, Siemiatycki J, 2015. Silverman DT, Simonato L, Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stayner LT, Steenland K, Stenzel M, Stewart BW, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. IARC monographs: 40 years of evaluating carcinogenic hazards to humans. Environmental Health Perspectives, 123, 507–514.
- Raffaele KC, Vulimiri SV and Bateson TF, 2011. Benefits and barriers to using epidemiology data in environmental risk. The Journal of Epidemiology, 4, 99–105.
- Raphael K, 1987. Recall bias: a proposal for assessment and control. International Journal of Epidemiology, 16, 167–170.

Rappaport SM, 2012. Biomarkers intersect with the exposome. Biomarkers, 17, 483–489.

Reich CG, Ryan PB and Schuemie MJ, 2013. Alternative outcome definitions and their effect on the performance of methods for observational outcome studies. Drug Safety, 36(Suppl 1), S181–S193.

Rothman KJ, 2002. Epidemiology – An Introduction. Oxford University Press, Oxford.

Rothman KJ and Greenland S, 1998. Modern Epidemiology. 2. Philadelphia: Lippincott Williams & Wilkins, 27 pp.

Rothman KJ, Greenland S and Lash TL, 2008. *Modern Epidemiology, 3rd Edition*. Lippincott Williams & Wilkins, Philadelphia, PA, USA.

Rushton L, 2011. Should protocols for observational research be registered? Occupational and Environmental Medicine, 68, 84–86.

Salerno J, Knoppers BM, Lee LM, Hlaing WW and Goodman KW, 2017. Ethics, big data and computing in epidemiology and public health. Annals of Epidemiology, 27, 297–301. https://doi.org/10.1016/j.annepidem. 2017.05.002

Santacatterina M and Bottai M, 2015. Inferences and conjectures in clinical trials: a systematic review of generalizability of study findings. Journal of Internal Medicine, 279, 123–126. https://doi.org/10.1111/joim.12389

SCENIHR, 2012. Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty.

Simera I, Moher D, Hoey J, Schulz KF and Altman DG, 2010. A catalogue of reporting guidelines for health research. European Journal of Clinical Investigation, 40, 35–53.

Skelly AC, 2011. Probability, proof, and clinical significance. Evidence-Based Spine-Care Journal, 2, 9–11.

Spiegelman D, 2016. Evaluating Public Health Interventions: 4. the nurses' health study and methods for eliminating bias attributable to measurement error and misclassification. American Journal of Public Health, 106, 1563–1566.

Stang PE, Ryan PB, Dusetzina SB, Hartzema AG, Reich C, Overhage JM and Racoosin JA, 2012. Health outcomes of interest in observational data: issues in identifying definitions in the literature. Health Outcomes Research in Medicine, 3, e37–e44.

Thomas DC, 2009. Statistical Methods in Environmental Epidemiology. Oxford University Press, Oxford, UK.



- Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G, Croghan CW, Jones PA, Knott CE, Lynch CF, Sandler DP, Blair AE and Alavanja MC, 2010. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. Journal of Exposure Science & Environmental Epidemiology, 20, 559–569.
- Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod MR and Ioannidis JP, 2013. Evaluation of excess significance bias in animal studies of neurological diseases. PLoS Biology, 11, e1001609.
- Turner MC, Wigle DT and Krewski D, 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis.
- US EPA (United States Environmental Protection Agency), 2011. Chlorpyrifos: preliminary human health risk assessment for registration review, 30 June 2011, 159 pp.
- US-EPA (U.S. Environmental Protection Agency), 2010a. Framework for incorporating human epidemiologic & incident data in health risk assessment (draft). Office of Pesticide Programs. Washington, DC, 2010.
- US-EPA (U.S. Environmental Protection Agency), 2010b. Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. Arlington, Virginia, USA, April 22, 2010b. Available online: https://archive.epa.gov/scipoly/sap/meetings/web/ pdf/020210minutes.pdf
- US-EPA (U.S. Environmental Protection Agency), 2012. Guidance for considering and using open literature toxicity studies to support human health risk assessment. Office of Pesticide Programs. Washington, DC, 2012. Available online: http://www.epa.gov/pesticides/science/lit-studies.pdf
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides December 28, 2016. Avilable online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf
- Vandenberg LN, Ågerstrand M, Beronius A, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS, Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ and Rudén C, 2016. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environmental Health, 15, 74.
- van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A and Goldbohm A, 2002. The contribution of epidemiology. Food and Chemical Toxicology, 40, 387–424.
- Vinken M, 2013. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology, 312, 158–165.
- Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, Rothman N and Vermeulen R, 2010. Application of OMICS technologies in occupational and environmental health research: current status and projections. Occupational and Environmental Medicine, 67, 136–43.
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. EHC 240: principles and methods for the risk assessment of chemicals in food.
- Wilson SJ and Tanner-Smith EE, 2014. Meta-analysis in prevention science. In: Sloboda Z and Petras H (eds.). Defining prevention science. Advances in Prevention Science (vol. 1): Defining Prevention Science Springer, New York. pp. 431–452.
- Youngstrom E, Kenworthy L, Lipkin PH, Goodman M, Squibb K, Mattison DR, Anthony LG, Makris SL, Bale AS, Raffaele KC and LaKind JS, 2011. A proposal to facilitate weight-of-evidence assessments: harmonization of Neurodevelopmental Environmental Epidemiology Studies (HONEES). Neurotoxicology and Teratology, 33, 354–359.
- Zingone A and Kuehl WM, 2011. Pathogenesis of monoclonal gammopathy of undetermined significance and progression to multiple myeloma. Seminars in Hematology, 48, 4–12.

#### **Glossary and Abbreviations**

ADI	Acceptable daily intake. A measure of the amount of a pesticide in food or
	drinking water that can be ingested (orally) on a daily basis over a lifetime without an appreciable health risk.
ADME	Abbreviation used in pharmacology (and toxicology) for absorption, distribution, metabolism, and excretion of a chemical o pharmaceutical compound and
	describes its disposition within an organism.
AOP	Adverse Outcome Pathway. A structured representation of biological events
	leading to adverse effects relevant to risk assessment.
ARfD	Acute Reference Dose. An estimate of the amount a pesticide in food or drinking water (normally expressed on a body weight basis) that can be ingested in a
	period of 24 hours or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation.
Biomarker	Also known as 'biological marker'. A characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention



BMD	Benchmark Dose. A threshold dose or concentration that produces a predetermined change in response rate of an adverse effect (the benchmark response or BMR) compared to background. The lower 95% confidence limit is calculated (BMDL) to be further used as a point of departure to derive health-based reference values.
НВМ	Human biomonitoring. The measurement of a chemical and/or its metabolites in human biological fluids or tissues. Also referred as to the internal dose of a chemical resulting from integrated exposures from all exposure routes.
Human data	They include observational studies (also called epidemiological studies) where the researcher is observing natural relationships between factors and health outcomes without acting upon study participants. Vigilance data also fall under this concept. In contrast, interventional studies (also called experimental studies or randomised clinical trials), where the researcher intercedes as part of the study design, are outside the scope of this opinion.
IARC	International Agency for Research on Cancer. An agency of the World Health Organization whose role is to conduct and coordinate research into the causes and occurrence of cancer worldwide.
LOAEL	Lowest-observed-adverse-effect level. The lowest concentration or amount of a chemical stressor evaluated in a toxicity test that shows harmful effects (e.g. an adverse alteration of morphology, biochemistry, function, or lifespan of a target organism).
NOAEL	No observed-adverse-effect level. Highest dose at which there was not an observed toxic or adverse effect.
OR	Odds ratio. A measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.
PBTK-TD	Physiologically based toxicokinetic/toxicodynamic modelling is a mathematical modelling approach aimed at integrating <i>a priori</i> knowledge of physiological processes with other known/observed information to mimic the fates and effects of compounds in the bodies of humans, preclinical species and/or other
РРР	organisms. Plant Protection Product. The term 'pesticide' is often used interchangeably with 'plant protection product', however, pesticide is a broader term that also covers non plant/crop uses, for example biocides.
RR	Relative risk. Ratio of the probability of an event (e.g. developing a disease) occurring in an exposed group to the probability of the event occurring in a comparison, non-exposed group.
RMS	Rapporteur member state. The member state of the European Union initially in charge of assessing and evaluating a dossier on a pesticide active substance toxicological assessment.
Sensitivity	The ability of a test to correctly classify an individual as 'diseased'. Probability of being test positive when disease present.
Specificity	The ability of a test to correctly classify an individual as disease-free. Probability of being test negative when disease absent.
Surrogate endpoint	A biomarker intended to substitute for a clinical endpoint
AHS	Agricultural Health Study
ASHTIII	Alerting and Reporting System for Chemical Health Threats, Phase III
BEES-C	Biomonitoring, Environmental Epidemiology, and Short-Lived Chemicals
DAR	draft assessment report
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
EMA	European Medicines Agency
EPA US	Environmental Protection Agency
EQUATOR	Enhancing the QUAlity and Transparency Of health Research
EU-OSHA	European Agency for Safety and Health at Work
EWAS GIS	Exposome-wide association studies Geographical information systems



good laboratory practice
global positioning system
healthy worker effect
Integrated Approach on Testing and Assessment
International Classification of Diseases
International Health Regulations
French National Institute of Health and Medical Research
limit of quantification
monoclonal gammopathy of undetermined significance
molecular initiating event
mode of action
non-Hodgkin's lymphoma
National Institute for Occupational Safety and Health
Newcastle-Ottawa scale
Organisation for Economic Co-operation and Development
Office of Pesticide Programs
Poison Control Centre
personal protective equipment
Renewal Assessment Report
rapid alert system covering food and feed
Research Triangle Institute
structure-activity relationship
STROBE Extension to Genetic Association studies
STrengthening the Reporting of OBservational studies in Epidemiology
Term of Reference
uncertainty factor
World Health Organization
Weight-of-Evidence

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# Annex A – Pesticide epidemiological studies reviewed in the EFSA External Scientific Report and other reviews

The extensive evidence gathered by the EFSA External Scientific Report (Ntzani et al., 2013) highlights that there is a considerable amount of information available on pesticide exposure and health outcomes from epidemiological studies. Nonetheless, the quality of this evidence is usually low and many biases are likely to affect the results to an extent that firm conclusions cannot be made. In particular, exposure epidemiology has long suffered from poor measurement and definition and in particular for pesticides this has always been exceptionally difficult to assess and define.

#### A.1. The EFSA External scientific report

#### A.1.1. Methodological quality assessment

The External Scientific Report consists of a comprehensive systematic review of all the epidemiological studies published between 1 January 2006 and 30 September 2012, investigating the association between pesticide exposure and the occurrence of any human health-related outcomes.

The methodological assessment of eligible studies (to evaluate risk of bias associated with each study) was focused on: study design, study population, level of details in exposure definition and the methods of exposure measurement and the specificity of the measurement. Efforts undertaken to account for confounders through matching or multivariable models, blinded exposure assessment and well-defined and valid outcome assessment were considered.

The elements of the methodological appraisal were considered from the Research Triangle Institute (RTI; Research Triangle Park, NC, USA) item bank, a practical and validated tool for evaluating the risk of bias and precision of observational studies. Those elements are described below (Table A.1).

Table A.1:	Elements from the Research Triangle Institute (RTI; Research Triangle Park, NC, USA)
	item bank for methodological appraisal of epidemiological studies

Question	High risk	Low risk
Study design (prospective, retrospective, mixed, NA)	Retrospective, mixed, NA	Prospective
Inclusion/exclusion criteria clearly stated (yes, partially, no)	No	Yes
Authors mention power calculations (yes, no)		Yes
Level of detail in describing exposure (high, medium, low)	Low	High
Robust measurement of exposure. (biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	No	Yes
Were measures of exposure specific? yes; based on broader, chemically- related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	No	Yes
Attempt to balance the allocation between the groups (e.g., through stratification, matching)	No	Yes
Adjustment performed for potential confounders (yes, some, no)	No	Yes
Assessors blinded to exposure status (for cohort studies)	No	Yes
Outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	No	Yes
Sample size	Low	Тор
Rough quality assessment	>6 answers high risk	>6 asnwers low risk

Quantitative synthesis of the results was attempted when there were 5 or more eligible studies per examined outcome and when there was no substantial heterogeneity among the published evidence. Publication bias was assessed using funnel plots which allowed to visually inspect asymmetry when more than 10 studies were included in the meta-analysis.

Toxicological data was not reviewed or discussed in the External Scientific Report.

#### A.1.2. Inclusion/exclusion criteria

All types of pesticides, including those banned in the EU, were considered to enhance the totality of the epidemiological evidence available at the time of the review.



Exclusion criteria:

- Studies without control populations (case reports, case series) and ecological studies
- Pesticide poisoning or accidental high dose exposure
- Studies with no quantitative information on effect estimates
- Studies with different follow-up periods and examining the same outcome, only the one with the longest follow-up was retained to avoid data duplication.
- Studies referred to the adverse effects of substances used as therapy for various medical conditions (e.g. warfarin-based anticoagulants)
- Studies on solvents and other non-active ingredients (e.g. co-formulants) in pesticides
- Studies examining the association between exposure and biomarkers of exposure were not considered eligible as they do not examine health outcomes
- Studies/analyses investigating exposure to pesticides: arsenic, hexachlorocyclohexane (HCH)  $\alpha$  or  $\beta$ , lead, dioxins and dioxin-like compounds including polychlorinated biphenyls (PCBs) were not considered
- Narrative reviews were excluded but not systematic reviews or meta-analyses.

Publications reporting series of acute poisonings or clinical cases, biomonitoring studies unrelated to health effects, or studies conducted on animals or human cell systems were not included; only epidemiological studies addressing human health effects were selected. Publications that lacked quantitative data for measuring associations were also excluded.

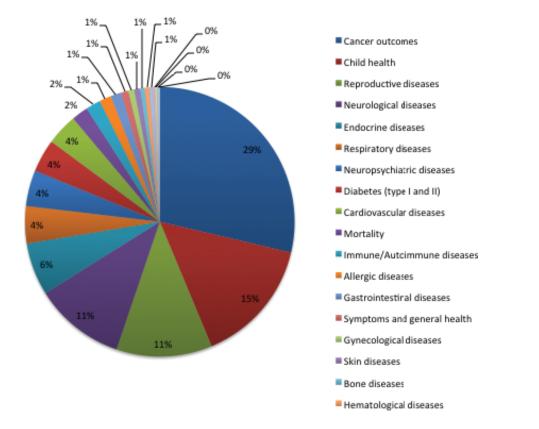
Cohort studies, case–control studies and cross-sectional studies were included. Each study underwent an assessment of its eligibility based on a method including 12 criteria such as study design, precise description of the inclusion/exclusion criteria, level of detail in describing exposure, robustness in the measurement of exposure, adjustment for potential confounding factors, method of assessment of the health outcome, sample size, etc. Among these 12 criteria, three were related to the degree of precision in the description/measurement of exposure, which may explain why a large number of epidemiological studies were not selected.

#### A.1.3. Results

Overall, 602 individual publications were included in the scientific review. These 602 publications corresponded to 6,479 different analyses. The overwhelming majority of evidence comes from retrospective or cross-sectional studies (38% and 32%, respectively) and only 30% of studies had a prospective design. Exposure assessment varied widely between studies and overall 46% measured biomarkers of pesticides exposure and another 46% used questionnaires to estimate exposure to pesticides. Almost half of the studies (49%) were based in America. Most studies examined associations between occupational exposure to pesticides and health effects. The entire spectrum of diseases associated with pesticides has not been studies before. The report examined a wide variety of outcomes (Figure A.1). The largest proportion of studies pertains to cancer outcomes (N = 164) and outcomes related to child health (N = 84).

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**Figure A.1:** Major outcome categories and corresponding percentage of studies examining those outcomes among the publications reviewed by the EFSA external scientific report (Ntzani et al., 2013)

Despite the large volume of available data and the large number (> 6,000) of analyses available, firm conclusions were not made for the majority of the outcomes studied. This was due to several limitations of the data collected as well as to inherent limitations of the review itself. As mentioned above, the review studied the whole range of outcomes examined in relation to pesticides during an approximately 5 years' period. Thus, only recent evidence was reviewed and the results of the meta-analyses performed should be cautiously interpreted as they do not include all the available evidence. It is therefore capable of highlighting outcomes which merit further in-depth analysis in relation to pesticides by looking at the entire literature (beyond 5 years) and by focusing on appraising the credibility of evidence selected. The limitations of the studies itself are in line with other field of environmental epidemiology and focus around the exposure assessment, the study design, the statistical analysis and reporting. In particular:

a) **Exposure assessment**: The assessment of exposure is perhaps the most important methodological limitation of the studies reviewed in the ESR. Studies used different methods for exposure assessment and assignment. Most studies were based on self-reported exposure to pesticides, defined as 'ever versus never' use or as 'regular versus non-regular' use. Such methods suffer from high misclassification rates and do not allow for dose–response analysis. This is especially the case for retrospective studies where misclassification would be differential with higher exposures reported in participants with disease (recall bias) (Raphael, 1987). While questionnaires might be capable of differentiating subjects with very high and very low exposure levels, they are not capable of valid exposure classification across an exposure gradient, thus not allowing the study of dose–response relationships. Also, questionnaire for exposure assessment need to be validated for use in epidemiological studies. Nonetheless, a vast proportion of studies use in house version of non-validated questionnaires which may suffer from content (the questionnaire does not cover all sources of exposure to the hazard of interest) or criterion validity (e.g. through inaccurate recall or misunderstanding of questions) (Coggon, 1995).

Although the range of categories of pesticide studied is wide, studies very often concentrate on a broadly defined pesticide category, so that it is difficult to know what type of pesticide the population is exposed to.



Exposure to pesticides was defined as reported use of pesticides by the study participant or by government registry data. These derive from self-administered questionnaires, interviewer administrated questionnaires, job exposure matrices (JEM), by residential status (proximity to pesticide exposure), by detecting biomarkers associated with pesticide exposure or by other means as defined by each study.

Studies often examine pesticides that have already been banned in western populations and the EU. The use of biomarkers as means of exposure assessment is infrequent, but still available in almost half of the studies.

b) **Study design**: As mentioned above, the majority of evidence comes form case-control studies and cross-sectional studies. Cross-sectional, and in part also case-control studies, cannot fully assess the temporal relationships and thus are less able to provide support regarding the causality of associations.

c) **Outcomes examined**: The definition of clinical outcomes displayed large variability in eligible epidemiological studies, which can further cause the variability in results. Perhaps most important in this setting is the use of a great number of surrogate outcomes examined. Surrogate outcomes are biomarkers or physical measures that are generally accepted as substitutes for, or predictors of, specific clinical outcomes. However, often these surrogate outcomes are not validated and do not meet the strict definitions of surrogate outcomes. Such outcomes can be defined as possible predictors of clinical outcomes but do not fulfil the criteria for a surrogate outcome. It is essential to appraise the evidence around non-validated surrogate outcomes by taking into account the implicit assumptions of these outcomes.

A great variety of assessed outcomes covering a wide range of pathophysiologies was observed. 'Hard' clinical outcomes as well as many surrogate outcomes included in the database reflect the different methodologies endorsed to approach the assessed clinical research questions. The different outcomes were divided into 23 major disease categories, with the largest proportion of studies addressing cancer and child health outcomes.

The adverse health effects assessed included:

- a) major clinical outcomes, such as cancer, respiratory (allergy), reproductive (decreased fertility, birth defects) and neurodegenerative (Parkinson's disease);
- b) clinical surrogate outcomes, e.g. neurodevelopmental impairment (assessed by neurocognitive scales);
- c) laboratory surrogate outcomes (e.g. liver enzyme changes).

For many adverse health effects attributed to pesticide exposure, there exist contradictory or ambiguous studies. Whether this results from lack of consistency or real heterogeneity warrants further clarification.

#### d) Statistical analysis:

Simultaneous exposure to multiple agents (heavy metals, solvents, suspended particulate matter etc.) from different sources is common. It may introduce further bias in the results as all of them may produce adverse health outcomes. Thus, it is essential to account for confounding from exposure to multiple agents in order to delineate true associations but this has not been possible in the overwhelming majority of evidence assessed in the EFSA external scientific report.

In addition, the evidence collected and appraised in the EFSA external scientific report (Ntzani et al., 2013) is likely to suffer from selective reporting and multiple testing. The studies reported a very wide range of analyses; 602 publications resulted in 6,000 analyses. The amount of multiple hypothesis testing is enormous. These analyses need to be adjusted for multiple hypothesis testing else, otherwise the results suffer from high false positive rate. Even when studies present only one analysis, selective reporting is always a possibility as has been shown in other epidemiological fields as well. In addition, when interpreting results one should also take into account that, especially for certain outcomes (e.g. cancers), the majority of evidence comes from single study populations and the Agricultural Health Study in particular.

#### A.1.4. Conclusion of the EFSA External Scientific Report

Regardless of the limitations highlighted above, the External Scientific Report (Ntzani et al., 2013) showed consistent evidence of a link between exposure to pesticides and Parkinson's disease and childhood leukaemia, which was also supported by previous meta-analyses. In addition, an increased risk was also found for diverse health outcomes less well studied to date, such as liver cancer, breast cancer and type II diabetes. Effects on other outcomes, such as endocrine disorders, asthma and allergies, diabetes and obesity showed increased risks and should be explored further.



Childhood leukaemia and Parkinson's disease are the two outcomes for which a meta-analysis after 2006 was found consistently showing an increased risk associated with pesticide exposure. Nonetheless, the exposure needs to be better studied to disentangle the effect of specific pesticide classes or even individual pesticides. Significant summary estimates have also been reported for other outcomes (summarised in Table A.2). However, as they represent studies from 2006 onwards results should be regarded as suggestive of associations only and limitations especially regarding the heterogeneity of exposure should always been taken into consideration. Data synthesis and statistical tools should be applied to these data in relation to specific outcomes, after the update of the results to include publications before 2006, in order to quantify the amount of bias that could exist and isolate outcomes where the association with pesticides is well supported even when estimates of bias are taken into account. Similarly, outcomes where further evidence is needed to draw firm conclusions need to be highlighted.

Health outcome		Meta-analysis results	I <sup>2</sup>
Leukaemia	6	1.26 (0.93; 1.71)	59.4%
Hodgkin lymphoma	7	1.29 (0.81–2.06)	81.6%
Childhood leukaemia (exposure to pesticides during pregnancy)	6	1.67 (1.25–2.23)	81.2%
Childhood leukaemia (exposure to insecticides during pregnancy)	5	1.55 (1.14–2.11)	65%
Childhood leukaemia (exposure to insecticides during pregnancy – update Turner, 2010)	9	1.69 (1.35–2.11)	49.8%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy)	5	2.00 (1.73–2.30)	39.6%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy – update Turner, 2010)	11	1.30 (1.06–1.26)	26.5%
Childhood leukaemia (exposure to pesticides during childhood)	7	1.27 (0.96–1.69)	61.1%
Childhood leukaemia (exposure to insecticides during childhood – update Turner, 2010)	8	1.51 (1.28–1.78)	0%
Childhood leukaemia (exposure to unspecified pesticides during childhood – update Turner, 2010)	11	1.36 (1.19–1.55)	0%
Breast cancer (DDE exposure)	5	1.13 (0.81–1.57)	0%
Breast cancer	11	1.24 (1.08–1.43)	0%
Testicular cancer (DDE exposure)	5	1.40 (0.82–2.39)	59.5%
Stomach cancer	6	1.79 (1.30–2.47)	0%
Liver cancer	5	2.50 (1.57–3.98)	25.4%
Cryptorchidism	8	1.19 (0.96–1.49)	23.9%
Cryptorchidism (DDT exposure)	4	1.47 (0.98–2.20)	51%
Hypospadias (general pesticide exposure)	6	1.01 (0.74–1.39)	71.5%
Hypospadias (exposure to specific pesticides)	9	1.00 (0.84–1.18)	65.9%
Abortion	6	1.52 (1.09–2.13)	63.1%
Parkinson's disease	26	1.49 (1.28–1.73)	54.6%
Parkinson's disease (DDT exposure)	5	1.01 (0.78–1.30)	0%
Parkinson's disease (paraquat exposure)	9	1.32 (1.09–1.60)	34.1%
Amyotrophic lateral sclerosis	6	1.58 (1.31–1.90)	10%
Asthma (DDT exposure)	5	1.29 (1.14–1.45)	0%
Asthma (paraquat exposure)	6	1.40 (0.95–2.06)	53.3%
Asthma (chlorpyrifos exposure)	5	1.03 (0.82–1.28)	0%
Type 1 diabetes (DDE exposure)	8	1.89 (1.25–2.86)	49%
Type 1 diabetes (DDT exposure)	6	1.76 (1.20–2.59)	76.3%
Type 2 diabetes (DDE exposure)	4	1.29 (1.13–1.48)	0%

 Table A.2:
 Summary of meta-analyses performed in the report

N = number of studies considered for the meta-analysis; in the column of meta-analysis results, the numbers represent the statistical estimate for the size of effect (odds ratio (OR), or relative risk (RR)) with the corresponding 95% confidence interval (CI). I<sup>2</sup> represents the percentage of total variation across studies that is due to heterogeneity.



#### A.2. The INSERM report

In September 2013, the French National Institute of Health and Medical Research (INSERM) released a literature review carried out with a group of experts on the human health effects of exposure to pesticides.<sup>22</sup> Epidemiological or experimental data published in the scientific literature up to June 2012 were analysed. The report was accompanied by a summary outlining the literature analysis and highlighting the main findings and policy lines, as well as the recommendations.

The INSERM report is composed of four parts: (1) exposure assessment, with a detailed description of direct and indirect methods to assess exposure in epidemiological studies; (2) epidemiology, with an inventory and analysis of epidemiological studies available in the literature up to 2012, and a scoring system to assess the strength of presumed association; (3) toxicology, with a review of toxicological data (metabolism, mode of action and molecular pathway) of some substances and assessment of biological plausibility; (4) recommendations.

The vast majority of substances identified by the INSERM report as having a presumed moderate or strong association with the occurrence of health effects are chemicals that are now prohibited. This is mainly driven by the fact that the majority of the diseases examined are diseases of the elderly; therefore, the studies performed to date are based on persons who were old at the time of the study and exposed many years ago. By definition, it is not yet possible to investigate the potential long term effects of many of the more recent products.

These substances belong to the group of organochlorine insecticides, such as DDT or toxaphene, or insecticides with cholinesterase-inhibiting properties, such as terbufos or propoxur.

Of the seven approved active substances identified by the INSERM expert appraisal report (the herbicides 2,4-D, MCPA, mecoprop, glyphosate, the insecticide chlorpyrifos, and the foliar fungicides mancozeb and maneb), all had a presumed moderate or weak association with haematopoietic cancers. Two of them (the foliar fungicides mancozeb and maneb) had a presumed weak association with Parkinson's disease and two (chlorpyrifos and glyphosate) had a presumed association with developmental impairment identified as weak or moderate in the expert appraisal.

## A.2.1. Description of methods to assess exposure in epidemiological studies

Different methods (direct and indirect) have been developed to assess exposure, such as biological or environmental monitoring data, ad hoc questionnaires, job- or crop-exposure matrices, analysis of professional calendars, sales data, land use data, etc. According to the authors, these various tools can be combined with each other but, to date none has been validated as a reference method for estimating exposure in the context of occupational pesticide exposure assessment.

#### A.2.2. Epidemiology

The group of experts from INSERM carried out an inventory and analysis of epidemiological studies available in the literature, examining the possible association between pesticide exposure and health outcomes: eight cancer sites (non-Hodgkin lymphoma, leukaemia, lymphoma, multiple myeloma, prostate, testis, brain, melanoma), three neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis), cognitive or depressive disorders, effects on reproductive function (fertility, pregnancy and child development) and childhood cancers. These are health outcomes that have been identified in previous studies as potentially related to pesticide exposure.

Epidemiological studies addressing primarily farmers, pesticide applicators and workers of the pesticide manufacturing industries, as well as the general population when it was relevant, were selected.

The INSERM group of experts established a hierarchy in the relevance of the studies, placing the meta-analysis at the top, then the systematic review, then the cohort study, and finally, the case-control study. Based on this hierarchy, a scoring system was defined to assess the strength of presumption of the association between exposure and the occurrence of health outcomes from the analysis of the study results; for each disease or pathological condition investigated, this score may vary depending on the quality, type and number of available studies, as, for example:

(++): strong presumption: based on the results of a meta-analysis, or several cohort studies or at least one cohort study and two case–control studies, or more than two case–control studies;

<sup>&</sup>lt;sup>22</sup> INSERM. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.



(+): moderate presumption: based on the results of a cohort study or a nested case–control study or two case–control studies;

 $(\pm)$ : weak presumption: based on the results of one case–control study. This synthesis takes the work beyond the status of a simple mapping exercise.

#### A.2.3. Toxicological data

Toxicological data that were considered in the literature review were mainly those regarding metabolism, mode of action and molecular pathways. None of the studies provided as part of the procedures for placing products on the market were considered except if they were published in the open literature.

When substances were clearly identified in the epidemiological studies, a scoring system was defined to assess the biological plausibility from the study results: coherence with pathophysiological data and occurrence of health outcome.

(++): hypothesis supported by 3 mechanisms of toxicity;

(+): hypothesis supported by at least one mechanism of toxicity.

#### A.2.4. Findings

The major results of the INSERM report are summarised in Tables A.3-A.6.

### **Table A.3:** Statistically significant associations between occupational exposure to pesticides and health outcomes in adults (health outcomes that were analysed in the review)

Health outcome	come Type of population with significant risk excess	
NHL	Farmers, operators, manufacturing plant personnel	++
Prostate cancer	Farmers, operators, manufacturing plant personnel	++
Multiple myeloma	Farmers, operators	++
Parkinson's disease	Occupational and non-occupational exposure	++
Leukaemia	Farmers, operators, manufacturing plant personnel	+
Alzheimer's disease	Farmers	+
Cognitive disorders <sup>(b)</sup>	Farmers	+
Fertility and fecundability disorders	Occupational exposure	+
Hodgkin lymphoma	Agricultural workers	±
Testicular cancer	Agricultural workers	±
Brain cancer (glioma, meningioma)	Agricultural workers	±
Melanoma	Agricultural workers	±
Amyotrophic lateral sclerosis	Farmers	±
Anxiety, depression <sup>(b)</sup>	Farmers, farmers with a history of acute poisoning, operators	±

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ).

(b): Almost all pesticides were organophosphates.



**Table A.4:** Associations between occupational or home use exposure to pesticides and cancers or developmental impairment in children (health outcomes that were analysed in the review) (only statistically significant associations are shown)

Health outcome	Health outcome Type of exposure and population with significant risk excess		
Leukaemia	Occupational exposure during pregnancy, prenatal exposure (residential)	++	
Brain cancer	Occupational exposure during pregnancy	++	
Congenital malformation	Occupational exposure during pregnancy; Residential exposure during pregnancy (agricultural area, home use)	++ +	
Fetal death	Occupational exposure during pregnancy	+	
Neurodevelopment	Residential exposure during pregnancy (agricultural area, home use, food) <sup>(b)</sup> ; Occupational exposure during pregnancy	++ ±	

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ). (b): Organophosphates.

### **Table A.5:** Findings related to approved active substances: epidemiological assessment and biological plausibility

Active substance Classification		Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>	
Organophosphates Insecticide				
Chlorpyrifos	Acute Tox cat 3	Leukaemia (+)	Yes (++)	
		Neurodevelopment (+)	Yes (++)	
		NHL ( $\pm$ )	Yes (++)	
Dithiocarbamates <i>Fungicid</i> e				
Mancozeb/Maneb	Repro cat 2	Leukaemia (+)	?	
		Melanoma (+)	?	
		Parkinson's disease	Yes (+)	
		(in combination with paraquat) ( $\pm$ )		
Phenoxy herbicides Herbicide	5		·	
2,4-D	Acute Tox cat 4	NHL (+)	?	
MCPA	Acute Tox cat 4	NHL (±)	?	
Mecoprop	Acute Tox cat 4	$NHL(\pm)$	?	
Aminophosphonate <i>Herbicide</i>	glycine			
Glyphosate		NHL (+)	?	
<i>,</i> ,		Fetal death $(\pm)$	?	

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ).

(b): Scoring system: (++): hypothesis supported by 3 different known mechanisms of toxicity, (+): hypothesis supported by at least one mechanism of toxicity.



Table A.6:	Findings related to non-approved active substances: epidemiological assessment and
	biological plausibility

Active substance	Ban in the EU	IARC classification	Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>
Dieldrin	1978	3 or 2 (US-EPA)	$\operatorname{NHL}^{(c)}(\pm)$ Prostate cancer (±) Parkinson's disease (±)	Yes (+) Yes (+) ?
DDT/DDE	1978	2B	NHL (++) Testicular cancer (+) Child growth (++) Neurodevelopment (±) Impaired sperm parameters (+)	Yes (+) ? ? ?
Chlordane	1978	2B	NHL (±) Leukaemia (+) Prostate cancer (±) Testicular cancer (+)	Yes (+) Yes (+) Yes (+) ?
Lindane (γ-HCH)	2002/2004/2006/2007	2B <sup>(d)</sup>	NHL (++) Leukaemia (+)	Yes (++) Yes (++)
β- <b>ΗCH</b>	2002/2004/2006/2007	2B <sup>(d)</sup>	Prostate cancer ( $\pm$ )	?
Toxaphene	2004	2B	NHL <sup>(c)</sup> (±) Leukaemia (+) Melanoma (+)	Yes (++) Yes (++) Yes (+)
Chlordecone	2004	2B	Cancer prostate (++) Impaired sperm parameters (+) Neurodevelopment (+)	Yes (+) ? ?
Heptachlor	1978	2B	Leukaemia (+)	Yes (+)
Endosulfan	2005	Not classified	?	Yes (+)
Hexachlorobenzene (HCB)	1978	2B	Child growth (+)	?
Terbufos	2003/2007		NHL (+) Leukaemia (+)	? ?
Diazinon	2008		NHL (+) Leukaemia (+)	? ?
Malathion	2008	3	NHL (++) Leukaemia (+) Neurodevelopment (+) Impaired sperm parameters (+)	Yes (+) Yes (+) ? ?
Fonofos	2003		NHL (±) Leukaemia (+) Prostate cancer (+)	? ? ?
Parathion	2002	3	Melanoma (+)	?
Coumaphos	Never notified and authorised in the EU		Prostate cancer (+)	?
Carbaryl	2008	3	NHL (±) Melanoma (+) Impaired sperm parameters (+)	? ? ?
Propoxur	2002		Neurodevelopment (+) Fetal growth (+)	? ?
Carbofuran	2008		NHL (±) Prostate cancer (+)	? ?
Butylate	2003		NHL (+) Prostate cancer (+)	? ?
EPTC	2003		Leukaemia (+)	?



Active substance	Ban in the EU	IARC classification	Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>
Atrazine	2005	3	NHL (±) Fetal growth (+)	Yes (+) ?
Cyanizine	2002/2007		NHL <sup>(c)</sup> (±)	?
Permethrin	2002	3	Prostate cancer (+)	Yes (+)
Fenvalerate	1998	Not classified	Impaired sperm parameters (+)	?
Methyl bromide	2010	3	Testicular cancer (+)	?
Dibromoethane	Banned	2A	Impaired sperm parameters (+)	?
Dibromochloropropane (DBCP)	Banned	2B	Impaired sperm parameters/impaired fertility (+++) (causal association)	Yes (+++) (mode of action elucidated)
Paraquat	2007		Parkinson's disease (+)	Yes (++)
Rotenone	2011		Parkinson's disease (+)	Yes (++)
Alachlor	2008		Leukaemia (+)	Yes (++)

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ).

(b): Scoring system: (++): hypothesis supported by 3 mechanisms of toxicity, (+): hypothesis supported by at least one mechanism of toxicity.

(c): Population with t(14,18) translocation, only.

(d): Technical mixture ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH).

#### A.2.5. Recommendations

The analysis of the available epidemiological and mechanistic data on some active substances suggests several recommendations for developing further research:

a) Knowledge on population exposure to pesticides should be improved

- 1) Collect information about use of active substances by farmers
- 2) Conduct field studies to measure actual levels of exposure
- 3) Monitor exposure during the full occupational life span
- 4) Measure exposure levels in air (outdoor and indoor), water, food, soil
- 5) Collect information on acute poisonings
- 6) Improve analytical methods for biomonitoring and external measurements
- 7) Allow researchers to have access to extensive formulation data (solvents, co-formulants, etc.).
- b) Research potential links between exposure and health outcomes
  - 1) Characterise substances or groups of substances causing health outcomes
  - 2) Focus on susceptible individuals or groups of individuals (gene polymorphism of enzymes, etc.)
  - 3) Focus on exposure windows and susceptibility (pregnancy, development)
  - 4) Bridge the gap between epidemiology and toxicology (mode of action)
  - 5) Improve knowledge on mixture toxicity
  - 6) Foster new approaches of research (in vitro and in silico models, omics, etc.).

#### A.3. Similarities and differences between the EFSA External Scientific Report and the INSERM report

The two reports discussed herein have used different methodologies. Yet, their results and conclusions in many cases agree. The INSERM report is limited to predefined outcomes and it attempted to investigate the biological plausibility of epidemiological studies by reviewing toxicological data as well, meanwhile the EFSA report is a comprehensive systematic review of all available epidemiological studies that were published during an approximately 5 year window.

The differences between the reports are shown in Table A.7 and are related to the time period of search (i.e. both reports did not assess the same body of published data), different criteria for eligibility of studies and different approaches to summarising the evidence across and within outcomes.



Overall, the INSERM report identified a greater number of associations with adverse health effects than the EFSA report. However, a well-documented association with pesticide exposure was claimed by both reports for the same health outcomes (childhood leukaemia, Parkinson's disease).

Table A.7:	Comparison	between	methods	used	in	the	EFSA	External	Scientific	Report	and	the
	INSERM Rep	ort										

	EFSA External report	INSERM report	
Articles reviewed	602/43,000	NR	
Language	Yes	NR	
Search strategy (key words, MeSH)	Yes	NR	
Search database	Yes (4)	NR	
Years of publication	2006–2012 (Sep)	? to 2012 (Jun)	
Type of epi studies assessed	Cross-sectional	Cross-sectional	
	Case-control	Case-control	
	Cohort	Cohort	
Inclusion criteria	Yes	NR	
Exclusion criteria	Yes	NR	
Methodological quality assessment	Yes (12 criteria)	NR	
Exposure groups <sup>(a)</sup>	Yes	Yes	
Exposure assessment	Yes	Yes	
Quantitative synthesis (meta-analysis)	Yes	No	
Qualitative synthesis <sup>(c)</sup>	Yes	Yes	
Supporting Toxicological data	NI	Yes	
Associations with individual pesticides	Yes	Yes	
Health outcomes studied			
Haematological cancer	Yes	Yes	
Solid tumours	Yes	Yes	
Childhood cancer	Yes	Yes	
Neurodegenerative disorders	Yes	Yes	
Neurodevelopmental outcomes	Yes	Yes	
Neuropsychiatric disturbances <sup>(b)</sup>	No	Yes	
Reproductive and developmental	Yes	Yes	
Endocrine	Yes	NI	
Metabolism	Yes	Yes	
Immunological	Yes	NI	
Respiratory	Yes	NI	

NR: not reported; NI: not investigated.

(a): Exposure type (environmental, occupational, etc.) and period (general population, children, etc.).

(b): E.g. depressive disorders.

(c): Add explanation.

# A.4. The Ontario College of Family Physicians Literature review (OCFPLR)

In 2004, the Ontario College of Family Physicians (Ontario, Canada) reviewed the literature published between 1992 and 2003 on major health effects associated with pesticide exposure. The authors concluded that positive associations exist between solid tumours and pesticide exposures as shown in Table A.8. They noted that in large well-designed cohort studies these associations were consistently statistically significant, and the relationships were most consistent for high exposure levels. They also noted that dose–response relationships were often observed, and they considered the quality of studies to be generally good.



Endpoint	Associations identified by the Ontario College, pesticide (if differentiated), study type, (no. of studies/total no. of studies)				
A) Cancer					
1. Lung	-ve cohort (1/1) +ve case-control (1/1) +ve carbamate, phenoxy acid, case-control (1/1)				
2. Breast	+ve case-control (2/4) +ve ecological (1/1) +ve triazine, ecological (1/1) -ve atrazine, ecological (1/1)				
3. Colorectal					
4. Pancreas	+ve cohort (1/1) +ve case_control (2/2)				
5. Non-Hodgkin's lymphoma	+ve cohort (9/11) +ve case_control (12/14) +ve ecological (2/2)				
6. Leukaemia	+ve cohort (5/6) +ve case_control (8/8) -ve ecological (1/1) +ve lab study (1/1)				
7. Brain	+ve cohort (5), similar case-control (5)				
8. Prostate	+ve cohort (5/5) case-control (2/2) ecological (1/1)				
9. Stomach					
10. Ovary					
11. Kidney	+ve pentachlorophenol cohort (1/1) +ve cohort (1/1) +ve case-control (4/4)				
12. Testicular					
B) Non-Cancer					
1) Reproductive effects	+ve glyphosate				
Congenital malformations	+ve pyridyl derivatives				
Fecundity/time to pregnancy	Suggest impaired				
Fertility					
Altered growth	Possible +ve association, but further study required				
Fetal death	Suggested association				
Mixed outcomes					
2) Genotoxic/immunotoxic Chromosome aberrations	+ve Synthetic pyrethroids (1) +ve organophosphates (1) +ve fumigant and insecticide applicators				
NHL rearrangements	+ve fumigant and herbicide applicators				
3) Dermatologic					
4) Neurotoxic Mental & emotional impact	+ve				
Functional nervous system impact	+ ve organophosphate/carbamate poisoning				
Neurodegenerative impacts (PD)	+ve cohort (4/4) +ve case_control (2/2) +ve ecological (1/1)				

+ve: positive; -ve: negative.

The report concluded that there was compelling evidence of a link between pesticide exposure and the development of non-Hodgkin's lymphoma (NHL), and also clear evidence of a positive association between pesticide exposure and leukaemia. The authors also claimed to have found consistent findings of a number of nervous system effects, arising from a range of exposure time courses.



Such strong conclusions found favour with Non-Governmental organisations (NGOs) and raised questions among some Regulatory Authorities. The Advisory Committee on Pesticides (ACP), at that time an UK government independent advisory committee, was asked to provide an evaluation of the outcome of the Ontario College review. The committee membership included one epidemiologist and the committee consulted five other epidemiologists involved in providing independent advice to other government committees. They all agreed that the review had major shortcomings (e.g. exact search strategy and selection criteria not specified, selective reporting of results, inadequate understanding and consideration of relevant toxicology, insufficient attention to routes and levels of exposure, not justified conclusions, etc.). Overall, the conclusions of the Ontario College review were considered not to be supported by the analysis presented. In 2012, the Ontario review authors published an update of their evaluation; in their second report they used a very similar approach but offered more detail concerning the inclusion criteria used. This example is a reminder of the risk of over interpretation of epidemiological studies. In particular, a causal inference between exposure and the occurrence of adverse health effects is often made, but this represents an association that should be further assessed.

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#### Annex B – Human biomonitoring project outsourced by EFSA<sup>23</sup>

In 2015, EFSA outsourced a project to further investigate the role of HBM in occupational health and safety strategies as a tool for refined exposure assessment in epidemiological studies and to contribute to the evaluation of potential health risks from occupational exposure to pesticides. It was in fact recognised that exposure assessment is a key part of all epidemiological studies and misclassification of exposure and use of simple categorical methods are known to weaken the ability of a study to determine whether an association between contact and ill-health outcome exists; at present, this limits integration of epidemiological findings into regulatory risk assessment.

The consortium formed by Risk & Policy Analysts Limited (RPA), IEH Consulting Limited (IEH) and the Health&Safety Laboratory (HSL) carried out a systematic literature review for the period 1990–2015 with the aim to provide an overview on the use of HBM as a tool for occupational exposure assessment refinement, identifying advantages, disadvantages and needs for further development (first objective). The search identified 2096 publications relating to the use of HBM to assess occupational exposure to pesticides (or metabolites). The outcome of the search (Bevan et al., 2017) indicated that over the past 10–20 years there has been an expansion in the use of HBM, especially into the field of environmental and consumer exposure analysis. However, further improvement of the use of HBM for pesticide exposure assessment is needed, in particular with regards to: development of strategies to improve or standardise analytical quality, improvement of the availability of reference material for metabolites, integration of HBM data into mathematical modelling, exposure reconstruction, improvements in analytical instrumentation and increased availability of human toxicology data.

The contractors performed a review of available HBM studies/surveillance programmes conducted in EU/US occupational settings to identify pesticides (or metabolites) both persistent and not persistent, for which biomarkers of exposure (and possibly effect) were available and validated (second objective). A two-tiered screening process that included quality scoring for HBM, epidemiological and toxicological aspects, was utilised to identify the most relevant studies, resulting in 178 studies for critical review. In parallel with the screening of identified studies, a Master Spreadsheet was designed to collate data from these papers, which contained information relating to: study type; study participants; chemicals under investigation; biomarker quality check; analytical methodology; exposure assessment; health outcome/toxicological endpoint; period of follow-up; narrative of results; risk of bias and other comments.

HBM has been extensively used for monitoring worker exposure to a variety of pesticides. Epidemiological studies of occupational pesticide use were seen to be limited by inadequate or retrospective exposure information, typically obtained through self-reported questionnaires, which can potentially lead to exposure misclassification. Some examples of the use of job exposure or crop exposure matrices were reported. However, little validation of these matrix studies against actual exposure data had been carried out. Very limited data was identified that examined seasonal exposures and the impact of PPE, and many of the studies used HBM to only assess one or two specific compounds. A wide variety of exposure models are currently employed for health risk assessments and biomarkers have also often been used to evaluate exposure estimates predicted by a model.

From the 178 publications identified to be of relevance, 41 individual studies included herbicides, and of these, 34 separate herbicides were identified, 15 of which currently have approved for use in the EU. Similarly, of the 90 individual studies that included insecticides, 79 separate insecticides were identified, of which 18 currently have approved for use in the EU. Twenty individual studies included fungicides, with 34 separate fungicides being identified and of these 22 currently have approved for use in the EU. The most studied herbicides (in order) were shown to be: 2,4-D > atrazine > metolachlor = MCPA > alachlor = glyphosate. Similarly, the most studied insecticides (in order) were: chlorpyrifos > permethrin > cypermethrin = deltamethrin > malathion, and the most studied fungicides were: captan > mancozeb > folpet.

Current limitations comprised the limited number of kinetic data from humans, particularly with respect to the ADME of individual pesticides in human subjects, which would allow more accurate HBM sampling for all routes of exposure. A wider impact of this is on the development of PBPK models for the risk assessment of pesticides, which rely on toxicokinetic data, and on validation of currently used exposure assessment models. Further limitations currently impacting on the use of HBM in this field are a lack of large prospective cohort studies to assess long term exposure to currently used pesticides.

<sup>&</sup>lt;sup>23</sup> Bevan et al. (2017).



The evidence identified has been used to help formulate recommendations on the implementation of HBM as part of the occupational health surveillance for pesticides in Europe. Some key issues were considered that would need to be overcome to enable implementation. These included the setting of priorities for the development of new specific and sensitive biomarkers, the derivation and adoption of health-based guidance values, development of QA schemes to validate inter-laboratory measurements, good practice in field work and questionnaire design, extension of the use of biobanking and the use of HBM for post-approval monitoring of pesticide safety.

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# Annex C – Experience of international regulatory agencies in regards to the integration of epidemiological studies for hazard identification

#### C.1. WHO-International Agency for Research on Cancer (IARC)

The IARC Monographs on the Evaluation of Carcinogenic Risks to Humans of the International Agency for Research on Cancer (IARC) is a programme established four decades ago to assess environmental exposures that can increase the risk of human cancer. These include individual chemicals and chemical mixtures, occupational exposures, physical agents, biological agents and lifestyle factors.

IARC assembles international interdisciplinary Working Groups of scientists to review and assess the quality and strength of evidence from scientific publications and perform a hazard evaluation to assess the likelihood that the agents of concern pose a cancer risk to humans. In particular, the tasks of IARC Working Group Members include the evaluation of the results of epidemiological and other experimental studies on cancer, to evaluate data on the mechanisms of carcinogenesis and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The Monographs are widely used and referenced by governments, organisations, and the public around the world to set preventive and control public health measures.

The Preamble<sup>24</sup> to the IARC Monographs explains the scope of the programme, the scientific principles and procedures used in developing a Monograph, the types of evidence considered and the scientific criteria that guide the evaluations. The scope of the monographs broadened to include not only single chemicals but also groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. Thus, the title of the monographs reads 'Evaluation of carcinogenic risks to humans'.

Relevant epidemiological studies, cancer bioassays in experimental animals, mechanistic data, as well as exposure data are critically reviewed. Only reports that have been published or accepted for publication in the openly available scientific literature are included. However, the inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results. Qualitative aspects of the available studies are carefully scrutinised.

Although the Monographs have emphasised hazard identification, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–response relationship. A Monograph may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies.

The structure of a Monograph includes the following sections:

- 1) Exposure data
- 2) Studies of cancer in humans
- 3) Studies of cancer in experimental animals
- 4) Mechanistic and other relevant data
- 5) Summary
- 6) Evaluation and rationale.

Human epidemiological data are addressed in point 2, where all pertinent epidemiological studies are assessed. Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

The IARC evaluation of epidemiological studies includes an assessment of the following criteria: types of studies considered (e.g. cohort studies, case–control studies, correlation (or ecological) studies and intervention studies, case reports), quality of the study (e.g. bias, confounding, biological variability and the influence of sample size on the precision of estimates of effect), meta analysis and pooled analyses, temporal effects (e.g. temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure), use of biomarkers in epidemiological studies (e.g. evidence of exposure, of early effects, of cellular, tissue or organism responses), and criteria for causality.

With specific reference to causality, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group

<sup>&</sup>lt;sup>24</sup> http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf



considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality. However, it is recognised that weak associations may be important when the disease or exposure is common. Associations that are replicated in several studies of different design under different exposure conditions are more likely to represent a causal relationship than isolated observations from single studies. In case of inconsistent results among different investigations, possible reasons (e.g. differences in exposure) are sought, and high quality studies are given more weight compared to less methodologically sound ones. Risk increasing with the exposure is considered to be a strong indication of causality, although the absence of a clear dose–response effect is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure also supports a causal interpretation of the findings. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall data are considered. Biomarkers information may be used in an assessment of the biological plausibility of epidemiological observations. Randomised trials showing different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement of lack of carcinogenicity can be made. In those cases, studies are scrutinised to assess the standards of design and analysis described above, including the possibility of bias, confounding or misclassification of exposure. In addition, methodologically sound studies should be consistent with an estimate of effect of unity for any observed level of exposure, provide a pooled estimate of relative risk near to unity, and have a narrow confidence interval. Moreover, no individual study nor the pooled results of all the studies should show any increasing risk with increasing level of exposure. Evidence of lack of carcinogenicity can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years, and latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation which summarises the results of epidemiological studies, the target organs or tissues, dose–response associations, evaluations of the strength of the evidence for human and animal data, and the strength of the mechanistic evidence.

At the end of the overall evaluation, the agent is assigned to one of the following groups: Group 1, the agent is carcinogenic to humans; Group 2A, the agent is probably carcinogenic to humans; Group 2B, the agent is possibly carcinogenic to humans; Group 3, the agent is not classifiable as to its carcinogenicity to humans; Group 4, the agent is probably not carcinogenic to humans.

The categorisation of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data. These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency).

For example, Group 1: The agent is carcinogenic to humans. This category is used when there is sufficient evidence of carcinogenicity in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Although widely accepted internationally, there have been criticisms of the classification of particular agents in the past, and more recent criticisms have been directed at the general approach adopted by IARC for such evaluations possibly motivating publication of a rebuttal (Pearce et al., 2015).

## C.2. The experience of US-EPA in regards to the integration of epidemiological studies in risk assessment

The US Environmental Protection Agency's Office of Pesticide Programs (OPP) is the governmental organisation in the US responsible for registering and regulating pesticide products.<sup>25</sup> As part of this activity and prior to any permitted use of a pesticide, OPP evaluates the effects of pesticides on human health and the environment. EPA receives extensive hazard and exposure information to characterise

<sup>&</sup>lt;sup>25</sup> See https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks for general information on pesticide science and assessing pesticide risks.



the risks of pesticide products through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Information on the toxic effects of pesticides is generally derived from studies with laboratory animals conducted by pesticide registrants and submitted to EPA.

In the past, information from well-designed epidemiology studies on pesticides has not been typically available to inform EPA's evaluations of potential risks that might be associated with exposure to pesticides. With an increasing number of epidemiology studies entering the literature which explore the putative associations between pesticides exposure and health outcomes, EPA is putting additional emphases on this source of information. This is especially true for the wealth of studies deriving from the Agricultural Health Study<sup>26</sup> (AHS), a large, well-conducted prospective cohort study following close to 90,000 individuals over more than 20 years and from the Children's Environmental Health and Disease Prevention Research Centers.<sup>27</sup> EPA intends to make increasing use of these epidemiology studies in its human health risk assessment with the goal of using such epidemiological information in the most scientifically robust and transparent way.

#### C.2.1. OPP Epidemiological Framework Document

As an early first step in this process, EPA-OPP developed a proposed epidemiological framework document released as a draft in 2010, 'Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment' (US-EPA, 2010a). The 2010 draft framework was reviewed favourably by the FIFRA Scientific Advisory Panel (SAP) in February, 2010 (US-EPA, 2010b). This document was recently updated in 2016 to the 'Office of Pesticide Programs' Framework Document for Incorporating Human Epidemiology and Incident Data in Risk Assessments for Pesticides' (US-EPA, 2016). The revised and updated 2016 Framework document proposes that human information like that found in epidemiology studies (in addition to human incident databases, and biomonitoring studies) along with experimental toxicological information play a significant role in this new approach by providing insight into the effects caused by actual chemical exposures. In addition, epidemiological/ molecular epidemiological data can guide additional analyses, identify potentially susceptible populations and new health effects and potentially confirming existing toxicological observations. The concepts in the 2016 Framework are based on peer-reviewed robust principles and tools and rely on many existing quidance documents and frameworks (Table C.1) for reviewing and evaluating epidemiology data. It is also consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action (MoA)/human relevance framework which highlight the importance of problem formulation and the need to integrate information at different levels of biological organisation (Meek et al., 2014). Furthermore, it is consistent with recommendations by the National Academy of Sciences' National Research Council (NAS/NRC) in its 2009 report Science and Decisions (NRC, 2009) in that the framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage is envisioned as starting with a planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern, exposure pathways, and duration along with key gaps in data or scientific information.

<sup>&</sup>lt;sup>26</sup> See https://aghealth.nih.gov/

<sup>&</sup>lt;sup>27</sup> See https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers

	1983	Risk Assessment in the Federal Government. Managing the Process	
NAS	1994		
NAS		Science and Judgement	
	2007	Toxicity testing in the 21st Century	
	2009	Science and Decisions: Advancing Risk Assessment	
WHO/ IPCS	2001–2007	Mode of Action/Human Relevance Framework	
	2005	Chemical Specific Adjustment Factors (CSAF)	
	2014	New Development in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis	
ΕΡΑ	1991–2005	Risk Assessment Forum Guidance for Risk Assessment (e.g. guidelines for carcinogen, reproductive, developmental, neurotoxicity, ecological, and expose assessment, guidance for benchmark dose modelling, review of reference dos and reference concentration processes) http://www.epa.gov/risk_assessment/guidance.htm	
	2000	Science Policy Handbook on Risk Characterisation http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=40000006.txt	
	2006	Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data for Risk Assessment	
	2014	Framework for Human Health Risk Assessment to Inform Decision-making	
	2014	Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Inter-species and Intra-species Extrapolation	
	2001	Aggregate Risk Assessment https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf	
OPP	2001 and 2002	Cumulative Risk Assessment http://www.epa.gov/ncer/cra/	
OECD	2013	Organisation for Economic Co-operation and Development Guidance Document on Developing and Assessing Adverse Outcome Pathways	

 Table C.1:
 Key guidance documents and frameworks used by OPP (from US-EPA, 2016)

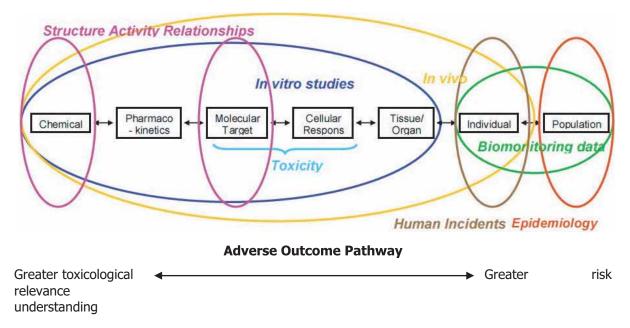
Briefly, this EPA Framework document describes the scientific considerations that the Agency will weigh in evaluating how such epidemiological studies and scientific information can be integrated into risk assessments of pesticide chemicals and also in providing the foundation for evaluating multiple lines of scientific evidence in the context of the understanding of the adverse outcome pathway (or MoA). The framework relies on and espouses standard practices in epidemiology, toxicology and risk assessment, but allows for the flexibility to incorporate information from new or additional sources. One of the key components of the Agency's framework is the use the MoA framework/adverse outcome pathway concept as a tool for organising and integrating information from different sources to inform the causal nature of links observed in both experimental and observational studies. MoA (Boobis et al., 2008; Simon et al., 2014; Meek et al., 2014) and adverse outcome pathway (Ankley et al., 2010) provide important concepts in the integrative analysis discussed in the Framework document. Both a MoA and an adverse outcome pathway are based on the premise that an adverse effect caused by exposure to a compound can be described by a series of causally linked biological key events that result in an adverse human health outcome, and have as their goal a determination of how exposure to environmental agents can perturb these pathways, thereby causing a cascade of subsequent key events leading to adverse health effects.

A number of concepts in the Framework are taken from two reports from the National Academies, *Science and Decisions: Advancing Risk Assessment* (NAS 2009) and *Toxicity Testing on the 21st Century* (NAS 2007). These two NRC reports advocate substantial changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made. In particular, the 2007 report on 21st century toxicity testing advocates a decided shift away from the current focus of using apical toxicity endpoints to using toxicity pathways to better inform toxicity testing, risk assessment, and decision-making.

The MoA framework begins with the identification of the series of key events that are along the causal path and established on weight of evidence using criteria based on those described by Bradford Hill taking into account factors such as dose–response, temporal concordance, biological plausibility, coherence and consistency. Specifically, the modified Bradford Hill Criteria (Hill, 1965) are used to evaluate the experimental support that establishes key events within a MoA or an adverse outcome pathway, and explicitly considers such concepts as strength, consistency, dose response, temporal



concordance, and biological plausibility in a weight of evidence analysis. Using this analytic approach, epidemiological findings can be evaluated in the context of other human information and experimental studies to evaluate consistency, reproducibility, and biological plausibility of reported outcomes and to identify areas of uncertainty and future research. Figure C.1 below (adapted from NRC, 2007) suggests how different types of information relate to each other across multiple levels of biological organisation (ranging from the molecular level up to population-based surveillance) and is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function in humans.



### Figure C.1: Source to Outcome Pathway: Chemical effects across levels of biological organisation (adapted from NRC, 2007)

#### C.2.2. Systematic reviews: Fit for purpose

The National Academies' National Research Council (NRC) in its review of EPA's IRIS program defines systematic review as 'a scientific investigation that focuses on a specific question and uses explicit, prespecified scientific methods to identify, select, assess, and summarise the findings of similar but separate studies'.<sup>28</sup> In recent years, the NRC has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision-making.<sup>29</sup>

Consistent with NRC's recommendations, EPA-OPP employs fit-for-purpose systematic reviews that rely on transparent methods for collecting, evaluating and integrating the scientific data supporting its decisions. As such, the complexity and scope of each systematic review will vary among risk assessments. EPA-OPP starts with scoping/problem formulation followed by data collection, data evaluation, data integration and summary findings with critical data gaps identified.

Systematic reviews often use statistical (e.g. meta-analysis) and other quantitative techniques to combine results of the eligible studies, and can use a semi-quantitative scoring system to evaluate the levels of evidence available or the degree of bias that might be present. For EPA's Office of Pesticide Programs, such a Tier III (systematic review) assessment conducted as part of its regulatory review process would involve review of the pesticide chemical undergoing review and a specific associated suspected health outcome (as suggested by the initial Tier II assessment).

A number of federal and other organisations in the US are evaluating or have issued guidance documents for methods to conduct such systematic reviews and a number of frameworks have been

<sup>&</sup>lt;sup>28</sup> http://dels.nas.edu/Report/Review-Integrated-Risk/18764

<sup>&</sup>lt;sup>29</sup> NRC, 2011. 'Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde' available for download at https://www.nap.edu/catalog/13142/review-of-the-environmental-protection-agencys-draft-iris-assessment-of-formaldehyde; See also NRC, 2014. 'Review of EPA's Integrated Risk Information System (IRIS) Process' available for download at https:// www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process



developed. These include the EPA IRIS programs' approach,<sup>30</sup> the National Toxicology Programs' Office of Health Assessment and Translation (NTP/OHAT) approach<sup>31</sup> the Cochran Collaboration's approach,<sup>32</sup> the Campbell Collaboration and the Navigation Guide,<sup>33</sup> with this latter described in a series of articles in the journal *Environmental Health Perspectives*. Each broadly shares four defined steps: data collection, data evaluation, data integration, and summary/update. For example, The Cochrane Collaboration in its Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine lists a number of the important key characteristics of a systematic review to be (from US-EPA, 2016):

- a clearly stated set of objectives with predefined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings from the identified studies;
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

As described and elaborated in the following sections of this Annex, OPP's approach to review and integration of epidemiological data into pesticide risk assessments takes a tiered approach which each tier appropriately fit-for-purpose in the sense that is considers 'the usefulness of the assessment for its intended purpose, to ensure that the assessment produced is suitable and useful for informing the needed decisions (US-EPA, 2012) and that required resources are matched or balanced against any projected or anticipated information gain from further more in-depth research. A Tier 1 assessment is either a scoping exercise or an update to a scoping exercise in which a research and evaluation is limited to studies derived from the AHS. A Tier II assessment involves a broader search of the epidemiological literature, comprehensive data collection, and a deeper, more involved data evaluation and is more extensive but is generally limited in scope to epidemiology and stops short of multidisciplinary integration across epidemiology, human poisoning events, animal toxicology and adverse outcome pathways. A Tier III assessment is a complete systematic review with data integration and more extensive data evaluation and extraction and may involve more sophisticated epidemiological methods such as meta-analysis and meta-regression, causal inference/causal diagrams, and quantitative bias and sensitivity analyses, among others.

#### C.2.3. Current and Anticipated Future EPA Epidemiology Review Practices

### C.2.3.1. Tier I (Scoping & Problem Formulation) and Tier II (more extensive literature search)

Currently at EPA, epidemiology review of pesticides is conducted in a tiered process as the risk assessment develops, as briefly described above. The purpose of this early Tier I/scoping epidemiology report is to ensure that highly relevant epidemiology studies are considered in the problem formulation/scoping phase of the process and, if appropriate, fully reviewed in the (later) risk assessment phase of the process. In Tier I, EPA-OPP focuses on well-known high quality cohort studies which focus on pesticide issues, particularly the Agricultural Health Study (AHS). The AHS is a federally funded study that evaluates associations between pesticide exposures and cancer and other health outcomes and represents a collaborative effort between the US National Cancer Institute (NCI), the National Institute of Environmental Health Sciences (NIEHS), CDC's National Institute of Occupational Safety and Health (NIOSH) and the US EPA. The AHS participant cohort includes more than 89,000 licensed commercial and private pesticide applicators and their spouses from Iowa and North Carolina. Enrolment occurred from 1993 to 1997, and data collection is ongoing. The AHS maintains on its website a list of publications associated with and using the AHS cohort (see https://aghealth.nih.gov/ news/publications.html).

If the pesticide of interest has been investigated as part of the AHS (www.aghealth.org), a preliminary (Tier I/scoping) review of these studies is performed early on in the evaluation as the

<sup>&</sup>lt;sup>30</sup> See https://www.epa.gov/iris/advancing-systematic-review-workshop-December-2015

<sup>&</sup>lt;sup>31</sup> See http://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html and NTP's 'Handbook for Conducting a Literature-based Assessment Using OHAT Approach for Systematic Review and Evidence Integration' at https://ntp.niehs.nih.gov/ntp/ohat/pub s/handbookjan2015\_508.pdf

<sup>&</sup>lt;sup>32</sup> See http://handbook.cochrane.org/

<sup>&</sup>lt;sup>33</sup> See http://ehp.niehs.nih.gov/1307175/



docket (or 'dossier') is opened as part of EPA's 'Scoping' analysis. In this early Tier I/scoping phase, basic epidemiological findings and conclusions from the Agricultural Health Study are described in a Tier I/scoping document which is designed to simply summarise in brief form the pertinent conclusions of various AHS study authors if there are AHS findings relevant to a the pesticide undergoing review; this Tier I scoping review is not designed to offer detailed content, critical evaluation, or evidence synthesis, and may only touch on summarised highlights of the relevant AHS -related journal articles. If other high-quality non-AHS studies are available like those from the Children's Environmental Health and Disease Prevention Research Centres, these may be similarly summarised in this Tier I/scoping epidemiological review as well. Again, no critique or synthesis of the literature is offered. In some cases, the Tier I/scoping review may conclude that no additional epidemiological review of available evidence is further required. Alternatively, it may recommend that further review is necessary as part of a more involved Tier I/update or Tier II assessment.

A <u>Tier I/update assessment</u> is generally completed 1" to 3 years following the completion of the Tier I/scoping assessment and is issued, like the Tier II discussed below, along with and as part of the Draft Human Health Risk Assessment. Tier I/update assessments perform a thorough review of the available literature in the AHS. A Tier I/update assessment reviews, summarises and evaluates in a qualitative, narrative summary (including reported measures of association), the applicable studies that are listed on the AHS website.<sup>34</sup> Reviews are generally in the form of a narrative, focusing on the key aspects of studies and their conclusions and include EPA OPP commentary along with summary EPA OPP conclusions and recommendations for further study, if necessary.

#### C.2.3.2. Tier II (more extensive literature search)

A Tier II assessment is a more complete review of the available epidemiological evidence and is generally done only if the earlier Tier I/scoping document suggests a potential for a specific concern (e.g. a specific and credible exposure-disease hypothesis has been advanced and needs to be further evaluated as part of a more detailed assessment). A Tier II epidemiology assessment, similar to the Tier I/update, is generally completed 1" to 3 years following the completion of the Tier I assessment and is issued along with and as part of OPP's Draft Human Health Risk Assessment; the Tier II evaluation is considered to be a qualitative narrative review that incorporates certain elements of a systematic review. For example, a Tier II assessment will include a thorough and complete literature search that is broader than that of the Tier I/update, including not only the AHS database, but also such databases as PubMed, Web of Science, Google Scholar and Science Direct, and sometimes others using standardised, transparent and reproducible query language for which specialised professional library and information science support is obtained.<sup>35</sup> Evidence synthesis by EPA – albeit generally in a qualitative and narrative form – also occurs in a Tier II assessment, and overall conclusions regarding the body of epidemiological literature are made. In addition, the Tier II assessment may indicate areas in which further epidemiological data and studies with respect to specific hypothesised exposure-health outcome is of interest for future work. The Tier II assessment document will not generally attempt to integrate the epidemiological findings with other lines of evidence such as that from animal toxicology studies or information from MoAs/AOPs which may be done (separately) to some degree as part of the risk assessment. To the extent that the Tier II assessment identifies specific health outcomes putatively associated with a given pesticide, further investigation and integration across disciplines can subsequently be done as part of a more comprehensive Tier III assessment (see below).

#### C.2.3.3. Tier III (Full Systematic Review with Data Integration)

While a Tier II assessment examines a wide range of health outcomes appearing in the epidemiological literature that are hypothesised to be associated with a given pesticide chemical, a Tier III assessment might encompass a broader (multidisciplinary) and sometimes more quantitative/statistical evaluation of at the epidemiological evidence for the association of interest, and it attempts to more

<sup>&</sup>lt;sup>34</sup> https://aghealth.nih.gov/news/publications.html

<sup>&</sup>lt;sup>35</sup> Additional searches conducted under the rubric of epidemiology and biomonitoring/exposure could be done using the NHANES Exposure Reports (http://www.cdc.gov/exposurereport/); TOXNET (http://toxnet.nlm.nih.gov/); CDC NBP Biomonitoring Summaries (http://www.cdc.gov/biomonitoring/biomonitoring\_summaries.html); ICICADS (http://www.inchem.org/pages/cicad s.html); ATSDR Toxicological Profiles (http://www.atsdr.cdc.gov/toxprofiles/index.asp); IARC Monographs (http://monographs. iarc.fr/ENG/Monographs/PDFs/; EFSA's Draft Assessment Report Database (http://dar.efsa.europa.eu/dar-web/provision); and Biomonitoring Equivalents (https://blog.americanchemistry.com/2014/07/biomonitoring-equivalents-a-valuable-scientific-toolfor-making-better-chemical-safety-decisions/



formally integrate this with animal toxicology and MoA/AOP information. Such a Tier III assessment could take the form of a systematic review of the epidemiological literature which would be performed together with evaluation of toxicity and adverse outcome pathways. For pesticide chemicals from AHS, a Tier III analysis would also ideally incorporate the results of evaluations from other high-quality epidemiological investigations and incorporate 'Weight of the Evidence' to a greater degree to reflect a more diverse set of information sources. Results from these investigations would be used to evaluate replication and consistency with results from the AHS. Early AHS findings in a number of cases were based on only a small number of participants that had developed specific outcomes or a relatively few number of years over which the participants have been followed. As the AHS cohort ages, the release of second evaluations of some chemicals from AHS will be based on additional years of follow-up and a greater number of cases that are expected to provide a more robust basis for interpreting positive and negative associations between exposure and outcome. In addition, the AHS is increasingly generating a substantial amount of biochemical, genetic marker, and molecular data to help interpret results from the epidemiological studies. Such results may further clarify AHS findings, provide evidence for a biological basis linking exposures to outcomes, or suggest additional laboratory and observational research that might strengthen evidence for mechanisms underlying causal pathways. In addition, Tier III analyses also may take advantage of efforts to bring together information and results from international cohort studies in the International Agricultural Cohort Consortium (AgriCOH) in which AHS is a member. AgriCOH is actively working to identify opportunities and approaches for pooling data across studies, and the availability of these other cohort data should aid in assessing reproducibility and replication of exposure-outcome relationships as EPA considers, evaluates and weighs the epidemiological data.

# C.2.4. OPP's open literature searching strategies and evaluation of study quality

An important aspect of the systematic review approach is the thorough, systematic, and reproducible searching of the open epidemiological literature such that much of the literature that meets the established eligibility criteria can be located.<sup>36</sup> OPP uses specific databases as part of their literature search and has specific guidance on their conduct (for example, OPP's open literature search guidance for human health risk assessments<sup>37</sup>). Evaluation of all relevant literature, application of a standardised approach for grading the strength of evidence, and clear and consistent summative language will typically be important components (NRC, 2011). In addition, a high quality exposure assessment is particularly important for environmental and occupational epidemiology studies.

A second important component of the above systematic review approach is the assessment of the validity of the findings from the identified studies. Generally speaking, the quality of epidemiological research, sufficiency of documentation of the study (study design and results), and relevance to risk assessment will be considered when evaluating epidemiology studies from the open literature for use in agency risk assessments. When considering individual study quality, various aspects of the design, conduct, analysis and interpretation of the epidemiology studies are important. These include (from US-EPA, 2016):

- 1) clear articulation of the hypothesis, or a clear articulation of the research objectives if the study is hypothesis-generating in nature;
- adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment;
- 3) reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population);
- 4) appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias;
- 5) adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed.

<sup>&</sup>lt;sup>36</sup> Some advocate looking at the grey or unpublished literature to lessen potential issues associated with publication bias.

<sup>&</sup>lt;sup>37</sup> See https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open and specifically p. 10 of the document 'Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment' dated 28.8.2012 at https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf for Special Notes on Epidemiologic Data.



- overall characterisation of potential systematic biases in the study including errors in the selection of participation and in the collection of information, including performance of sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented;
- 7) adequate statistical power for the exposure–outcome assessment, or evaluation of the impact of statistical power of the study if under-powered to observed effects, and appropriate discussion and/or presentation of power estimates; and
- 8) use of appropriate statistical modelling techniques, given the study design and the nature of the outcomes under study.

## References

- Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730–741.
- Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36, 781–792.
- Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C, 2008. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Critical Reviews in Toxicology, 38, 87–96.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295–300.
- Meek, ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J and Vickers C, 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. Journal of Applied Toxicology, 34, 595–606.
- Meek, ME, Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of Applied Toxicology, 34, 1–18.
- NAS (National Academy of Sciences), 2007. Toxicity Testing on the 21st Century: A Vision and a Strategy. Board on Environmental Studies and Toxicology. Available online: https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a
- NAS (National Academy of Sciences), 2009. Science and decisions: advancing Risk Assessment. Board on Environmental Studies and Toxicology. Available online: http://dels.nas.edu/Report/Science-Decisions-Advanc ing-Risk-Assessment/12209
- NAS (National Academy of Sciences), 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Board on Environmental Studies and Toxicology. Available online: https:// www.nap.edu/download/13142
- Simon TW, Simons SS, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Crisp PF, McMullin TS, McQueen CA and Rowlands JC, 2014. The use of mode of action information in risk assessment: Quantitative key events/dose response framework for modelling the dose-response for key events. Critical Reviews in Toxicology, 44 (Suppl 3), 17–43.
- US-EPA (Environmental Protection Agency), 2010a. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment. Presented to FIFRA Scientific Advisory Panel on February 2-4 2010a. January 7. Available online: https://www.regulations.gov/document?D=EPA-HQ-OPP-2009-0851-0004
- US-EPA (Environmental Protection Agency), 2010b. Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. MEMORANDUM dated 22 April, 2010b. SAP Minutes No. 2010-03. Available online: https://www.re gulations.gov/document?D=EPA-HQ-OPP-2009-0851-0059
- US-EPA (Environmental Protection Agency), 2012. Office of the Science Advisor. Risk Assessment Forum. Draft Framework for Human Health Risk Assessment to Inform Decision Making. July 12, 2012.
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic and Incident Data in Risk Assessments for Pesticides. December 28, 2016. Available online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf



## Annex D – Effect size magnification/inflation

As described in the main text of this document, a potential source of bias may result if a study has low power. This lesser known type of bias is known 'effect size magnification'. While it is as widely known that, generally small, low-powered studies can result in false negatives since the study power is inadequate to reliably detect a meaningful effect size, it is less well known that these studies can result in inflation of effect sizes if those estimated effects are required to pass a statistical threshold (e.g. the common p < 0.05 threshold used for statistical significance) to be judged important, relevant, or 'discovered'. This effect – variously known as effect size magnification, the 'winners curse', truth inflation, or effect size inflation – is a phenomenon by which a 'discovered' association (i.e. one that has passed a given threshold of statistical significance to be judged meaningful) from a study with suboptimal power to make that discovery will produce an observed effect size that is artificially and systematically inflated.

Such truth inflation manifests itself as (systematic) bias away from the null in studies that achieve statistical significance in instances where studies are underpowered (Reinhart, 2015). This is because low-powered (and thus generally smaller) studies are more likely to have widely varying results and thus be more likely to be affected by random variation among individuals than larger ones. More specifically, the degree of effect size magnification that may be observed in any study depends, in part, on how widely varying the results of a study is expected to be and this depends on the power of the study; low powered studies tend to produce greater degrees of effect size magnification in results that are found to be statistically significant (or pass other threshold criteria) than higher powered studies.

As an example of this 'effect size magnification' concept and why it may come about, it is useful to imagine a trial run thousands of times with variable sample sizes. In this case, there will be a broad distribution of observed effect sizes. While the observed medians of these estimated effect sizes are expected to be close to the true effect size, the smaller trials will necessarily systematically produce a wider variation in observed effect sizes than larger trials. However, in low powered studies, only a small proportion of observed effects will pass any given (high) statistical threshold of significance and these will be only the ones with the greatest of effect sizes. Thus, when these generally smaller, low powered studies with greater random variation do indeed find a significance-triggered association as a result of passing a given statistical threshold, they are more likely to overestimate the size of that effect. What this means is that research findings of low-powered and statistically significant studies are biased in favour of finding inflated effects. As summarised by Gelman and Carlin (2014): 'when researchers use small [underpowered]<sup>38</sup> samples and noisy measurements to study small effects..., a significant result is often surprisingly likely to be in the wrong direction and to greatly overestimate an effect'. In general, it can be shown that low background (or control or natural) rates, low effect sizes of interest, and smaller sample sizes in the study end to produce lower power in the study and this leads to a greater tendency towards and magnitude of (any) inflated effect sizes.

It is important to note that the effect size inflation phenomenon is a general principle applicable to discovery science in general and is not a specific affliction or malady of epidemiology (Ioannidis, 2005; Lehrer, 2010; Button, 2013; Button et al., 2013; Gelman and Carlin, 2014; Reinhart, 2015). It is often seen in studies in pharmacology, in gene studies, in psychological studies, and in much of the most-often cited medical literature. When researchers have limited ability to increase the sample size such as in most epidemiological studies, effect size magnification is not a function or fault of the research or research design, but rather a function of how that the results of that research are interpreted by the user community. Thus, unlike other possible biases such as selection or information bias in epidemiology studies, the bias is not intrinsic to the study or its design, but rather characteristic of how that study is interpreted.

In order to determine (and quantify) the potential degree of effect size magnification for any given study that produces a statistically significant result, the reviewer must perform various power calculations. More specifically, when the association between a chemical exposure and a disease is found to be statistically significant, a power analysis can be done to determine the degree to which the statistically significant effect size estimate (e.g. odds ratio, relative risk or rate ratio) may be artificially inflated.

<sup>&</sup>lt;sup>38</sup> [italics added]

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In order to perform the requisite power calculation, the reviewer must know or obtain four values:

- 1) the number of subjects in non-exposed group;
- 2) the number of subjects in the exposed group;
- 3) the number of individuals with the disease of interest (or cases) in the non-exposed group; and
- 4) a target value of interest to detect a difference of a given (predetermined) size in a comparison of two groups (e.g. exposed vs. not exposed)

The first three listed values are provided in or must be obtained from the publication while the target value of interest (typically an OR or RR in epidemiology studies) is selected by the risk managers (and is ultimately a policy decision).<sup>39</sup> This Annex examines this effect size inflation phenomenon in a quantitative way using simulations. The annex uses two example published studies and simulations of hundreds of trials to evaluate the degree to which effect size magnification may play a role in producing biased effect sizes (such as odds ratios, rate ratios or relative risks) due to low power.

The first example uses data from Agricultural Health Study prospective cohort publication examining diazinon exposure and lung cancer and illustrates the effect size magnification issue for a calculated RR. The second example uses ever-never data from a case–control study studying malathion exposure and NHL and illustrates the effect size magnification concept from the point of view of an estimated OR.

#### An Example Illustrating Effect Size Magnification and Relative Risk (Jones et al. (2015))

The power associated with a comparison between those that are not exposed to diazinon to those that are exposed at the highest tertile (T) can be computed from the information provided in the AHS study publication 'Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study - an updated analysis' by Jones et al. (2015) for lung cancer. The number of subjects at each exposure level was provided in the article (non-exposed group: N = 17710, and T(ertile)1, T2 and T3 were categorised based on exposure distribution; specifically: N of each tertile = (2,350 + 2,770)/3 = 1,710 from the publication's Table 1 where: (a) the value of 2,350 represents the number in the lowest exposed *level* and (b) the value of 2,770 represents the number of the two highest exposed levels when the exposed subjects were dichotomously categorised. Since we have (i) the number of subjects in the reference non-exposed group = 17,710; (ii) the number of subjects in each of the exposed groups (tertiles) = 1710; and (iii) the number of diseased individuals (lung cancer) in the reference non-exposed group = 199 (from Table 3 of the cited publication), we can calculate the power of the comparisons between T1 vs non-exposed, T2 vs non-exposed and T3 vs non-exposed that were presented in the article, given the assumption that any true Rate Ratio = 1.2, 1.5, or 2.0, etc.

Here, we are interested in evaluating the power associated with the estimated background rate of 199/17710 (= 0.011237), and, as a form of sensitivity analysis, one half of this background rate (or 0.005617), and twice this rate (0.022473) for detecting (admittedly arbitrary) relative rates of (possible regulatory interest of) 1.2, 1.5, 2.0 and 3.0 among the subjects in each tertile of the diazinon exposed individuals. This analysis was performed using Stata statistical software and is shown below in both tabular and graphical format for true Rate Ratios of 1.2, 1.5, 2.0 and 3.0 for

<sup>&</sup>lt;sup>39</sup> This target value is an effect size of interest, often expressed as either a relative risk (for cohort studies) or an odds rate (for case control studies). That is, the target value is generally an OR or RR of a given magnitude that the risk manager desires to detect with a given degree of confidence. The higher the OR or RR, the greater the magnitude of the estimated association between exposure and the health outcome. While there are not strict guidelines about what constitutes a 'weak' association vs a 'strong' one - and it undoubtedly can be very context-dependent - values less than or equal to about 1 (or sometimes  $\leq$  1.2) are considered to be `null' or `essentially null' (this ignores the possibility of a protective effect which in some contexts – for example, vaccination efficacy - may be appropriate to consider). Values less than 2 or 3 are often considered by some as 'weak'. Values greater than 2 (or 3) and up to about 5 might be considered 'moderate', and values greater than 5 are considered by some to be 'large'. Monson (1990) describes as a guide to the strength of association a rate ratio of 1.0–1.2 as 'None', of from 1.2 to 1.5 as 'Weak', of from 1.5 to 3.0 as 'Moderate', and of 3.0-10.0 as 'Strong'. Other authors use Cohen's criteria to describe ORs of 1.5 as 'small' and 5 as 'large', with 3.5 as 'medium' in epidemiology (Cohen and Chen, 2010). Others describe 1.5 as 'small', 2.5 as 'medium' or 'moderate', 4 as 'large' or 'strong' and 10 as 'very large' or 'very strong' (Rosenthal, 1996) Taube (1995) discusses some of the limitations of environmental epidemiology in detecting weak associations (also see invited commentary illustrating counter-arguments in Wynder (1997). It should be recognized that none of the demarcation lines are 'hard' and there can be legitimate disagreements about where these are drawn and how these are considered and interpreted. Regardless, these can be very much context-dependent and the above demarcations should not be regarded as in any way official or definitive.



1/2x-, 1x- (shown below in bold/shaded) and 2x- the (observed) background rate of 199 diseased individuals/17,710 persons<sup>40</sup>:

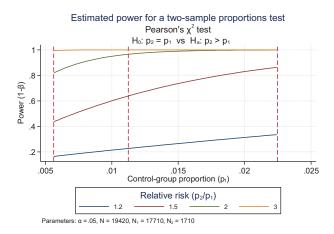
Results of p	Results of power analysis for a one-sided, two-sample proportions test ( $\alpha = 0.05$ ) <sup>(a)</sup>						
N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Relative risk	Power		
17,710	1,710	0.00562	0.00674	1.2	0.1634		
17,710	1,710	0.00562	0.00843	1.5	0.4353		
17,710	1,710	0.00562	0.01124	2.0	0.8182		
17,710	1,710	0.00562	0.01685	3.0	0.9935		
17,710	1,710	0.01124	0.01348	1.2	0.2259		
17,710	1,710	0.01124	0.01685	1.5	0.6379		
17,710	1,710	0.01124	0.02247	2.0	0.9652		
17,710	1,710	0.01124	0.03371	3.0	1		
17,710	1,710	0.02247	0.02697	1.2	0.3353		
17,710	1,710	0.02247	0.03371	1.5	0.8632		
17,710	1,710	0.02247	0.04495	2.0	0.9991		
17,710	1,710	0.02247	0.06742	3.0	1		

Stata code used to generate the above power calculation results: power two proportions ('= 0.5 \* 199/ 17710'= 199/17710'= 2 \* 199/17710), test(chi2) RR (1.2 1.5 2.0 3.0) n1(17710) n2 (1710) one-sided table(N1: 'N control' N2: 'N exposed' p1: 'proportion control' p2: 'proportion exposed' RR: 'relative risk' power: 'power').

(a): One-sided test  $\alpha = 0.05$  Ho: p2 = p1 vs Ha: p2 > p1; N<sub>controls</sub> = 17,710, N<sub>exposed</sub> = 1,710; Number of Iterations = 1,000 (data sets).

(b): Representing 1/2x-, 1x- and 2x- the observed background rate of lung cancer of 199/17710 in Jones et al. (2015). Highlighted/bolded region in table above represents power associated with this 1x observed background rate of lung cancer in cited study.

These values can be graphed as shown below<sup>41</sup>:



Graph showing estimated power for a (one-sided) two-sample proportions test evaluating power as a function of control-group proportion at true RRs of 1.2-, 1.5-, 2.0- and 3.0. Dashed red vertical lines represent control group proportions at 1/2x of that observed, 1x of that observed and 2x of that observed and illustrate sensitivity of the power to these background rate assumptions.

<sup>41</sup> Stata code for generating the above graph: power twoproportions (' = 0.5 \* 199/17710'(0.0001) '= 2 \* 199/17710'), test(chi2) rrisk(1.2 1.5 2.0 3.0) n1(17710) n2(1710)graph (recast(line) xline('= 0.5 \* 199/17710' '=199/17710' '= 2 \* 199/17710', lpattern (dash)) legend(rows(1)size(small)) ylabel(0.2(0.2)1.0)) one sided.

<sup>&</sup>lt;sup>40</sup> The RRs of 1.2, 1.5, 2.0 and 3.0 were selected somewhat arbitrarily to illustrate the power associated with a series of relative risks that might be of interest to the risk manager/decision-maker. The values of RR or OR = 2.0 and 3.0 are considered by some to be a demarcation between weaker effect sizes and stronger effect sizes. The RR value of 1.2 is what some consider 'near to or essentially null', and the RR of 1.5 is an intermediate value between these. In determining whether the epidemiological evidence suggests a relationship between an exposure and a health outcome, a risk manager might consider the 'essentially null' RR of 1.2 from a robust study with acceptable statistical power (generally considered 80–90%) as sufficient evidence for failing to find an association and, in effect, may provide supporting evidence for a conclusion of no observable association between the exposure and the outcome.



As can be seen in the above table and graph, this study had a power of about 23% at 1x the background rate (control-group proportion, equal to 199 diseased individuals/17,710 subjects = 0.011237) to detect a RR of 1.2. To detect an RR of 1.5, there is about 64% power. If the true background rate were in reality twice the observed background rate ( $2 \times 0.011237 = 0.022473$ ), we would have about 86% power to be able to detect a RR of 1.5 and essentially 100% power to detect an RR of 2.0.<sup>42</sup>

Given the above, SAS was used to simulate the degree to which there may be effect size magnification (aka effect size inflation) given *true* relative risks of 1.2, 1.5, 2.0 and 3.0. The table below illustrates the power analysis for diazinon and lung cancer which shows the extent of the effect size magnification from the simulation results. The analysis presented in the table below parallels that done by Ioannidis (2008) and presented in his Table 2 for a set of hypothetical results passing the threshold of formal statistical significance to illustrate the effect size magnification concept.

SAS simulation results illustrating effect size magnification given true odds ratios of 1.2, 1.5, 2.0	
and 3.0 <sup>(a)</sup>	

True values Proportion of diseased individuals in control RR				Distribution of observed significant RRs				
		N analysed data sets	Power <sup>(b)</sup>	N	10th percentile	Median (% inflation)	90th percentile	
0.005617	1.2	1,000	0.16	157	1.6	1.7 (42)	2.0	
(1/2 $\times$ background)	1.5	1,000	0.40	401	1.6	1.8 (20)	2.3	
	2	1,000	0.82	823	1.7	2.1 (5)	2.8	
	3	1,000	1	997	2.3	3.0 (0)	3.9	
0.011237	1.2	1,000	0.22	224	1.4	1.6 (33)	1.8	
(1 $\times$ background)	1.5	1,000	0.63	627	1.4	1.6 (7)	2.0	
	2	1,000	0.98	977	1.6	2.0 (0)	2.5	
	3	1,000	1	1,000	2.5	3.0 (0)	3.6	
0.022473	1.2	1,000	0.33	331	1.3	1.4 (17)	1.6	
(2 $\times$ background)	1.5	1,000	0.87	871	1.3	1.5 (0)	1.8	
	2	1,000	1	1,000	1.7	2.0 (0)	2.3	
	3	1,000	1	1,000	2.6	3.0 (0)	3.4	

Poisson regression model was used to compare the rate of (relative risks) between the groups. The EXACT Test was used in the analysis of some data sets when the generalised Hessian matrix is not positive definite (due to a zero cases in one of the groups). (a): One-sided test,  $\alpha = 0.05$ , N Controls = 17,710, N diazinon Exposed = 1,710, Number of iterations = 1,000 (data sets).

(b): The power resulting from this simulation may be close but not precisely match the power calculated from built-in procedures in statistical software such as SAS (PROC POWER) or Stata (power two-proportion). This may be due to the number of data sets simulated being of insufficient size. However, 1,000 iterations is sufficient to adequately estimate the power and to illustrate the degree of effect size magnification given a statistically significant result (here,  $\alpha \le 0.05$ ).

Note that – given a statistically significant result at p < 0.05 – the percent effect size inflation at the median of the statistically significant results varies from 0% to 42% depending on both the rate of lung cancer among individuals not exposed to diazinon (i.e. proportion of diseased individuals in the non-exposed group) and the true relative risk (ranging from 1.2 to 3.0). For example, if the **true RR** of a tertile of exposed vs non-exposed were 1.2, where the non-exposed group has a rate of lung cancer of 0.011237 (bolded row in the above table), half of the **observed** statistically significant RRs would be above the median of 1.6 and half would be below 1.6; this represents a median inflation of 33% over the true RR of 1.2 used in the simulation.

For the background rate found in the Jones et al. (2015) study (0.011237), a true RR of 1.2 that was found to be statistically significant would instead were the study to be repeated be observed to vary from 1.4 (at the 10th percentile) to 1.8 (at the 90th percentile) with the aforementioned median of 1.6. When the **true RR** is 2 or 3, the power is greater than 80% (as seen in the above table) and the median of observed RR is close to the true RR and the range of observed RRs are narrow. As the true RR increases to 3, the study's power increases such that the effect size inflation disappears and the median from the simulations indeed reflects the true RR.

<sup>&</sup>lt;sup>42</sup> Said another way, if the true (but unknown) background rate were actually twice the observed background rate, we could reasonably conclude (with 86% confidence) if no statistically significant relationship was found that the true OR did not exceed 1.5.



# An Example Illustrating Effect Size Magnification and Odds Ratios in an Ever/Never Analysis (Waddell, et al. 2001)

Sometimes comparisons between exposed group vs non-exposed group are presented in an 'ever/never' comparison as opposed to a comparison based on some other categorisation or grouping such as terciles or quartiles. This exposure category-based analysis might be done because there are an insufficient number of cases to break the exposure categories into small (more homogenous) exposure classifications or groupings or because the measurements of exposure are not available or are less reliable (such as in case-control studies). In these situations, we similarly need (i) the total number of subjects in non-exposed group; (ii) the number of subjects in exposed group; (iii) the number of diseased individuals in the non-exposed group in order to calculate the power of the comparison between exposed group vs non-exposed group at some; (iv) given or preselected odds ratios.

To illustrate how a power and effect size magnification analysis might be done for a case–control study using ever-never exposure categorisations, a study investigating the association between malathion and NHL (Waddell et al., 2001) was selected. Here, we have (i) the number of subjects in the reference non-exposed group = 1,018 (from Table 1: non-farmers = 243 diseased individuals + 775 non-diseased individuals); (ii) the number of subjects in the exposed group = 238 (from Table 4: malathion exposed individuals = 91 exposed cases + 147 non-exposed controls); (iii) the number of diseased individuals in the reference non-exposed group = 243 (from Table 1: 243 diseased individuals in the non-farmer or non-exposed group), we can similarly calculate the power of the comparisons between the ever vs never exposed, given the assumption that any true OR = 1.2, 1.5, 2.0, etc.

As was described above for lung cancer and diazinon, we estimated a power of 30.5% to detect an OR of 1.2 at the study-estimated NHL proportion of 0.2387 among non-farmers (non-exposed), as illustrated in the table below:

Results of p	Results of power analysis for a one-sided, two-sample proportions test ( $\alpha = 0.05$ ) <sup>(a)</sup>					
N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Odds Ratio	Power	
1,018	238	0.1194	0.1399	1.2	0.2279	
1,018	238	0.1194	0.1689	1.5	0.647	
1,018	238	0.1194	0.2133	2.0	0.9693	
1,018	238	0.1194	0.2891	3.0	1	
1,018	238	0.2387	0.2734	1.2	0.3047	
1,018	238	0.2387	0.3199	1.5	0.8149	
1,018	238	0.2387	0.3854	2.0	0.9971	
1,018	238	0.2387	0.4847	3.0	1	
1,018	238	0.4774	0.523	1.2	0.3522	
1,018	238	0.4774	0.5781	1.5	0.8779	
1,018	238	0.4774	0.6463	2.0	0.9992	
1,018	238	0.4774	0.7327	3.0	1	

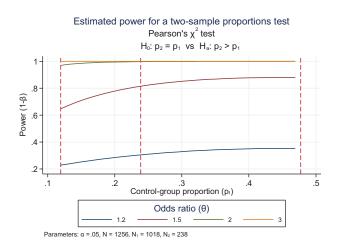
Stata code used to generate the above results: power two-proportions ('= 0.5 \* 243/1018' '= 243/ 1018' '= 2 \* 243/1018'), test(chi2) OR (1.21.52.03.0) n1(1,018) n2(238) one-side table(N1: 'N control' N2: 'N exposed' p1: 'proportion control' p2' proportion exposed' OR: 'odds ratio' power: 'power').

(a): One-sided test  $\alpha = 0.05$  Ho: p2 = p1 vs Ha: p2 > p1;  $N_{controls} = 1,018$ ,  $N_{exposed} = 238$ , Number of iterations = 1,000 (data sets). (b): Representing 1/2x-, 1x- and 2x- the observed background rate of lung cancer of 243/1018 in Waddell et al. (2001). Highlighted, bolded region in table above represents power associated with this 1x observed background rate of NHL in cited study.

Such power relations for malathion and NHL are graphed below<sup>43</sup> – as was done in the above AHS prospective cohort study for diazinon and lung cancer – with the middle vertical dotted line in the graph showing power at the NHL proportion of 0.2387 among non-farmers/non-exposed and the left-hand and right-hand vertical dashed lines representing a form of sensitivity analysis at one-half and twice the NHL proportion among non-farmers/non-exposed, respectively.

<sup>&</sup>lt;sup>43</sup> Stata code for generating the graph: power two proportions ('= 0.5 \* 243/1018'(0.01) '= 2 \* 243/1018'), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1018) n2(238)graph(recast (line) x-line('= 0.5 \* 243/1018' '= 243/1018' '= 2 \* 243/1018', lpattern(dash)) legend(rows(1)size(small)) y-label(0.2(0.2)1.0)) one sided.





Graph showing estimated power for a (one-sided) two-sample proportions test evaluating power as a function of control-group proportion at true RRs of 1.2-, 1.5-, 2.0- and 3.0. Dashed red vertical lines represent control group proportions at 1/2x of that observed, 1x of that observed and 2x of that observed and illustrates the sensitivity of the power to these background rate assumptions.

At the study-estimated NHL proportion of 0.2387 among non-farmers/non-exposed, the power (one-sided) to detect ORs of 1.2, 1.5, 2.0 and 3.0 is shown to be 30.5%, 81.5%, 99.7% and > 99.9%, respectively. Note that Waddell et al. (2001) reported an OR of 1.6 with a 95% CI of 1.2–2.2, based on 91 NHL cases who used malathion and 243 cases that were among non-farmers who did not.

Given the above, SAS was used to simulate the degree to which effect size magnification may exist given *true* odds ratios of 1.2, 1.5, 2.0 and 3.0. Below is a SAS-generated table for the power analysis for malathion and NHL showing the magnitude of the effect size magnification from the SAS-based simulation results.

True values			Distribution of observed significant ORs				
Proportion of diseased individuals in non-exposed group	OR	N analysed data sets	Power <sup>(b)</sup>	N	10th percentile	Median (% inflation)	90th percentile
0.1194 (1/2 background)	1.2	1,000	0.22	220	1.4	1.5 (25)	1.8
	1.5	1,000	0.66	661	1.5	1.7 (13)	2.0
	2	1,000	0.97	972	1.6	2.0 (0)	2.5
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7
0.2387 (1 $\times$ background)	1.2	1,000	0.32	323	1.3	1.4 (17)	1.6
	1.5	1,000	0.81	812	1.4	1.6 (7)	1.8
	2	1,000	1.0	997	1.6	2.0 (0)	2.4
	3	1,000	1.0	1,000	2.5	3.0 (0)	3.6
0.4774 (2 $\times$ background)	1.2	1,000	0.34	337	1.3	1.4 (17)	1.6
	1.5	1,000	0.87	872	1.3	1.5 (0)	1.8
	2	1,000	1.0	1,000	1.6	2.0 (0)	2.5
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7

SAS simulation results illustrating effect size magnification given true odds ratios of 1.2, 1.5, 2.0,	,
and 3.0 <sup>(a)</sup>	

The logistic regression model was used to compute the odds ratios for the two groups. The EXACT Test was used in the analysis of some data sets when the maximum likelihood estimate did not exist (perhaps due to a zero cases in one of the groups). (a): One-sided test,  $\alpha = 0.05$ , N non-exposed = 1,018, N malathion exposed = 238, N iterations = 1,000 (data sets).

(b): The power resulting from this simulation may be close but not match exactly with the power calculated from built-in procedures in statistical software such as SAS (PROC POWER) or Stata (power two-proportion). This may be due to number of data sets simulated being of insufficient size. However, 1,000 iterations are sufficient to adequately estimate the power and to illustrate the degree of effect size magnification given a statistically significant result (here,  $\alpha \leq 0.05$ ).



Note that – given a statistically significant result at p < 0.05 – the median effect size varies from 1.4 to 3, depending on the NHL proportion in the non-exposed group, and the true odds ratio (ranging from 1.2 to 3.0). For example, if the true OR for a NHL proportion among non-farmers of 0.2387 was 1.2 (bolded row in the table), half of the *observed statistically significant* ORs would be above the median of 1.4 and half would be below. Further, most (90%) of the statistically significant ORs would be observed to be above 1.3, and a few (10%) would be observed even to be above 1.6.

In sum, then, the power of an epidemiological study is an important factor that should considered by regulators and others evaluating such studies. A study that is sufficiently powered will not only be more likely to detect a true effect of a given size if it is indeed present (the classic definition of power which relates to the issue of a Type II error or a false negative) but will also be less likely to magnify or exaggerate the effect if it is not there but (by chance) crosses a preselected threshold (such as the 0.05 level for statistical significance). If a study is suitably powered (say, 80% or more), the observed effect size is more likely to be a reflect a true effect size and any observed chance variation in this effect size will reflect a distribution symmetrically centred around the unknown true value. The take home message from these simulations and the original work by Ioannidis and extensions by Gelman and Carlin (2014) is that a study should be not only suitably powered to avoid a false negative (Type II error) but also suitably powered to avoid a magnification of the effect size for those effect sizes that are statistically significant (or pass some other threshold). Gelman and Carlin (2014) go further, stating that such 'retrospective design calculations may be more relevant for statistically significant findings than for nonsignificant findings. The interpretation of a statistically significant result can change drastically depending on the plausible size of the underlying effect'. Note that if a study is suitably powered, there is NO systematic risk inflation, but the effect estimates for underpowered studies that produce statistically significant effects are prone to what might be substantial risk inflation, the interpretation of which depends on realistic estimates of the true (underlying) effect.

Ideally, then, published literature studies should conduct and document power analyses. Short of that, published literature should provide adequate information for the reader to perform such power calculations (or, as Gelman and Carlin (2014) term them: (retrospective) design calculations). In the two examples provided above, the authors did provide sufficient information for the reader to calculate power and the potential for effect size magnification. This is not always the case. Sometimes information used for power calculations are only partially provided in the publications or provided information was structured in a way that does not permit such calculations.<sup>44,45</sup> For example, if authors use number of cases instead of level of exposure to determine tertiles or quartiles (which would be evidenced by a constant number of cases between groups) or if authors group multiple cancer outcomes together and use that number to determine tertiles, then the power (or design) calculations illustrated here are not possible since the required inputs are not able to be derived. Since the counts and data which are tabulated and reported are not necessarily standardised among authors and publications, one strong recommendation would be for publications to require reporting (even if in supplementary or online data) the necessary information to estimate power such that such evaluations can be done by both peer reviewers and interested readers.

<sup>&</sup>lt;sup>44</sup> For example, in the review of the association between malathion exposure vs aggressive prostate cancer presented in the publication 'Risk of Total and Aggressive Prostate Cancer and Pesticide Use in the Agricultural Health Study' by Stella Koutros et al. (2012), the Panel was not able to calculate the power of the comparison between the malathion-exposed groups vs non-exposed group because critical information was not provided in the published article. From the publication and the supplemental document of the publication, we were able to easily find the number of <u>cases</u> in the non-exposed group (Table 2 in the main article), but the number of <u>subjects</u> in the non-exposed group or at each exposed level (i.e., quartile) appeared not to be available. We attempted to derive the number of subjects in the non-exposed group and number of subjects in each quartile from the information in Table 1 of the supplemental document of the article but were not able to do so since the information in Table 1 was presented in a way that was not consistent with many other AHS publications in that the exposed subjects were categorized into groups based on the quartiles of number of cases.

<sup>&</sup>lt;sup>45</sup> Sometimes, information used for power calculations may have only been <u>partially</u> provided in the publications. For example, we calculated the powers associated with various thyroid cancer comparisons from the information provided in the AHS study publication 'Atrazine and Cancer Incidence Among Pesticide Applicators in the Agricultural Health Study (1994–2007)', by Laura Beane-Freeman et al. (2011). In this publication, the authors did not categorize the subjects into quartiles based on exposure but instead categorized or grouped the subjects based on the total number of *all* cancer *cases <u>combined</u>*. In this way, the number of cases of all types of cancer was the same between categorized groups and thus both the number of *cases* of any specific cancer of interest (e.g. thyroid, here) was not the same between groups and the number of *subjects* was not the same between groups. In this example, the publication provided (i) the reference Q1: N = 9,523, (ii) total subjects in Q2, Q3 and Q4: N = 26,834 (Table 1) and (iii) the number of thyroid cancer cases in the reference Q1 = 3 (Table 2). The exact number of subjects in each of the compared groups (Q2, Q3 or Q4) was, however, not available.



While the above analysis suggests that potential implications of the effect size inflation phenomenon are important considerations in evaluating epidemiological studies, it is important to remember a number of caveats regarding the phenomenon and how its consideration should enter into any interpretation of epidemiological studies.

- First, while this phenomenon would tend to inflate effect sizes for underpowered studies for which the effect of interest passes a statistical (or other) threshold, there are other biases that may be present that bias estimates in the other direction, *towards* the null. This bias might be referred to as effect size *suppression*. Perhaps, the most well-known of these is non-differential misclassification bias discussed in the main body of the text. This can commonly (but not always) produce predictable biases towards the null, thereby systematically under-predicting the effect size. Recognising that this is not always true and there are potentially countervailing or counteracting factors like effect size magnification (at least for small underpowered studies) is an important step forward. Specifically, underpowered studies can result in biased estimates in a direction away from the null to a degree that that can potentially offset (and possibly more than offset) any biases towards the null that may result, for example, from non-differential misclassification bias. Regardless, what is of critical importance is to recognise that adequately powered studies are necessary to be able to have at least some minimal degree of confidence in the estimate of the effect size for a statistically significant result.
- Secondly and as stated in the main body of the text effect size magnification is linked to a focused effort on the part of the researcher (or regulators interpreting such a study) on identifying effects that pass a given threshold of significance (e.g. p < 0.05) or achieve a certain size (e.g. OR > 3) when that study is underpowered. This phenomenon, then, is of most concern when a 'pre-screening' for statistical significance (or effect size). To the extent that regulators, decision-makers and others avoid acting by focusing on only those associations that 'pass' some predetermined statistical threshold and then use that effect size to evaluate and judge the magnitude of the effect without acknowledging that it might be inflated if the study is underpowered, the phenomenon is of lesser concern. Note that effect size magnification is not a function or fault of the research or research design, but rather a function of how that research is interpreted by the user community.

Unfortunately, there is sometimes a tendency for attention to focus on effect sizes that are greater than a given size or that pass a certain statistical threshold and are as such 'discovered'. As recommended by Ioannidis with respect to how these 'discoveries' should be considered (Ioannidis, 2008):

'At the time of the first postulated discovery, we usually cannot tell whether an association exists at all, let alone judge its effect size. As a starting principle, one should be cautious about effect sizes. Uncertainty is not conveyed simply by CIs (no matter if these are 95%, 99% or 99.9%).

For a new proposed association, credibility and accuracy of the proposed effect varies depending on the case. One may ask the following questions: does the research community in the field adopt widely statistical significance or similar selection thresholds for claiming research findings? Did the discovery arise from a small study? Is there room for large flexibility in the analyses? Are we unprotected from selective reporting (e.g. was the protocol not fully available upfront?). Are there people or organisations interested in finding and promoting specific "positive" results? Finally, are the counteracting forces that would deflate effects minimal?'

Thirdly, it should be remembered that the effect size inflation phenomenon is a general principle applicable to discovery science in general and is not a specific affliction or malady of epidemiology (Ioannidis, 2005; Lehrer, 2010; Button, 2013; Button et al., 2013; Reinhart, 2015). As indicated earlier, it is often seen in studies in pharmacology, in gene studies, in psychological studies, and in much of the most-often cited medical literature. Such truth inflation occurs in instances where studies are small and underpowered because such studies have widely varying results. It can be particularly problematic in instances where many researchers are performing similar studies and compete to publish 'new' or 'exciting' results (Reinhart, 2015).

#### Summary and Conclusions

Effect size magnification or 'truth inflation' is a phenomenon that can result in exaggerated estimates of odds ratios, relative risks or rate ratios in those instances in which these effect measures



are derived from underpowered studies in which statistical or other thresholds need to be met in order for effects to be 'discovered'. The phenomenon is not specific to epidemiology or epidemiological studies, but rather to any science in which studies tend to be small and predetermined thresholds such as those relating to effect sizes or statistical significance are used to determine whether an effect exists. As such, it is important that users of epidemiological studies recognise this issue and its potential interpretational consequences. Specifically, any discovered associations from an underpowered study that are highlighted or focused upon on the basis of passing a statistical or other similar threshold are systematically biased away from the null. While we cannot know if any specific observed effect size from a specific study is biased away from the null as a result of being a 'discovered' association that passes a statistical threshold (just as we can't say that a specific study showing non-differential misclassification will necessarily be biased towards the null), we do know that that chance favours such a bias to some degree as illustrated by the explications presented and simulations performed here. Said another way: by choosing to focus on, report, or act upon effect sizes on the basis of those effect sizes passing a statistical or other threshold, a bias is introduced since it is inevitably more likely to select those associations that are helped by chance rather than hurt by it (Yarkoni, 2009). Again, this is an issue related to how studies are interpreted by users, not one that is intrinsic to the study design nor one that is related to good scientific principles or practices.

One (partial) solution to the above issue is for the reader to cautiously interpret effect sizes in epidemiological studies that pass a prestated threshold or are statistically significant if they arise from an underpowered study, recognising that the observed effect sizes can be systematically biased away from the null. Such an approach would require that either the authors report the power of the study or that the authors provide sufficient information for the reader to do so. Effects sizes from studies with powers substantially less than 80% should be interpreted with an appropriate degree of scepticism, recognising that these may be inflated – perhaps substantially so (particularly if the power is less than 50%). The potential degree of this inflation will depend on a number of issues including background rate of the health outcome of interest, the sample size of the study and the effect size of interest. More specifically, when (a) the smaller the background rate of the health outcome of interest is low, (b) the sample size of the study is small and (c) the effect size of interest is weak, then the power of the study (to detect that effect size) will be low and the tendency towards inflated effect sizes in statistically significant results will be high. Low power studies investigating small or weak effects in populations that have a low background rate of the health outcome of interest will tend towards the greatest degree of effect size inflation. As a result, the PPR Panel recommends that epidemiological publications either incorporate such calculations or include key information such that those calculations can be performed by the reader. Specifically:

When the association between a given pesticide exposure and a disease is found to be statistically significant, particularly in (presumed) low powered studies, data user should perform various power calculations (or a power analysis) to determine the degree to which the statistically significant effect size estimate (OR or RR) may be artificially inflated or magnified. This requires three values to be clearly reported by epidemiological studies: (i) the number of subjects in the non-exposed group (including diseased and non-diseased individuals); (ii) the number of subjects in the exposed group (including diseased and non-diseased individuals); and (iii) the number of diseased subjects in the non-exposed group. Risk managers can then select the target value of interest (typically an OR or RR) to detect a difference of a given (predetermined) effect size between the exposed and non-exposed subjects, and evaluate the degree to which effect size magnification could potentially explain the effect size that was estimated in the study of interest.

Since it appears that (i) many epidemiological studies are frequently underpowered; (ii) it is not common for authors to provide either power calculations or (sometimes) the information in publications required to do them, and (iii) the phenomenon of effect size magnification generally appears to be little recognised in the epidemiological field, the above PPR Panel recommendation will require effort on the part of researchers/grantees, publishers, and study sponsors to implement. While the above suggests that the current state of practice in this area may leave one pessimistic, an opinion piece on this topic by researcher Kate Button (Button, 2013) describing her work in Nature Reviews Neuroscience (Button et al., 2013) offered guarded reasons for optimism:

'Awareness of these issues is growing and acknowledging the problem is the first step to improving current practices and identifying solutions. Although issues of publication bias are difficult to solve overnight, researchers can improve the reliability of their research by adopting well-established (but



often ignored) scientific principles: Also, researchers can improve the usefulness/reliability of their research by adopting well-established (but often ignored) scientific principles:

- 1) Consider statistical power in the design of our studies, and in the interpretation of our results;
- 2) Increase the honesty with which we disclose our methods and results.
- 3) Make our study protocols, and analysis plans, and even our data, publically available; and
- 4) Work collaboratively to pool resources and increase our sample sizes and power to replicate findings.'

Although the above set of recommendations and thoughts were set in the context of sample size and neurotoxicology, they have broad applicability to any discovery science, including epidemiology. In sum, while there is much room for improvement in the conduct and reporting of epidemiological studies for them to be useful to regulatory bodies in making public health-based choices, the issues are beginning to be better defined and recognised and – going forward – there is reason for optimism.

## References

- Beane Freeman, LE, Rusiecki, JA, Hoppin, JA, Lubin, JH, Koutros, S, Andreotti, G, Hoar Zahm, S, Hines, CJ, Coble, JB, Barone Adesi, F, Sloan, J. Sandler, DP, Blair, A, and Alavanja, MCR. Atrazine and cancer incidence among pesticide applicators int eh agricultural health study (1994–2007). Environ Health Perspect, 119, 1253–1259.
- Button K, 2013. Unreliable neuroscience? Why power matters. *The Guardian* newspaper (UK). 10 April 2013 Available online: https://www.theguardian.com/science/sifting-the-evidence/2013/apr/10/unreliable-neuroscie nce-power-matters [Accessed 6 September 2017]
- Button K, Ioannidis JPA, Mokrysz C, Nosek BA, Flink J, Robinson ESJ and Munafo MR, 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nature Reviews Neuroscience, 14, 365–376.
- Cohen P and Chen S, 2010. How big is a big odds ratio: interpreting the magnitudes of odds ratios in epidemiological studies. Communications in Statistics: Simulation and Computation, 39, 860–864.
- Gelman A and Carlin J, 2014. Beyond power calculations: assessing type S (sign) and type M (magnitude) errors. Perspectives on Psychological Science, 9, 641–651.
- Ioannidis JP, 2005. Why most published research findings are false. PLoS Med, 2, e124.
- Ioannidis JP, 2008. Why most discovered true associations are inflated. Epidemiology, 19, 640-648.
- Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC and Beane Freeman LE. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. Occupational and Environmental Medicine, 72, 496–503.
- Koutros, S, Beane Freeman, LE, Lubin, JH, Heltshe, SL, Andreotti, G, Hughes-Barry, K, DelllaValle, CT, Hoppin, JA, Sandler, DP, Lynch, CF, Blair, A and Alavanja, MCR, 2013. Risk of total and aggressive prostate cancer and pesticide use in the agricultural health study. American Journal of Epidemiology, 177, 59–74.
- Lehrer J, 2010. The truth wears off: is there something wrong with the scientific method. New Yorker. 13 December, 2010. Available online: http://www.newyorker.com/magazine/2010/12/13/the-truth-wears-off [Accessed September 2017]
- Reinhart A, 2015. Statistics Done Wrong: the woefully complete guide. No Starch Press (San Francisco, CA).
- Rosenthal JA, 1996. Qualitative descriptors of strength of association and effect size. Journal of Social Service Research, 21, 37–59.
- Taubes G, 1995. Epidemiology faces its limits. Science, 269, 164–169.
- Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP and Blair A, 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control, 12, 509–517.
- Wynder EL, 1997. Epidemiology Faces its Limits Reply. Invited Commentary: Response to Science Article, "Epidemiology Faces Its Limits". American Journal of Epidemiology, 143, 747–749.
- Yarkoni T, 2009. Ioannidis on effect size inflation, with guest appearance by Bozo the Clown. 21 November 2009. Available online: http://www.talyarkoni.org/blog/2009/11/21/ioannidis-on-effect-size-inflation-with-guest-appearance-by-bozo-the-clown/ [Accessed on 6 September 2017]

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## 「農薬へのばく露と健康影響に関連する疫学研究の文献レビュー」 の結果のフォローアップ(追跡調査)に関する PPR パネルの意見書

植物保護製剤(農薬)とその残留物に関する EFSA パネル(PPR)

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#### 抄録

2013年にEFSAは、2006年から2012年までに発表された疫学研究の包括的なシステマティックレビューを発表 し、農薬ばく露と多くの健康影響との関連性を調査した。かなりの量の疫学的情報が得られたにもかかわらず、これらの エビデンスの多くはかなり質が低く、多くの制限が結果に影響している可能性が高いため、確固たる結論を出すことは できなかった。このように、規則(EU)No 1107/2009 に記載されている「認可基準」を満たしていない研究は、リスク評 価には適していない。この科学的意見書では、植物保護製剤(農薬)とその残留物に関するEFSAパネル(PPRパネ ル)は、農薬疫学研究の方法論的限界を評価するよう求められており、その主な限界はばく露の特徴付けが不十分で あることが原因であることが判明した。また、前向き研究ではなく症例対照研究を頻繁に使用していることも限界と考え られた。健康影響の不適切な定義や不正確さは避ける必要があり、結果の報告はいくつかのケースで改善される可能 性がある。PPRパネルは、これらの限界を克服し、リスク評価への適切な利用を促進するために、農薬疫学研究の質と 信頼性を向上させる方法についての勧告を提案した。パネルは、農薬の潜在的な有害性、ばく露シナリオ、ばく露評 価の方法、ばく露一反応特性、リスク特性を理解するための有用な方法として、農薬観察研究のシステマティックレビュ ーとメタアナリシスの実施(必要に応じて)を推奨した。最後に、PPRパネルは、農薬のリスク評価のために疫学的デー タを含む複数のエビデンスを統合し、重み付けする方法論的アプローチを提案した。生物学的妥当性は因果関係の 立証に寄与することができる。

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キーワード:疫学、農薬、リスク評価、品質評価、エビデンスの統合、複数のエビデンス、エビデンスの重み付け

要求者:欧州食品安全機関(European Food Safety Authority) 課題番号:EFSA-Q-2014-00481 対応:<u>pesticides.ppr@efsa.europa.eu</u> 謝辞:パネルは、本研究成果にサポートを提供してくれた以下の EFSA スタッフに感謝の意を表する。Andrea Terron、Andrea Altieri、Arianna Chiusolo。パネルと EFSA は、以下のヒアリング専門家の意見に謝意を表する。(1) David Miller (US-EPA)は US-EPA の経験を共有し、効果量の算出を行った。(2) 農業健康調査のための Kent Thomas (US-EPA), (3) the INSERM Report のための Marie Christine Lecomte (INSERM), Sylvaine Cordier (INSERM) and Alexis Elbaz (INSERM), (4) エクスポゾームおよびメタボロミクスのための Toby Athersuch (Imperial College), (5) 農薬に職業的にばく露された人間のバイオモニタリングのデータ収集のための Peter Floyd (Risk & Policy Analysts Ltd), Ruth Bevan (IEH Consulting Ltd), Kate Jones (UK Health & Safety Laboratory)。 最後に、EFSA は、意見書の改訂と提供された意見に対して、科学委員会とAMU ユニットに感謝する。

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#### 概要

欧州食品安全機関(EFSA)は、植物保護製剤(農薬)とその残留物に関するパネル(PPR パネル)に、外部科学報 告書「農薬へのばく露と健康影響を関連付ける疫学研究の文献レビュー」(Ntzani ら、2013 年)の結果のフォローアッ プ(追跡調査)に関する科学的意見書の作成を依頼した。この報告書は、2006 年から 2012 年の間に発表された疫学 研究のシステマティックレビューとメタアナリシスに基づいており、農薬ばく露と 23 の主要なカテゴリーのヒト健康影響と の間に見出された関連性をまとめたものである。最も関連性が高いのは、肝臓がん、乳がん、胃がん、筋萎縮性側索硬 化症、喘息、II型糖尿病、小児白血病、パーキンソン病であった。評価された疫学研究に内在する弱点があるため、因 果関係についての結論を導き出すことはできないが、システマティックレビューでは、特定の複雑なヒトの健康に関する 影響について情報を提供するための規制研究の適合性についての懸念が提起された。

PPR パネルは、農薬に関する疫学研究の質に影響を与える方法論的限界に対処するために、科学的意見書を作成した。この意見書は、規制(EC)1107/2009 の下での農薬の更新時のピアレビュープロセスを支援することのみを目的としており、あらゆる種類のヒトばく露による臨床例や中毒事故(入手可能であれば)を加えた疫学的研究の評価データが必要である。欧州における農薬へのばく露に関する疫学的データは、有効成分の最初の認可前には入手できないため、評価報告書草案(DAR)に提供することは期待できない。しかし、他の管轄区域では有効成分の使用について先行して認可されている可能性があり、その地域の疫学的データが適切だと考えられる。規則(EC)No 1107/2009 では、既存の疫学研究を含む学術的に査読された公表文献を検索することを要求している。このタイプのデータは、「更新のために提出された書類には、有効成分に関連する新しいデータと新しいリスク評価を含めるべきである」という規則(EC)1141/2010にも準拠しており、有効成分の更新プロセスに適している。

本意見書では、疫学データをリスク評価に適切に活用するための農薬有効成分に特化した方法論的アプローチを 提案し、農薬の疫学研究の質と信頼性を向上させるための提言を行った。さらに、PPR パネルは、農薬のリスク評価プ ロセスを改善するために、疫学的と実験毒性学のデータを統合するための方法論を議論し、提案した。

まず、本意見書では、観察による疫学研究1の基本的な要素を紹介し、因果関係を推論するための条件が通常満た されていることから、疫学研究において最も信頼性の高いエビデンスを提供すると考えられている介入研究との対比を 行っている。主な観察による研究の計画については、農薬ばく露の詳細な記述の重要性、有効な健康影響の使用及 びばく露と健康影響の関係をモデル化するための適切な統計解析の重要性が説明されている。また、外部及び内部 研究の妥当性については、結果における偶然の役割を説明し、ばく露以外の要因が発見された関連性を歪めないか どうかを確認するために取り上げられてもいる。いくつかの種類のヒトのデータは、農薬のリスク評価プロセス、特にハザ ードの特定をサポートするのに貢献することができる。正式な疫学研究以外にも、症例集積、疾病登録、毒物管理セン ター情報、労働衛生監視データ、市販後の監視プログラムなどのヒトのデータの他の情報源は、特に急性の特定の健 康影響の場合には、ハザードの特定に有用な情報を提供することができる。

しかし、農薬ばく露と健康影響に関する既存の疫学研究の多くは、さまざまな方法論の限界や不完全性に悩まされ ている(Terms of Reference(ToR)1)。パネルは、ヒトの観察環境における農薬ばく露と健康影響との関連を研究する ことは複雑で、疫学の他の多くの分野よりも困難であると指摘している。この複雑さは、市場に出回っている有効成分の 数の多さ(欧州連合 EU で使用が認可されているものは約 480 種類)、ばく露の測定の難しさ、個々の農薬へのばく露 に関する定量的及び定性的データが頻繁に欠如していることなど、農薬疫学の分野におけるいくつかの特殊な特徴に 起因する。EFSA の外部科学報告書(Ntzani ら、2013 年)で実施された疫学的証拠の系統的評価では、多くの方法 論的限界が指摘されている。特定の農薬に対する直接かつ詳細なばく露評価が行われていない(例えば、ジェネリック 農薬の使用に対して情報不足)ため、主にばく露の特徴付けが不十分であることが、ほとんどの既存の研究の主な限 界となっている。前向き研究ではなくて症例対照研究を頻繁に使用していることも限界となっている。健康影響の不十 分な定義、統計解析がないこと、研究結果の質の低い報告が、いくつかの農薬疫学研究の他の限界として確認されて いる。これらの限界は、因果関係に関する強固な結論を導き出すことを困難にするデータの不均一性や矛盾の原因と

<sup>&</sup>lt;sup>1</sup>本意見書は、観察研究(疫学研究ともいう)と警戒データのみを扱う。これに対し、介入研究(無作為化臨床試験などの実験研究とも呼ばれる)は本意見書の対象外である。

なっている。Ntzaniら、(2013年)が取り上げたほとんどの健康影響の効果量が小さいことを考えると、研究デザインにおけるバイアスの寄与が一役買っている可能性がある。

PPR パネルはまた、リスク評価に有益な将来の農薬疫学研究を改善するための多くの再修正(ToR 2)と勧告(ToR 3)も提供している。疫学研究の質と妥当性は、以下によって高められる。(a)ばく露の適切な評価、好ましくは個人のば く露モニタリングや特定の農薬(または農薬の組み合わせ)のバイオマーカー濃度を個人レベルで使用し、ばく露の誤 分類を最小化し、用量反応評価を可能にする方法で報告すること、(b)十分に有効で信頼性の高い健康影響(アウトカ ム)の評価(十分に定義された臨床データまたは有効な代替物)、(c)交絡変数(健康影響(アウトカム)に影響を与える 他の既知のばく露を含む)を適切に考慮すること、(d)サブグループ解析(例:性別、年齢などによる層別解析)を実施 し、報告すること、環境疫学の研究のために特別に開発された多くの報告ガイドラインとチェックリストは、農薬ばく露を 評価する疫学研究にとっても有用なものである。これは、修正された STROBE(STrengthening the Reporting of OBservational studies in Epidemiology)基準の拡張版が特に該当し、観察による研究の正確で完全な報告書に 何を含めるべきかについての推奨事項が含まれている。

ばく露評価は、個人レベル(バイオモニタリングのような他の直接的な手段で補足することができる信頼性の高い線 量計を目的とする農薬に使用することにより、特定の農薬に対する直接かつ詳細なばく露評価を行う)で改善すること ができる。さらに、登録されたデータを電子カルテにリンクさせることにより集団レベルでのばく露を評価することができ る。これにより、これまでにないサンプルサイズの研究が可能となり、ばく露とその後の疾患に関する情報を得ることがで きるようになる。地理情報システム(GIS)や小規模地域調査も、住居ばく露の推定値を提供するための追加的な方法と して役立つかもしれない。これらのより一般的なばく露評価は、一般的なリスク因子を特定する可能性があり、規制政策 全体への情報提供と、さらなる疫学研究の対象を特定することの両方で重要になる可能性がある。オミクス技術の開発 はまた、生物学的マトリックス中の外来物質や代謝物(メタボロミクス)からDNAやタンパク質との複合体(アダクトミクス) まで、幅広い分子の測定を通じてばく露評価を改善するための興味深い可能性を提示している。オミクスは、複雑な化 学物質の混合物への累積ばく露に対する生物学的反応の特性やシグネチャーを測定する可能性があり、生物学的経 路の理解を深めることができる。つまり、ばく露レベルに関連して、健康障害に関連する生化学的、生理学的、またはそ の他の変化を定量化できるバイオマーカーを使用することで、健康影響を再発見することができ、また、病気の発生の メカニズムを理解するのに役立つ。

規制リスク評価(ToR 4)に疫学的研究を組み込むことは、科学者、リスク評価者、リスク管理者にとって大きな課題で ある。様々な疫学研究の知見は、潜在的な健康被害と有害な健康影響との関連性を評価するために使用することがで き、その結果、リスク評価のプロセスに貢献することができる。農薬ばく露とヒト健康影響との関連性に関する利用可能 なデータが大量にあるが、それにもかかわらず、規制上のリスク評価へのこのような研究の影響はまだ限られている。ヒ トのデータはリスク評価の多くの段階で利用できるが、同じ農薬有効成分に関する他の研究がない場合には、単一の (反複されていない)疫学研究は、質が高く、規則(EU)No 1107/2009 に記載されている「認可基準」を満たしていな い限り、ハザードの特性評価に利用すべきではない。これらの「認可基準」は同規則には詳述されていないため、農薬 の規制評価を支援するための疫学的研究の最適な計画と報告のために、多くの勧告が考慮されるべきである。さらなる 特定のガイダンスが有用であるが、これは本意見書の ToR の範囲を超えている。システマティックレビューやメタアナリ シス(必要に応じて)などのエビデンス統合技術が有用なアプローチを提供する。これらのツールは、選択基準を満た すすべての個々の研究の結果を組み合わせることで、要約データを生成し、統計検出力を高め、リスク推定の精度を 向上させることができるが、個々の研究の方法論的な欠陥やバイアスを克服することはできない。観察による研究のシ ステマティックレビューやメタアナリシスは、これらのツールが農薬の潜在的なハザード、ばく露シナリオ、ばく露評価の 方法、ばく露-反応特性、リスク特性に関する理解を強化する情報を提供するため、リスク評価に大きな影響を与える 能力を持っている。システマティックレビューもまた、毒性学的な課題に答えるための潜在的なツールと考えられている が、その方法論は、異なるエビデンスの系統に合わせて対応させる必要がある。

研究の評価はベストエビデンス統合の枠組みの中で行われるべきであり、それによって各特定の研究が持つ可能性のあるバイアスの特性と疫学的データベースの全体的な整合性の評価が示される。本意見書は、単一の疫学研究で

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評価すべき研究の質のパラメータと、各パラメータの関連する程度(低、中、高)を報告している。ヒトのデータをバイア スのリスクと品質に関して整理するための第一段階として、3つの基本的なカテゴリーが提案されている。(a)バイアスの リスクが低く、信頼性が高い/中程度、(b)バイアスのリスクが中程度で、信頼性が中程度、(c)バイアスのリスクが高く、 信頼性が低いのは、結果の妥当性を低下させたり、潜在的な因果関係をほとんど解釈できないような重大な方法論的 限界や欠陥があるためである。これらのカテゴリーは、EFSAの有効成分のピアレビューに基づく各エビデンスの信頼 性と妥当性の評価(受容可能、補足的、許容できない)と並行して行うことを意図している。規則(EU) No 1107/2009 ヒ トの健康リスクを適切に記載されている「認可基準」を満たすために、明確なデータ品質基準を満たさない疫学研究の 結果に基づいてリスク評価を行うべきではない。

疫学研究は補完的なデータを提供するものであり、農薬リスク評価のために in vivo の実験動物試験、in vitro のメ カニズムモデル及び in silico 技術から得られるデータと統合することができる(ToR 4)。これらすべてのエビデンスを 組み合わせることで、判断の改善を目的としたヒトの健康リスクの特性評価におけるエビデンスの重み付け(WOE, Weight-of-Evidence)解析に貢献することができる。異なるデータセットは補完的であり、結論を出すことができ、その 結果、1 つのエビデンス系統の別のエビデンス系統との整合性を強化するのに役立つが、それらは個別には不十分で あり、ヒトの健康リスクを適切に特性評価するにあたっての課題となる可能性がある。したがって特にばく露されたヒト集 団で臨床的に発現するまでに数十年かかる可能性がある農薬の慢性的な健康影響については、4 つのエビデンス(疫 学、動物実験、in vitro、in silico)は強力な組み合わせとなる。

最初に検討すべき事項は、対象となる健康影響が、農薬に関する既存の毒性学的・疫学的研究でどれだけカバー されているかということである。既知の健康影響/エンドポイントについて両方のタイプの研究が利用可能な場合、リス ク評価に使用する前に、両方の研究の長所と短所を評価すべきである。利用可能なとトのエビデンス(観察疫学及び監 視データ)、実験的エビデンス(動物及び in vitro のデータ)、非試験データ(in silico 研究)の信頼性が評価されたら、 次のステップでは、これらのデータソースに重み付けを行う必要がある。この意見書では、リスク評価をより適切にサポ ートするために、すべてのエビデンスを全体的な WOE フレームワークの中で考慮する統合的なアプローチを提案して いる。このフレームワークは、ある系統が他の系統よりも優先されるべき時を強調するいくつかの原則に基づいている。 どのデータセットを優先すべきかを決定するために、ヒトのデータと実験データの一致や不一致を評価すべきである。 エビデンスの全体性を評価すべきであるが、データがヒトと実験のどちらから来たものであるかに関わらず、より信頼性 の高いデータがより重要視されるべきである。より困難な状況は、研究結果が一致しない場合である。このような場合に は、相違の理由を検討し、矛盾の生物学的根拠の理解をより深く理解する努力をすべきである。

農薬に関するとトのデータは、標的臓器、用量反応関係、毒性影響の可逆性に関する完全な毒性学的データベースからの外挿に基づいて行われた推定値の妥当性を検証するのに役立ち、基準値の定義に直接的な影響を与えることなく、外挿の過程を再確認するのに役立つ。このように、農薬疫学的データは、根本的な因果関係の可能性を高めるための組織的なツールとして、改訂 Bradford Hill 基準を使用して、利用可能なデータの全体である WOE の一部を形成することができる。

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#### 1. 序章

#### 1.1. 農薬リスク評価におけるとトの健康に関する規制データ要求

先進国の規制当局は、指定された試験プロトコールに基づいて実施される義務づけられた毒性学的研究と、ヒトへのばく露の可能性の推定値に基づいて、登録された各農薬について正式なヒトのリスク評価を行っている。

欧州連合(EU)では、植物保護剤(農薬)(PPP)の上市手続きは、欧州委員会規則第 1107/2009 号<sup>2</sup>で規定されて いる。欧州委員会規則第 283/2013 号<sup>3</sup>及び第 284/2013 号<sup>4</sup>では、有効成分及びその製剤の評価及び再評価のため のデータ要求が定められている。

有効成分の哺乳類毒性に関するデータ要求は、化学有効成分については欧州委員会規則(EU)No 283/2013 の Part A に、ウイルスを含む微生物については Part B に記載されている。農薬有効成分の要求事項に関しては、ヒトの データ使用に関する言及は、異なるエンドポイントに関連する第5章の異なる章で見られる。例えば、ヒト由来物質(ミク ロソームまたは無処置の細胞システム)を対象とした in vitro 代謝試験を含む毒物動態及び代謝に関するデータは、 哺乳類における吸収、分布、代謝及び排泄に関する研究を扱う第5.1章に属し、ヒト由来物質を対象とした in vitro 遺 伝毒性試験は、遺伝毒性試験に関する第5.4章に、ヒトのボランティアにおけるアセチルコリンエステラーゼ阻害などの 特殊な研究は、神経毒性試験に関する第5.7章に記載されている。5.8章では、有効成分に関する補足的な試験や、 薬理学的、免疫学的な試験などのいくつかの特殊な試験について言及している。

農薬の評価プロセスは主に実験研究に基づいているが、ヒトのデータはそのプロセスに関連する情報を追加すること ができる。ヒトのデータに関する要求は、主に規則(EU) No 283/2013 の第 5.9 章「医療データ」にある。これには、偶 発的な職業上のばく露や自傷/自殺の後の医学的報告書や、製造工場の従業員の監視などのモニタリング調査が含 まれる。情報は、国の毒物管理センターからの報告書や、公表文献に掲載されている疫学的研究によって生成され、 報告される。同規則は、ヒトへのばく露の影響に関する「意味のある」情報が入手可能な場合には、ばく露に関する外 挿法の妥当性や、標的臓器、用量反応関係、毒性影響の可逆性に関する結論を導き出すために使用することを要求 している。

規則(EU) No 1107/2009も同様に、「入手可能で、ばく露レベルとばく露期間に関するデータが裏付けされており、 公認の基準に従って実施されている場合、疫学的研究は特に価値があり、提出しなければならない」としている。しかし、 承認または更新プロセス中の有効成分に特化した疫学的研究を実施する義務が申請者にはないことは明らかである。 むしろ、規則(EC) No 1107/2009 によると、有効成分の承認のための書類(ドシエ)を提出する申請者は、「科学的な ピアレビューを受けた公的に利用可能な文献[......]」を提出しなければならない。これは、健康への副作用を扱った有 効成分及びその関連代謝物に関するものであり、書類(ドシエ)提出日前の過去 10 年以内に発表されたものでなけれ ばならない[......]。

特に、農薬に関する疫学的研究は、「規則(EC) No 1107/2009 の下での農薬有効成分の承認のための科学的根拠に基づいた公表文献の提出」(EFSA、2011 年 a)と題する EFSA ガイダンス「政策決定を支援するための食品・飼料安全性評価へのシステマティックレビュー方法論の適用」(EFSA、2010 年 a)の原則に沿って、文献から検索する必要がある。EFSA ガイダンスに示されているように、「有効成分、その代謝物、または植物保護製剤(農薬)のための科学的に査読された公表文献を特定し、選択するプロセス」は、アプローチが体系的な文献レビューに基づいている。

ヨーロッパにおける申請者による疫学研究やより一般的なヒトデータの提出は、特にこれまでに、不完全であったり、 現行の EFSA ガイダンス(EFSA、2011 年 a)に準拠していなかったりすることがあった。これは、特定の EFSA ガイダ ンスに従って(疫学的)文献検索を行うことを義務付けることが比較的最近になって導入(例えば AIR-3 物質に対し)さ

<sup>&</sup>lt;sup>2</sup> 植物保護製剤(農薬)の上市と理事会指令 79/117/EEC 及び 91/414/EEC の廃止に関する 2009 年 10 月 21 日の欧州議会及び理事会の規則(EC)No 1107/2009。OJL 309, 24.11.2009, p. 1-50.

<sup>&</sup>lt;sup>3</sup> 活性物質のデータ要求を定めた 2013 年 3 月 1 日の欧州委員会規則(EU) No 283/2013。 植物保護製剤(農薬)の上市に関する欧州議会の規則(EC) No 1107/2009 に基づく。OJ L 93, 3.4.2013, p. 1-84.

 <sup>&</sup>lt;sup>4</sup> 植物保護製剤(農薬)のデータ要求を定めた 2013 年 3 月 1 日の欧州委員会規則(EU) No 284/2013。
 植物保護製剤(農薬)の上市に関する欧州議会及び理事会の規則(EC) No 1107/2009 に基づく。OJ L 93, 3.4.2013, p. 85-152

れたことによるものであろう(規則 AIR-3: Reg. (EU) No 844/2012;ガイダンス文書 SANCO/2012/11251-rev.4)。

EU における農薬のピアレビュープロセスにおける疫学的データと毒性学的結果の統合評価は奨励されるべきであ るが、まだ不足している。最近の大きな議論となった例としては、グリホサートの評価に関連したものがあり、リスク評価 に疫学的研究を含めるために多大な努力がなされたが、結論としては、これらの研究はグリホサートと健康影響との間 の関連性を示す非常に限定的なエビデンスを提供したに過ぎず、十分なエビデンスは得られなかった。

2,4-D のピアレビューの場合、疫学的データのほとんどはリスク評価には使用されなかった。結論として、欧州の規制 システムの中では、疫学的データが農薬有効成分の承認に影響を与えた例はない。

疫学研究を含む文献検索が義務化され、ガイダンスが整備された現在(EFSA、2011 年 a)、より一貫したアプロー チにより、リスク評価が容易になると考えられる。しかし、規制プロセスにおいて、このような疫学的情報をどのように評価 するかについての枠組みは確立されていない。特に、これらの研究の評価に用いられる古典的な基準は、現在の規制 の枠組みには含まれていない(例:研究デザイン、オッズ比と相対リスクの使用、潜在的な交絡因子、多重比較、因果 関係の評価)。評価報告書草案(DAR)や更新評価報告書(RAR)の作成とピアレビューの過程で、疫学的知見を適 切に使用するための特定の基準や指針が必要である。EFSA ステークホルダーワークショップ(EFSA、2015 年 a)で は、ばく露に関する正確な情報を提供する上で、より強固で方法論的に健全な研究が利用可能になれば、EU におけ る農薬規制の向上が図れると予想している。

もう一つの潜在的な課題は、有効成分の更新プロセスと疫学研究の成果との同期化である。実際、疫学研究の計画、 実施、解析には多くの場合、特にデータの解釈が複雑な場合には相当な時間を必要とする。

#### 1.2. 依頼者から提供された背景と委託条件

2013 年、欧州食品安全機関(EFSA)は、イオアニナ大学医学部が実施した外部科学報告書「農薬へのばく露と健 康影響に関連する疫学的研究に関する文献レビュー」を発表した(Ntzani ら、2013 年)。この報告書は、2006 年から 2012 年の間に発表された疫学研究のシステマティックレビューに基づき、農薬ばく露と調査したあらゆる健康影響(ヒト の健康影響の 23 の主要カテゴリー)との関連性をまとめたものである。特に、農薬ばく露と以下の健康影響(肝臓がん、 乳がん、胃がん、筋萎縮性側索硬化症、喘息、II 型糖尿病、小児白血病、パーキンソン病)との間の統計学的に有意 な関連性が、固定効果及びランダム効果のメタアナリシスによって観察された。

膨大な数の研究論文と解析(6,000 件以上)が利用可能であるにもかかわらず、報告書の著者は、大部分の健康影響については何ら確かな結論を見出すことができなかった。この観察結果は、農薬の使用ととト健康への悪影響の発生との関連性を評価したこれまでの研究と一致しており、そのような疫学研究は多くの限界とデータの大きな不均一性が問題となっていることを認めている。著者らは特に、疫学研究における農薬の広範な定義がメタアナリシスの結果の価値を制限していることを指摘している。また、本報告書の範囲では、農薬ばく露と特定の健康影響との間の詳細な関連付けを行うことができなかった。しかし、報告書では、農薬ばく露との関連性の可能性について、より詳細な結論を出すためにさらなる研究が必要とされる多くの健康影響を強調している。

とはいえ、外部科学報告書の結果は、ヨーロッパで発表された他の同様の研究<sup>5.6</sup>と一致しており、農薬ばく露とヒト 健康影響との関連性について、多くの疑問や懸念を投げかけている。さらに、本報告書の結果は、疫学研究の結果を どのように農薬リスク評価に統合するかについての議論の道を開くものである。このことは、EU 規則 No 283/2013 に 従って疫学的結果を評価する必要がある植物保護製剤(農薬)の承認評価を扱う EFSA のピアレビューチームにとっ て特に重要である。同規則では、申請者は、利用可能な場合には「意味のある」疫学的研究を提出しなければならない とされている。

この科学的意見書では、PPR パネルは、外部科学報告書(Ntzani ら、2013 年)で観察された農薬ばく露とヒト健康

<sup>5</sup> フランス。INSERM レポート 2013。 農薬 – 人に及ぼす影響

<sup>6</sup> 英国。COTレポート2011年。農薬への准職業上ばく露とがん以外の健康影響に関する疫学的文献のシステマティックレビューに関する声明及び COT 報告書 2006。農薬散布と住民や居合わせただけの者 (bystanders)の健康に関する環境汚染に関する王立委員会報告書に関する共同声明。

影響との関連性と、これらの結果が規制上の農薬リスク評価の背景でどのように解釈されるかを議論する。したがって、 PPR パネルは、報告書で収集された疫学研究を体系的に評価し、研究の主要なデータギャップと限界に対処し、関連 する勧告を提言する。

PPR パネルは特に以下を行う。

- 1)利用可能な疫学研究の質と妥当性に関して外部科学報告書で明らかにされたものに基づいて(必ずしもこれに 限定されないが)、ギャップと限界のすべての情報源を収集し、レビューする。
- 2)上記 1)項で特定されたギャップと限界に基づき、調査結果の質、妥当性、信頼性を向上させ、それが農薬リス ク評価にどのように影響を与えるかについて、将来の疫学調査のための潜在的な改善点を提案する。これには、 研究デザイン、ばく露評価、データの質と評価、健康影響の診断分類、統計解析が含まれる。
- 3) 情報及び/または基準が不十分または不足している分野を特定し、リスク評価への適用を改善し最適化するために、農薬疫学的研究をどのように実施するかについての提言を行う。これらの推奨事項には、第1)項で明らかになったギャップと限界に基づいて、ばく露評価(バイオモニタリングデータの利用を含む)、脆弱な集団のサブグループ及び/または対象となる健康影響(生化学的、機能的、形態学的、臨床的レベルでの)の調和を含む。
- 4)評価報告書草案のピアレビューの過程で、疫学的知見を実験毒性学、有害転帰経路(AOP)、作用機序などのデータと統合するとともに、WOEなど、農薬のリスク評価に疫学的知見を適切に利用する方法を議論する。

PRAS ユニットは、リスク評価における疫学的研究の統合を含む EFSA の包括的な科学的分野7への合意に基づく アプローチについて、科学技術委員会に諮る。

#### 1.3. 委託条件の解釈

EFSA は、検討事項(ToR)の中で、2006 年から 2012 年の間に発表された農薬へのばく露ととトの健康影響を関連 付ける疫学的研究のシステマティックレビューの結果のフォローアップについて、PPR パネルに科学的意見書を作成 するよう要請している(Ntzani ら、2013 年)。EU 規則 No 283/2013 によると、疫学的データを農薬リスク評価に統合 することは、EU 承認のための有効成分の DAR と RAR 及び植物保護製剤(農薬)としての使用を目的とした有効成 分のピアレビュープロセスにとって重要であるとされている。

PPRパネルは、委託条件の解釈において、農薬の疫学的研究で明らかになった方法論的限界に対処し、規制上の 農薬リスク評価、特に承認後の物質のリスク評価への利用を容易にするためにどのように改善するかについて、そのよ うな研究のスポンサーに勧告を行うための科学的意見書を作成することになっている。PPRパネルは、実験的な毒性 試験にもその方法論と報告の質に関連した限界があることに留意しているが、これらの限界の評価は本意見書のToR の範囲を超えている。

この科学的意見書は、規制 1107/2009 に基づく農薬の更新時のピアレビュープロセスを支援することを目的として おり、疫学的研究の評価に加えて、なんらかのヒトばく露後の臨床症例や中毒事例(入手可能な場合)がデータ要求と なっている。欧州における農薬へのばく露に関する疫学的データは、有効成分の最初の承認前には入手できない(製 造過程で発生した事故を除いて、その可能性は非常に低いと予想される)ため、DAR に貢献することは期待できない だろう。しかし、他の管轄で有効成分の使用について先行承認を受けている可能性があり、その分野の疫学的データ が有用であると考えられる。EC 規則: (EC) No 1107/2009 では、既存の疫学的研究を検索することが期待される学術 的に査読された公表文献を検索することを要求している。したがって、疫学的研究が有効成分の更新プロセスにおい てより適していることが認識されており、「更新のために提出された書類には、有効成分が指令 91/414/EEC の付録 I に最初に含まれた時から、データ要求の変更や科学的・技術的知識の変化を再確認するために、有効成分に関連す る新しいデータと新しいリスク評価を含めるべきである」という EC 規則 1141/2010 の規定にも準拠している。

PPR パネルは具体的に以下のトピックに取り組む。

<sup>7</sup> 規則(EC)No 178/2002の第28条による。

- 1) 疫学研究の質に影響を与える固有の弱点(利用可能な農薬疫学研究のギャップと限界を含む)と、規制上の農 薬リスク評価との関連性を検討する。これらの弱点にはどのように対処できるか?
- 2)実験動物を用いた古典的な毒性学的研究を補完する疫学的研究は、農薬リスク評価の分野でどのような貢献 が期待できるか?
- 3) 農薬有効成分に特化した方法論的アプローチとして、疫学的研究をどのように適切に活用するかについて、指摘されたギャップや限界をどのように改善するかを中心に議論し、提案する。
- 4)リスク評価の目的で利用可能な疫学的証拠をより良く利用するための実践への再提案と推奨を提案する。疫学的情報と実験毒性学のデータを統合するための方法論を議論し、提案する。

本意見書、特にセクション 2-4 は、科学としての疫学の基礎を論じることを意図したものではない。疫学の科学的側面を深めたいと考えている読者には、疫学の一般的な教科書(例:Rothman ら、2008 年)を読むことを勧める。

本意見書は、EU 規制の背景における農薬疫学研究にのみ焦点を当てており、一般的な科学的観点からではない ことを考慮に入れるべきである。したがって、実験的な毒性試験の実際の限界と弱点については、ここでは触れていな い。

#### 1.4. 追加情報

上記のトピック 1-4(第 1.3 節)に完全に対応するために、疫学的研究の多くの関連するレビュー及び疫学の知識を 持つ他の国内外の機関の経験に注意を払い、疫学を農薬のリスク評価に特に適用した。付録 A ではこれらの研究に 詳細な注意を払い、この分野の理解に建設的に貢献してきた著者の経験に基づいている。また、付録 A では、いくつ かの公表された研究がどれほど役に立たないかを示すために、厳密さに欠けていると批判された公表情報を記録して いる。このような優れた(そしてあまり良くない)実践から得られた教訓は、附属書 A を相互に参照することで本文に組 み込まれている。このようにして、この科学的意見書(Scientific Opinion)は、それにもかかわらずアクセス可能なすべ ての裏付けとなるデータで読者を圧倒することなく、本文の議論を明確に抽出し、効果的に伝えることを目的としている。

さらに、付属書 B には、疫学研究におけるばく露評価のためのツールとしての労働安全衛生戦略におけるヒト生物 学的モニタリング(HBM)の役割をさらに調査し、農薬への職業上ばく露による潜在的な健康リスクの評価に貢献する ために、2015年に EFSA が委託したプロジェクトの主な成果の要約が含まれている(Bevan ら、2017年)。

### 2. 農薬に関する疫学研究の一般的枠組み

ここでは、農薬に関する疫学研究の基本的な要素を紹介し、他のタイプの研究との対比を行う。詳細については、疫 学の一般的な教科書を勧める(Rothman ら、2009 年)。

#### 2.1. 研究デザイン

疫学は、いつ、どこで、どのようにして疾患が発生したかを確認するために、ヒトまたは他の標的種の集団における健 康影響の分布と決定要因を研究する。これは観察による研究や介入研究(すなわち臨床試験)®によって行うことができ、 潜在的なリスク因子へのばく露が異なる研究グループを比較する。どちらのタイプの研究も、実験室よりも管理の行き届 いていない自然環境で実施される。

<sup>&</sup>lt;sup>8</sup> この見解では、「ヒトデータ」には、疫学研究とも呼ばれる観察研究が含まれ、研究者は研究参加者に影響を与えることなく、因子と健康影響との 間の自然な関係を観察している。警戒データもまた、この概念に該当する。これに対して、介入研究(実験研究ともいう)は本意見の対象外であ り、研究者が研究デザインの一部として介入することが大きな特徴である。

自然環境で発生した疾病の事例に関する情報は、ばく露者のみを対象とした症例報告や症例シリーズという形で体系的に記録することも可能である。症例報告や症例シリーズは、ばく露の違いによって研究グループを比較するものではないが、有用な情報、特に高濃度ばく露後の急性影響に関する情報を提供することができ、ハザードの特定に役立つ可能性がある。

無作為化臨床試験では、対象となるばく露が被験者に無作為に割り付けられ、可能な限り被験者は治療法を盲検 化し、それによって特定の治療法へのばく露に関する知識に起因する潜在的なバイアスを排除する。これが介入研究 と呼ばれる理由である。観察による疫学研究は臨床介入研究とは異なり、対象となるばく露が登録された被験者にラン ダムに割り付けられておらず、参加者はばく露について盲検化されていないことが多い。これが観察的研究と呼ばれる 理由である。その結果、無作為化臨床試験は平均的な治療効果のバイアスのない推定値を提供するため、計画の点 で上位にランクされている。

観察による研究におけるばく露の無作為割り付けがないことは、疾患の発生に関連する他のリスク因子がばく露者と 非ばく露者の間で不均等に分布している可能性があるため、重要な課題となる。これは、既知の交絡因子を測定して 説明する必要があることを意味する。しかし、未知の交絡因子は対処できないが、未知の交絡因子または測定されてい ない交絡因子が考慮されずに放置されている可能性が常にある。さらに、観察による研究で研究参加者が現在または 過去のばく露を知らないことが多い、またはこれらを正確に記憶していないことがある(例えば、副流煙、食事摂取量、 または職業上のハザード)という事実は、自己報告に基づいている場合、ばく露の推定値に偏りが生じる可能性がある。 例えば、がん症例と対照者が過去に農薬にばく露されたことがあるかどうかを尋ねられたとき、過去のばく露が両群間 で差がなかった場合でも、がん症例は対照者とは異なるばく露を報告する可能性は低くない。

伝統的に、観察による疫学研究計画は、生態学的研究、横断研究、症例対照研究、コホート研究のいずれかに分類される。このアプローチは、ばく露評価の質とばく露から結果への方向性を評価する能力に基づいている。これらの違いは、研究の質を大きく左右する(Rothman 及び Greenland、1998年; Pearce、2012年)。

- ・生態学的研究は観察研究であり、ばく露、影響(結果)、またはその両方を個人レベルではなく集団レベルで測定し、両者の相関関係を調べるものである。多くの場合、ばく露は集団レベルで測定されるが、健康登録を利用することで、個人レベルでの健康影響(がん、死亡率)を抽出することができる。これらの研究は、直接のばく露評価が困難な場合や、ばく露量の大きな対照が必要な場合(異なる国や職業間のレベルの比較)によく利用される。個人レベルでのばく露及び/または影響がないことを考えると、これらの研究は仮説を立てるのに有用であるが、一般的には、ヒトまたは実験動物を用いたより厳密な計画で結果をフォローアップ(追跡調査)する必要がある。
- ・横断研究では、ばく露と健康状態が同時に評価され、ばく露の程度が異なる群における有病率(または最近の限られた時間における罹患率)が比較される。このような研究では、現在のばく露が疾患の発症につながる関連時間枠ではないかもしれないので、ばく露と疾患の間の時間的関係は確立できない。有病率の高い症例を含めることは、(ほとんどの)横断研究の大きな欠点であり、特に慢性的な長期疾患の場合には注意が必要である。それでも、ばく露と影響が多かれ少なかれ同時に発生している場合や、ばく露が経時的に変化しない場合には、横断研究はリスク評価に有用であるかもしれない。
- ・症例対照研究では、すでに対象となる疾患(例:症例)と診断されている個人の過去のばく露の推定値と、そのような疾患のない同一集団の対照との間の関連を調べるものである。集団ベースの症例対照研究では、症例は十分に整備された集団から得られ、対照は症例が発生した時点で病気にかかっていない集団のメンバーから選ばれる。症例対照研究の利点は、前向き研究に比べてサンプル数、時間、供給源が少なくて済むことであり、ある種のがんのようなまれな疾患を研究する場合には、症例対照研究が唯一の実行可能な選択肢となることが多い。症例対照研究では、ほとんどの場合、過去のばく露は「直接的な」測定に基づいて評価されるのではなく、質問者または自己記入式のアンケートや職務記述書の肩書きや職務歴などの代用手段によって得られた想起など、より確実性の低い測定を介して評価される。症例対照研究は適切なばく露評価を可能にするかもしれないが、これらの研究はばく露を推定する際に想起バイアスに陥りやすい。その他の課題としては、適切な対照の

選択及び適切な交絡因子管理の必要性が挙げられる。

 コホート研究では、調査対象となる集団は、将来のある時点で特定の疾患や健康影響を発症するリスクがある 個人で構成されている。ベースライン時及びその後の追跡調査(前向きコホート研究)では、関連するばく露、 交絡因子及び健康影響が評価される。適切な追跡期間の後、以前に評価された対象となるリスク因子に異なる ばく露を受けた人々の間で、疾患の発生頻度が比較される。したがって、コホート研究は計画としては前向きで あり、対象となるリスク因子や共変量へのばく露の評価は健康影響が発生する前に測定される。したがって、コ ホート研究は、上記の他の計画と比較して、因果関係のより良いエビデンスを提供することができる。場合によっ ては、コホート研究が過去のばく露の推定値に基づいていることもある。このような回顧的ばく露評価は、直接測 定に比べて精度が低く、想起バイアスがかかりやすい。その結果、コホート研究から得られるエビデンスの質は、 ばく露を評価するために実際に使用された方法や共変量に関する情報が収集された詳細のレベルによって異 なる。コホート研究は、比較的一般的な健康影響に関する研究に特に有用である。規模の点で十分な検出力 があれば、比較的まれなばく露と健康影響に適切に対処するためにも利用できる。前向きコホート研究は、異な る臨界ばく露枠を研究するためにも不可欠である。その例として、成人になるまで一定間隔で子供を追跡する 縦断的出生コホート研究がある。コホート研究では、疾患発症前の潜伏期間が長い場合、長い観察期間を必要 とすることがある。このような研究は、実施するには複雑で費用がかかり、追跡調査の損失が生じやすい。

#### 2.2. 母集団とサンプルサイズ

疫学研究の主な強みは、代理種ではなく、結論を出すべき集団の中で病気を研究することである。しかし、全集団を 調査できることは稀であり、その代わりに研究の目的のために参照母集団からサンプルが引き出される。その結果、前 者が後者を正確に反映していない場合、研究母集団で観察された効果量は、母集団で観察された効果量と異なる可 能性がある。しかし、非代表的なサンプルで行われた観察は、そのサンプル内ではまだ有効であるかもしれないが、結 果を一般集団に外挿する際には注意が必要である。

研究のために対象個人をどのように選択するかを決定した後、最小で何人の参加者を登録すべきかを決定することも必要である。研究のサンプルサイズは、十分な統計的検出力を保証するのに十分な大きさでなければならない。標準検出力(感度とも呼ばれる)は80%で、これは、ある研究が、その効果が対象集団に実際に存在するときに、ある大きさの効果を検出する能力を意味する。言い換えれば、解析の結果から正しい結論を導き出せる確率は80%で、それに対応する確率は20%で、間違った結論を導き出して真の効果を見逃してしまう確率である。検出力解析は、与えられたサイズの効果を検出するのに必要な最小サンプルサイズを計算するためによく使用される。小規模サンプルは、非代表的サンプルを構成する可能性が高い。統計的検出力はリスク・インフレーション(risk inflation)と密接に関係しており、小規模または検出力不足の研究から統計的に有意な結果を解釈する際には特別な注意を払う必要がある(付属書Dを参照)。

疫学研究は、実験動物を用いた毒性学的研究と同様に、多くの場合は複数のエンドポイントを調査するように計画さ れているが、臨床試験は単一の仮説、例えば治療の有効性などを検証するために計画され、実施される。この点では、 実験動物の毒性試験プロトコールについては、OECDの農薬に関するガイダンスでは、各投与群に登録する動物の 最小数が規定されているので、同じ研究で試験される他の多数のエンドポイントのいずれに対しても、十分な検出力を 保証することはできない。したがって、疫学研究と実験室研究の両方を実施する際には、研究の検出力を適切に考慮 することが重要である。

#### 2.3. ばく露

ばく露測定の質は、ばく露(用量)と特定の毒性影響との間の因果関係を正確に確認する研究の能力に影響を与える。

実験動物を用いた毒性学的試験では、用量、頻度、期間、経路などの「試験実施計画」が事前に十分に定められており、その実施状況を確認することができる。これにより、例えば90日間の研究では、飼料中に存在する化学物質の目

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標とする(そして確認された)濃度と、試験動物が毎日摂取した飼料の量を掛け合わせることで、経口経路を介して毎日投与された外部ばく露量を把握することが可能になる。また、将来的には、重要な試験で内部ばく露量を決定しなければならない。

農薬の場合、ヒトの観察環境においてはばく露濃度、ばく露経路、ばく露期間が管理されておらず曖昧のため正確 なばく露量を推定することは困難である。

意味のある関連性を調べるためには、ばく露の強度、頻度、期間を測定することが必要であることが多い。ばく露に は、比較的短期間の高濃度ばく露もあれば、数週間から数年にわたる低レベルの長期ばく露もある。急性の高用量の 農薬ばく露の影響は数時間から数日以内に現れるかもしれないが、慢性の低用量ばく露の影響は数年後にならないと 現れないかもしれない。また、病気によっては最小限のばく露で発現することもあろうが、ばく露期間が長くなればその 確率は高くなる。

異なるばく露経路(経皮、吸入、経口)では、吸収と代謝に違いが生じる可能性がある。経皮または吸入が職業上の 環境でばく露される経路であることが多いが、一般集団では経口摂取(食品、水)が農薬ばく露の主要な経路である。ヒ トにおける薬物動態には個人差が存在するため、吸収された外部ばく露量が類似している場合でも、異なる全身ばく 露または組織/器官ばく露をもたらす可能性がある。

#### 2.4. 健康影響

健康影響という用語は、調査中の健康に関連する疾病状態、事象、行動、または状態を指す。健康影響とは、研究 の焦点となる臨床事象(通常は診断コード、すなわち国際疾病分類(ICD)10)または健康影響(すなわち死亡)として 表現されるものである。健康影響データを使用する際には、十分に詳細な症例定義、症例を報告し記録するシステム、 そしてこれらの事象の頻度を示す尺度が必要である。

明確な症例の定義は、どこで、いつ、誰によって診断されたかを問わず、一貫して診断されることを保証し、誤分類を 避けるのに必要である。症例定義には標準的な基準が必要であり、それは臨床症状や徴候の組み合わせであり、時に は感度と特異性が知られている診断検査によって補完されることもある。真の有病率または罹患率を推定するためには、 検査手順全体の検出感度(すなわち、健康状態の悪い人が本当に不健康と診断される確率)を認識する必要がある。

また、臨床基準には、疾患リスクの増加と関連する他の特性(例えば、年齢、職業)も含まれている。同時に、適切に 測定・定義された表現型あるいは困難な臨床結果は、調査結果の妥当性を高める。

疾患登録には、診断、治療、結果に関する患者の臨床情報が含まれている。これらの登録は定期的に患者情報を 更新しているため、疫学研究に有用なデータを提供することができる。死亡率、がん、その他の全国的な健康登録は、 一般的に症例定義の要件を満たしており、母集団内の偶発的な症例に関する(ほぼ)網羅的なデータを提供している。 これらの健康影響は、国民健康統計データベースに記録され、分類されているものの、内容的にまだ改善の余地が 多々あり、また、国ごとに異なる許容診断基準に依存していることも問題である。これは、社会的利益のために有効なデ ータを集積する試みを混乱させる可能性がある。登録データは有意義な解析を可能にするが、データの完全性と妥当 性の程度によっては、適切な推論を行うことを困難にするかもしれない。また、データベースの存続期間中におけるコ ーディング規約の変更は、後ろ向きデータベース研究に影響を与える可能性がある。

疾患状態は一般的に二分変数として表現されるが、順序変数(例えば、重度、中等度、軽度、無疾患)あるいは定量 的変数(例えば、標的臓器における毒性反応の分子バイオマーカーや血圧、脂質、特定タンパク質血清濃度などの生 理学的測定値)として測定されることもある。

データ収集の完全性とその一貫性は、研究の信頼性に大きく寄与する。診断基準、データ保存、有用性の調和は、 疫学研究の質に利益をもたらすであろう。

代替エンドポイント(surrogate endpoint)は、十分に定義された疾患エンドポイント、健康影響指標、一般的な臨床 検査値(反応のバイオマーカー)等の代替として使用される。これらの指標は、臨床事象の原因経路上にあると考えら れる。明白な臨床疾患とは対照的に、このような健康状態の生物学的マーカーは、微妙な不顕性の毒性力学的プロセ スを検出することができるかもしれない。このような健康影響のために、詳細な定量分析プロトコールは、研究室間での 比較や再現を可能にするために特定されるべきである。AOP の使用は、症例定義における違いを強調することができる。

代替健康影響(surrogate outcomes)は付加的な情報を提供するかもしれないが、検査された代替健康影響の適 合性は慎重に評価される必要がある。特に、代替健康影響の妥当性は、その主な使用制限となりうる(la Cour ら、 2010年)。したがって、妥当性が確認されていない代替エンドポイントの採択は避けるべきである。

健康状態が他の方法により、例えば自己記入式のアンケートや電話インタビュー、地域の記録(医療や行政のデー タベース)から得られた場合、あるいは臨床検査のみで収集された場合、これらは基礎となる症例の定義を正確に反映 していることを実証するために検証されるべきである。

#### 2.5. 統計的解析と報告

疫学研究の質を保証するためには、資料、方法、結果を詳細に報告し、適切な統計解析を行うことが重要である。統 計解析については、記述的統計及びばく露ー健康影響の関係のモデル化に分けることができる。

#### 2.5.1. 記述的統計

記述的統計は、ばく露尺度、健康影響、可能性のある交絡因子やその他の関連因子など、研究対象グループの重要な特徴を要約することを目的としている。記述的統計には、しばしば頻度表と調査したパラメータまたは変数の中心傾向(平均値や中央値など)及びばらつき度(分散や四分位数範囲など)の測定が含まれる。

#### 2.5.2. ばく露ー健康影響の関係のモデル化

ばく露ー健康の関係のモデル化は、検討中のばく露と健康影響との間に考えられる関係を評価することを目的としている。特に、この関係によってばく露の量や様式、その他の介入因子にどのように依存しているかを評価することができる。

統計的検定は、科学的研究で発見された結果が偶然の結果として起こった可能性があるかどうかを判定する。これは、個々の所見からの結果を要約し、データのランダムエラーを考慮した後、これらの要約推定値が、例えば、ばく露 群と非ばく露群の間で有意に異なるかどうかを評価することによって行われる。

二分された調査結果については、統計解析によりばく露群と対照群との間で疾患頻度に差があるかどうかを検索する。これは通常、相対的な尺度を用いて行われる。コホート研究における相対リスク(RR)は、ばく露群(または高ばく露 群)と非ばく露群(または低ばく露群)を比較して、ばく露と疾患との関連性の相対的な大きさを推定する。これは、ばく 露群では、非ばく露群(または低ばく露群)と比較して、病気を発症する可能性が高いことを示唆している。オッズ比 (OR)は、一般的に症例対照研究や横断研究における健康影響の指標であり、症例と対照(または横断研究では罹患 者と非罹患者)の間のばく露のオッズ比を表し、しばしば統計的検査で使用される相対的な尺度である。用量反応関 係については、異なるレベルまたは用量のばく露を比較することによって確認できる。連続的な健康影響測定の場合、 結果の平均値や中央値の変化は、分散分析やその他のパラメトリック統計を用いて、異なるばく露レベルにまたがって 検討されることが多い。

統計解析は、観察された変化に統計学的有意差があるか否かを確認することであるが、いずれの結果においても慎重な再検討が必要である(Greenland ら、2016年)。

統計的に有意差がないことの解釈。帰無仮説を棄却できなかったからといって、必ずしも関連性がないということで はなく、関連性の有無はその研究の検出力が十分か否かに起因する。検出力は以下の要因に依存する。

- ・ 標本サイズ:標本サイズが小さいと、たとえ真であっても統計的な有意差を検出するのは困難である。
- ・ 偶然性または非ランダムな要因による個々の反応や特性のばらつき: ばらつきが大きいほど、統計的な有意性 を示すのは難しい。
- 効果の大きさ、またはグループ間の観察された差の大きさ:効果の大きさが小さければ小さいほど、統計的有意 性を示すのは困難である。

統計的に有意な差の解釈。統計的有意差とは、観察された差が偶然性だけによるものではないことを意味する。しかし、そのような結果はまだ慎重に検討する必要性がある。

- ・ 生物学的関連性:帰無仮説の否定は、必ずしも関連が生物学的に意味のあるものであることを意味するわけではなく、関連が因果関係にあることを意味するわけでもない(Skelly、2011年)。重要な問題は、観察された差の大きさ(または「効果の大きさ」)が、生物学的に関連性があると考えられるほど大きいかどうかということである。このように、統計的に有意な関連性は、生物学的に関連性があるかもしれないし、ないかもしれないし、その逆もある。統計的に有意な疫学的結果は「生物学的に関連性がない」として却下されるかもしれないが、統計的に有意でない結果が「生物学的に関連性がある」と判断されることはめったにない。研究者や規制当局は、一般的に使用されている健康影響の指標について、統計的有意性を超えて「生物学的に重要な差が最小である」というエビデンスを求めているケースが増えている。生物学的有意性の関連性を研究デザインや検出力の計算に配慮した上で、統計的有意性と同様に生物学的有意性の観点から結果を報告することは、リスク評価においてますます重要になってくるであろう(Skelly、2011年)。これは、生物学的関連性を考慮する際に考慮すべき一般的な問題と基準を概説した EFSA Scientific Committee のガイダンス文書の対象となっている(EFSA Scientific Committee、2017年a);また、エビデンスを扱うプロセスに関連した三つの主要な段階で生物学的関連性を考慮するためのフレームワークが開発されている(EFSA Scientific Committee、2017年b)。
- ・偶発誤差(ランダムエラー):統計的精度の評価には、研究内の偶然誤差を考慮する必要がある。偶発誤差とは、研究における予見できない部分であり、その部分が偶然性に起因する。統計的検定は、科学的研究で発見された結果が偶然性の結果として発生した確率を決定する。一般的に、研究参加者の数が増えると、中心傾向(例えば 平均値)の推定値の精度(標準誤差として表現されることが多い)が上がり、研究グループ間に実際の差がある場 合、統計的に有意な差を検出する能力が向上する。しかし、少なくとも理論的には、観察された結果が偶然に起因 するものであり、比較されたグループ間に真の違いが存在しないという可能性が常にある(Skelly、2011 年)。多く の場合、この値は 5%(有意水準)に設定される。
- ・ 多重検定:サンプルサイズについて議論する際に前述したように、ばく露ー健康影響の関係のモデル化は、原則として仮説主導型であり、予め何を検索するかを研究目的に明記しておく必要がある。しかしながら、実際には、疫学的研究(及び実験動物を用いた毒性学的研究)では、多くの場合、同じばく露に関連して多くの異なる健康影響を調査している。多くの統計的検定が実施された場合、そのうちの 5%程度は偶然にも統計的に有意な結果が得られることがある。このような複数のエンドポイント(仮説)の検定は、偽陽性結果のリスクを高めるが、これはBonferroni、Sidak、あるいは Benjamini-Hochberg の補正や、他の適切な方法を使用することでコントロールすることができる。しかし、これはしばしば省略される。このように、研究者が同じデータセットを対象に多くの統計的検定を行った場合、実際には何も変化はないのにも拘わらず差があるように結論づけられてしまうことがある。したがって、多くの統計結果は、さらなる検証を必要とする予備的な指標と考えることが重要である。統計的有意性と生物学的有意性に関する EFSA の見解は、統計解析から導き出される仮定は、研究デザインに関連して採択すべきであることに注意を促している(EFSA、2011年b)。
- ・ 効果量の拡大:あまり知られていないとはいえ、バイアス追加の原因は、サンプルサイズが小さく、結果として統計 的検出力が低いことに起因する可能性がある。このあまり知られていないバイアスの種類として知られているのは、 低検出力研究から生じる「効果量の拡大」である。小規模で低検出力の研究では、研究の検出力が意味のある効 果量を確実に検出するには不十分であるため、偽陰性が生じる可能性があることは一般的に知られているが、推 定された効果が統計的閾値(例えば、統計的有意性の判定に使用される一般的な p<0.05 閾値)を通過した場合 に、これらの研究が効果量の誇張(インフレ)をもたらす可能性があることはあまり知られていない。この効果は、効 果量の拡大としても知られているが、これは、「発見された」関連性(すなわち、統計的有意性のある閾値を通過し たもの)を有効化することを目的とした最適下限の検出力を伴う研究から得られる現象であり、観察された効果量が、 人工的かつ系統的に誇張されることを意味する。これは、小規模で検出力の低い研究は、大規模な研究よりも個 人間のランダムな変動(ばらつき)の影響を受けやすいからである。数学的には、結果が統計的に有意であるという

あらかじめ決められた閾値を通過することを条件に、推定された効果量は真の効果量の偏った推定値となり、この 偏りの大きさは研究の検出力に反比例している。例えば、ある試験を何千回も実施した場合、観察された効果量に は広い分布があり、小規模な試験では大規模な試験よりも観察された効果量のばらつきが大きくなるが、これらの 推定効果量の中央値は真の効果量に近いものになる。しかし、小規模で低検出力の研究では、観察された効果 のうち、任意の(高い)統計的閾値を通過するのはごく一部であり、これらは最大の効果量を持つものだけである。 したがって、ランダム変動が大きい小規模で検出力の低い研究では、与えられた統計的閾値を通過した場合、実 際に有意性を重視することにより引き起こされる関連性については、その効果量を過大評価する可能性が高くなる。 このことが意味するものは、小規模研究における有意な研究結果は、効果を誇張して発見することに有利になるよ うに偏っているということである。一般的に、バックグラウンド(または対照または無処置)の割合が低く、対象となる 効果量が小さく、研究の検出力が低いほど、誇張効果量の増大傾向がみられる。

しかし、この現象は、統計的有意性のための「事前スクリーニング」が行われた場合にのみ存在することに注意する ことが重要である。要するに、オッズ比(OR)や相対リスク(RR)のような与えられた量を推定したい場合、統計的有 意性のために一連の効果量を「事前スクリーニング」すると、無効値から系統的に偏った(真の効果量よりも大きい) 効果量が得られるということである。規制当局、政策決定者、その他の人々がこの方法で行動している範囲では、 限りない比較と考えられるものの中から統計的に有意な結果を探し、効果の大きさを評価し判断するために統計的 有意性のある閾値を超えたものを使用しているため、仮定された関連の大きさを誇張した感覚になる可能性が高 い。追加の詳細といくつかの効果量のシミュレーションは、本書の付属書 D で提供されている。

**交絡**は、ばく露と疾病との関係が他のリスク因子の影響、すなわち交絡因子の影響にある程度起因している場合に 発生する。リスク因子が実際に交絡因子として作用するためには、McNamee(2003年)により提示(以下に図示)され、 従来から認識されているいくつかの要件がある。その因子は次のようなものでなければならない。

- ・ 被曝していない人に疾患を引き起こす原因、または原因の代替指標となること。この条件を満たす因子は「リスク 因子」と呼ばれる。
- 病気の存在とは無関係に、調査集団のばく露と正または負の相関があること。調査集団がばく露群と非ばく露群に 分類されている場合、その因子が2つの群で異なる分布(有病率)を持っていることを意味する。
- ・ ばく露と疾患の間の因果関係の経路に中間段階はない。

交絡は、ばく露と疾病の関係を過大または過小に評価する結果となり、2 つのリスク因子の影響が分離されていなかったり、「解放」されていなかったりするために起こる。実際には、十分に強固な場合、交絡はまた、見かけ上の関連性を逆転させることもある。例えば、農業上ばく露は多くの異なるばく露カテゴリーがあるため、農業従事者は、生物学的要因(土壌生物、家畜、農場動物)、花粉、粉塵、日光、オゾンなど、潜在的な交絡因子として作用する可能性のあるものを含む、多種多様なリスク因子に一般集団よりも多くばく露されている可能性が高い。

交絡を制御するために、研究の計画段階または解析段階の両方で、多くの手順が利用可能である。大規模な研究 では、計画段階でのコントロールが好ましいことが多い。計画段階では、疫学研究者は、研究者がコントロールしたい 特徴を共有する個人に研究集団を限定することができる。これは「限定」として知られており、実際には、その特性によ って引き起こされる交絡の潜在的な影響を取り除くことができる。研究者が交絡をコントロールするための計画段階での 2つ目の方法は、「マッチング」によるものである。ここでは、研究者は交絡変数に基づいて個人をマッチングさせ、交絡 変数が2つの比較グループ間で均等に分布するようにする。

計画段階を超えて、解析段階では、層別化または統計的モデリングのいずれかの方法で交絡をコントロールすること ができる。コントロールの1つの手段は、交絡変数(例えば、男性と女性、民族、または年齢グループ)のそれぞれの下 で、関連性が別々に測定される層別化によるものである。別々の推定値は、各層で測定された推定値を重み付けする ことによって、共通のオッズ比(OR)、相対リスク(RR)、または他の効果量を生成するために(必要に応じて)統計学的 に「集積する」ことができる(例えば、Mantel-Haenszel アプローチを使用する)。これは、分析のサンプルサイズを小さ くする代償として行うことができる。比較的簡単に実行できるが、この層別化が複数の交絡因子を同時に扱うことができ ないことに起因する困難が生ずるかもしれない。このような状況では、統計的なモデル化(例えば、多重ロジスティック 回帰)によってコントロールを達成することができる。

上述の研究の計画と解析の段階で交絡をコントロールするために利用可能なアプローチにかかわらず、研究者が計 画で考慮しなかった変数や、データを収集しなかった変数をコントロールすることができないため、この分野で疫学研 究を開始する前に、交絡因子を慎重に考慮することが重要である。

疫学研究は、公表されているかどうかにかかわらず、特定のリスク因子を誤って暗示したり、不適切に否定したりする 可能性のある潜在的な交絡因子を無視しているとして、しばしば批判される。このような批判にもかかわらず、そのよう な可能性のある交絡因子によるバイアスの影響の大きさについての議論が提示されることはほとんどない。交絡因子は、 リスク推定値に実質的な歪みを生じさせるためには、対象となるばく露に強く関連した疾患の比較的強いリスク因子で なければならないことを強調しなければならない。単に交絡の可能性を提起するだけでは十分ではなく、リスク因子が なぜ交絡因子になりやすいのか、その影響がどのようなものなのか、そしてその影響が結果の解釈にとってどれほど重 要なのかを説明する説得力のある議論をしなければならない。強い相対リスクは測定されていない交絡因子によるもの である可能性が低いのに対し、弱い交絡因子は、研究者が解析で測定または管理していない変数による残存交絡因 子によるものである可能性があるため、相対リスク(RR)、オッズ比(OR)、リスク比、回帰係数などで測定される交絡の 大きさを考慮することが重要である(US-EPA、2010年b)。

効果修飾。
とトの健康に対する農薬及びその他の化学物質の影響は、すべての個人で同一であるとは考えにくい。 例えば、ある特定の有効成分が成人の健康な被験者に及ぼす影響は、乳児、高齢者、妊婦に及ぼす影響と同じでは ない可能性がある。このように、ある化学物質にばく露された場合、ある集団の一部(サブセット)が感受性が高いことか ら疾患を発症する可能性が高くなる。このため、「脆弱な小集団」という用語が使用されているが、これは子供、妊婦、高 齢者、重病歴のある人に加え、環境化学物質へのばく露による特別な健康リスク(薬物代謝酵素、トランスポーター、ま たは生物学的標的の遺伝的多型のため)の対象となると同定された小集団を含む。平均効果とは、あるばく露の影響 をすべての小集団で平均化したものである。しかし、様々な小集団間の関連の強さには不均一性があるかもしれない。 例えば、化学物質 A へのばく露と健康影響 B との間の関連の程度は、健康な成人よりも子供の方が強く、また、ばく露 時に防護服を着用している人や遺伝子型の異なる人には同様な影響は見られないかもしれない。もし不均一性が本当 に存在するのであれば、全体的な関連性を示す単一の要約尺度は意味をなさず、誤解を招く可能性がある。不均質 性の存在は、様々な小集団における因子と効果の間に統計的に有意な相互作用があるかどうかを検定することによっ て評価される。しかし、実際には、これは大きな標本サイズを必要とする。

関連因子によって定義された小集団での効果を調査することは、対象となるリスク因子のヒトの健康への影響についての知識を前進させるかもしれない。

#### 2.6. 研究の妥当性

例えば、農薬ばく露と健康影響の間に統計的に有意な関連が観察された場合、またはそのような有意な関連が観察 されなかった場合には、真の関連を歪めたり、その解釈に影響を及ぼす可能性のある要因を評価して、調査研究の妥 当性も評価する必要がある。これらの不完全性は、ばく露と疾病の間の関連性を(系統的に)誤って推定することになる 系統的な誤差の原因に関係している。さらに、単一の研究から得られた結果は、病気を発症するリスクのある他の集団 で実施された独立した調査で再現された場合、より高い妥当性を持つことになる。

時間的シーケンス(順序)。因果関係の断定は、推定される効果に先行する原因を時間的に関与させなければならない。Rothman(2002年)は、時間性を真に因果関係がある唯一の基準と考え、時間性の欠如は因果関係を排除する。疫学的関連の時間的順序は、時間的にはばく露が結果(効果)に先行する必要性を示唆しているが、ばく露の測定が結果の測定に先行する必要はない。この要件は、ばく露が後ろ向きに評価される場合(症例対照研究)や、結果と同時に評価される場合(横断研究)よりも、前向き研究の計画(すなわちコホート研究)では容易に満たされる。しかし、前向き研究においても、疾患の発症が遅かったり、初期の疾患形態が測定しにくかったりすると、原因と結果の時間的な順序や時間的な方向性を確認することが困難になることがある(Höfler、2005年)。

研究の妥当性については、研究対象集団からより広い集団への結果の一般化可能性も考慮しなければならない。

前述したランダムエラーは精度の問題と考えられ、サンプリング変動の影響を受けるが、バイアスは妥当性の問題と考 えられている。より具体的には、バイアスの問題は一般的に、正しい母集団パラメータが推定されているかどうかに影響 を与える研究デザインまたは研究分析における方法論的な不完全性を伴う。バイアスの主なタイプには、選択バイアス、 情報バイアス(想起バイアス、質問者/オブザーバーバイアスを含む)、交絡因子がある。追加の潜在的なバイアスの 発生源は、すでに述べた効果量の規模である。

選択バイアスは、被験者を研究に参加させるために使用された手順や方法、被験者が研究から外れる方法や、そう でなければ研究への継続的な参加に影響を与える結果として発生する妥当性に関する系統的な誤差に関係している。

典型的には、このようなバイアスは、症例対照研究において、疾患に基づいて被験者を含める(または除外する)こと が、研究対象となる前のばく露状態と何らかの形で関連している場合に発生する。一例としては、ばく露と健康影響との 間に関連性が疑われることに対する初期の広報やメディアの注目が、ばく露を受けた人はばく露を受けていない人に 比べて優先的に診断される傾向があるかもしれない。選択バイアスはまた、コホート研究においても、例えば、研究から 外れた人(追跡調査に参加できなくなった人、離脱した人、無回答の人)と残された人の状態が異なる場合のように、ば く露群と非ばく露群が真に比較可能でない場合に発生しうる。また、横断研究では、生存者のみを研究対象とする選択 的生存により、選択バイアスが生じることがある。このようなタイプのバイアスは、一般的に研究の慎重な計画と実施によ って対処できる(第4節、第8節も参照)。

「健康労働者効果」(HWE)は、一般的に認識されている選択バイアスであり、職域疫学研究で起こりうる特定のバイ アスを示すものである:労働者は、労働力として雇用される必要があるため、一般集団からの個人よりも健康である傾向 があり、そのため、一般集団から得られた集団ベースのサンプルよりも好ましい健康状態を持つことが多い。このような HWE バイアスは、観察された関連性が真の効果に比べて隠されたり、軽減されたりすることがあり、その結果、化学物 質やその他の有害物質にばく露された労働者の死亡率や罹患率が低く見えることがある。

**情報バイアス**とは、ばく露または健康影響に関する情報が異なる研究グループから得られる方法に系統的な違いがあり、その結果、研究で測定される1つ以上の共変量に関して不正確な情報が得られたり、測定されたりする場合の系統的な誤差のことである。情報のバイアスは、結果として、ばく露または疾病状態のいずれかに関して誤った分類につながり、ORやRRのような疫学的効果の大きさの尺度にバイアスが生じる可能性がある。

ばく露状態の誤分類は、不正確、不十分、または不正確な測定値、被験者の不正確な自己申告、またはばく露デー タの不正確なコーディングに起因する可能性がある。

疾患状態の誤分類は、例えば、検査室のエラー、検出バイアス、データベース内の疾患状態の不正確な、または一 貫性のないコーディング、あるいは不正確な想起から生じることがある。想起バイアスは情報バイアスの一種であり、ば く露状態(またはその逆)に応じて疾患状態の報告が異なる場合の系統的な誤りに関係している。質問者・バイアスは、 質問者が個人のばく露状況を認識している場合に発生するもう一つの情報バイアスで、ばく露グループ間で意図して いるかどうかに関わらず、ばく露グループ間で異なる疾患状況に関する回答を求めてしまうことがある。なぜなら、病気 の被験者は、病気ではない被験者に比べて、より早い時期に発生したばく露を思い出す可能性が高いからである。こ れは、何らかの効果測定において帰無値(ばく露と疾病の間に関係がないという)から遠ざかるバイアスにつながる。

重要なことに、上述のような誤分類は、「差がある(differential)」場合と「差がない(non-differential)」場合があることである。これらは、(i)真にばく露されている(または病気にかかっている)人が、真にばく露されている、または病気にかかっていると正しく分類される度合いと、(ii)真にばく露されていない(または病気にかかっていない)人が、そのように正しく分類される度合いに関係している。前者は「感度」として知られているが、後者は「特異性」と呼ばれ、これらの両方がバイアスの存在と可能性のある方向性を決定する役割を果たしている。差異的誤分類とは、他の変数の値に依存しない誤分類を意味する。

疫学的観点から重要なことは、誤分類バイアス(差異的か非差異的か)は、そのようなばく露を分類するために使用された研究方法の感度と特異性に依存し、特定の(限定された)条件の下でバイアスの方向に予測可能な影響を及ぼすことができるということである。すなわち、研究の方法や解析の知識に基づくバイアスの方向性を特性評価する能力は、考慮される疫学的効果量(OR、RR など)が真の効果量の過小評価か過大評価かを政策決定者が判断することができ

るから、規制当局の政策決定に有用である。非差異的な誤分類バイアスが帰無値への予測可能なバイアスをもたらす (したがって、体系的に効果量を過小予測する)と一般的に想定されていますが、これは必ずしもそうではない。また、 誤分類は非差異的であるという疫学研究で時々みられる一般的な仮定(これは、非差異的な誤分類バイアスが常に帰 無(null)に向かっているという仮定と対になっていることもある)は、必ずしも正当化されているわけではない(例えば、 Jurekら、2005 年を参照)。

測定されていない交絡因子が結果に影響を与えると考えられる場合、研究者は感度分析を実施して、影響の範囲と その結果として生じる調整された効果測定値の範囲を推定すべきである(US-EPA、2010 年 b)。しかし、定量的感度 (またはバイアス)分析は、多くの疫学研究では一般的には行われておらず、ほとんどの研究者は、論文の考察で様々 な潜在的なバイアスを定性的に記述している。

疫学研究者は既知ではあるが測定されていないリスク因子によるばく露の誤分類や選択バイアスなどのバイアスの 影響を推定したり、見落としや考慮されていない交絡因子が観察された効果の大きさに及ぼす影響を示すために感度 分析を実施することが推奨されている(Lash ら、2009 年;Gustafson 及び McCandless、2010 年)。感度分析は、リ スク評価目的で疫学データをレビューする際の基準リストに組み込まれるべきである。

#### 3. 農薬に関する利用可能な疫学研究の主な限界事項

#### 3.1. EFSA 外部科学研究報告書の著者が指摘した限界

EFSA 外部科学報告書(Ntzani ら、2013 年;付属書 A に要約)では、多様な健康影響を調査する疫学的研究が 多数報告されている。疫学的証拠を体系的に評価する努力の中で、多くの方法論的限界が強調された。これらの限界 の存在下では、確固たる結論を導き出すことはできなかったが、疫学からの裏付けとなるエビデンスが存在する結果は、 今後の調査のために強調された。識別された主な限界は以下の通りである(Ntzani ら、2013 年)。

- 前向き研究の欠如と、バイアスがかかりやすい研究デザイン(症例対照研究と横断研究)の頻繁な使用。さらに、評価された研究の多くは、十分な検出力を持っていないようにみえる。
- 少なくとも疫学の他の多くの分野の疫学と比較して、詳細なばく露評価が欠如している。特定の農薬ばく露と混合ばく露に関する情報は、多くの場合、不足しており、適切なバイオマーカーはほとんど使用されていない。代わりに、多くの研究では、アンケート調査(多くの場合、妥当性が確認されていない)によって評価されたばく露の大まかな定義に頼っていた。
- ・結果評価の不備(おおまかな結果の定義、自己申告性の健康影響または代替健康影響の使用)。
- ・報告と解析の不備(効果推定値の解釈、交絡因子のコントロール、多重検定)。
- ・ 選択的な報告・出版バイアスと、その他のバイアス(例:利害の対立)。

各研究結果の中で観察された結果の不均一性は、しばしば大きなものであった。しかし、不均一性は常にバイアスの結果であるとは限らず、本物である可能性があり、先験的に定義されたサブグループ解析やメタ回帰を考慮することは、エビデンス統合の努力の一環であるべきである。農薬ばく露に関して特に重要な職域研究もまた、健康労働者効果の影響を受けやすく、このバイアスによって労働者の罹患率と死亡率が一般集団よりも低くなっている。健康労働者効果は、雇用期間と追跡調査の期間が長くなるにつれて低下する傾向がある。

+分な統計力を持ち、農薬ばく露の詳細な定義、多くの健康影響に関するデータ及び明白性な報告を備えている 研究は、農業健康調査(Agricultural Health Study:AHS)や他の同様の研究を除けば、稀である。これらの方法論 的限界のいくつかは、農薬ばく露研究に限定されたものではなく、最も重要なことは、疫学的には特異的なものではな く、動物試験を含む他の特異的な研究分野でも観察されていることに注意することが重要である(Tsilidisら、2013年)。

EFSA の外部科学報告書には様々に定義された広範囲の農薬が記載されているが、この情報を研究間で調和させることは困難である。研究間での結果の不均一性は、同質性と同じくらい有益な情報になるが、情報は、反復を評価したり、要約効果量を計算したりできるように調和されている必要がある。これは、真の不均一性がある場合、異なる研究をプールすることができないことを意味するものではない。単一の研究からは限られた結論を導き出すことができるのみである。それにもかかわらず、報告書では、さらなる検討と調査に値する農薬と健康影響との間の多くの関連性が強調

されている。興味深いのは、公表されている文献のかなりの割合が EU 及びほとんどの先進国で使用が認可されてい ない農薬に焦点を当てているという事実である(例えば、DDTとその代謝物のみに焦点を当てた研究は、対象となる研 究の10%近くを占めている(Ntzaniら、2013年)。これらの研究は、残留農薬として残留している可能性があることや、 開発途上国で使用され続けていることから、まだ適切であるかもしれない。また、報告書では、約5年の期間にわたる あらゆる健康影響に関連した疫学的証拠に焦点を当てている。この報告書は、農薬ー健康影響の関連性の疫学的評 価の分野を記述する上で有用なものではあるが、特定の疾患と農薬の問題に完全に答えることはできない。農薬ばく 露に関連する疾患エンドポイントのより詳細な解析が必要であり、そのような情報が入手できる場合には、EFSAの外 部科学研究報告書でカバーされている期間よりも前に発表された研究も含めるべきである。

#### 3.2. 研究デザインの限界

倫理的な理由から、EU では低用量の農薬ばく露の安全性を試験するための無作為化比較試験は認められていない。したがって、ヒトにおける潜在的な有害健康影響に関する情報は、観察による研究を用いて抽出する必要がある。

潜伏期間の長い疾患では、ある時点でのばく露量を測定しても、そのような疾患の発症に必要な長期ばく露量を正確に再現できない可能性がある。これは、生物学的サンプル中の濃度が一定ではなく、頻繁に変動する非持続性農薬の場合に特に重要である。したがって、尿サンプル中の単一の測定値と長期の潜伏期間の結果との間に関連性があると断定する研究は、慎重に解釈されるべきである。

Ntzani の報告書で検討された 795 件の研究のうち、38%が症例対照研究であり、32%が横断研究であった。その 結果、農薬ばく露による潜在的な有害健康影響のエビデンスは、少なくとも潜伏期間が長い結果については、前向き な計画を欠いた研究に大部分が基づいている。横断研究では方向性を評価することができず、観察された関連性はし ばしば逆因果関係(病気はばく露によって引き起こされたのか、それとも病気がばく露に影響を与えたのか)をもたらす 可能性がある。逆因果関係は多くの疫学の分野で横断研究の潜在的な問題であるが、農薬疫学では、ほとんどの場 合、病気が農薬へのばく露を引き起こすことはほとんどないため、問題にはならない。

いくつかのがんなどのまれな転帰に対しては症例対照研究が頻繁に用いられるが、その主な限界は、想起バイアス がかかりやすく、ばく露の後ろ向き評価に頼らなければならないことである。しかしながら、特にまれな転帰に対しては 有用な情報を提供することはできる。症例対照研究と前向き研究の結果が一致するかどうかを調べることが重要である。 例えば、トランス脂肪酸の摂取量と心血管疾患との関連を調べるために実施された研究(EFSA、2004 年)では、症例 対照研究と前向き研究の両方で一貫して正の関連が報告されている。2 つの研究デザイン間の効果推定値は控えめ な効果量が報告された前向き研究とは系統的に異なるが、どちらの研究デザインも同様の結論に達した。農薬に関し ては、研究デザインにかかわらず、パーキンソン病と農薬ばく露との間の関連の大きさについては、同様の値が観察さ れている(レビューは Hernández ら、2016 年)。

#### **3.3.** 研究対象の妥当性

個人がばく露される農薬の環境的に適切な用量は、動物モデルで観察された毒性を誘発するのに必要な用量よりも 低いため、関連する毒性影響は、小集団の感受性の違いとの関連で理解する必要がある。潜在的に脆弱な集団は、 健康な個人よりも低用量の農薬へのばく露に対してリスクが高く、時にはばく露の敏感な時期にばく露されることもある。 これは遺伝的感受性の場合であり、これはリスク評価のために説明されるべき重要な要因である(Gómez-Martín ら、 2015 年)。遺伝的感受性は、毒物動態に影響を与える機能的な遺伝的多型(例えば、異物代謝酵素及び膜トランスポ ーターをコードする遺伝子)及び/または毒力学に影響を与える機能的な遺伝的多型(例えば、異なる受容体遺伝子 多型)に大きく依存する。この遺伝的多様性は、妥当な科学的仮説に基づいて考慮されるべきである。

さまざまな障害、特に神経変性疾患(パーキンソン病、アルツハイマー病、筋萎縮性側索硬化症)は、環境因子(例 えば農薬)へのばく露と結び付けられてきたが、多くの場合、病気の遺伝子構造は考慮されていない。特定の集団では、 特定の遺伝子変異の有病率は5-10%に達し、時には症例の20%を超えることもある(Gibsonら、2017年)ので、農薬 ばく露とこれらの疾患の関連性は、研究対象となる集団内の遺伝的構造によって大きく影響を受ける可能性がある。こ れらの疾患の多くの効果量が小さいことを考えると、研究デザインでは考慮されていない特定の遺伝子の根本的な効果が、疾患リスクの推定値を修正する可能性がある。したがって、農薬ばく露との関連は、一連の神経変性疾患に関連することが知られている一般的な遺伝的変異に照らして評価する必要があるかもしれない。しかし、遺伝的変異はそれ自体が人々を農薬ばく露の増加に向かわせるものではない。

特に注目すべきサブグループは子供であり、彼らの代謝、生理、食生活、環境化学物質へのばく露パターンは成人 とは異なり、有害な影響を受けやすくなるからである。生物学的感受性の窓口はほとんどの場合不明のままであり、メカ ニズムによって異なると予想される。性別に基づく感受性は、農薬に関連した生殖毒性や内分泌かく乱の場合にも考 慮する必要がある。これらのサブグループは現在リスクアセスメントの過程で考慮されているが、追加的な保護を提供 するためには、より注意を払う必要があるかもしれない。

#### 3.4. ばく露評価における課題

農薬に関する疫学研究の主な限界は、ばく露評価の不確実性に由来する。現在認可されているほとんどの農薬は、 消失半減期が短い傾向があり、作物や季節に応じて様々な製剤を散布しなければならないという事実もその限界に含 まれている。その結果、正確な評価には、これらの非難分解性化学物質の間欠的な長期ばく露を把握し、個々の農薬 へのばく露を定量化する必要がある。

多くの研究では、大規模な農薬群に共通する尿中の非活性代謝物(例えば、有機リン酸塩の場合はジアルキルリン酸塩、ピレスロイドの場合は 3-フェノキシ安息香酸、ネオニコチノイドの場合は 6-クロロニコチン酸)を測定することで内部ばく露を評価している。これらのデータを、以下の理由からリスクを推測するために利用すべきではない。(a)これらの代謝物の一部は、親化合物を摂取するのではなく、食品やその他の供給源から事前に生成された代謝物を摂取することで直接ばく露を反映する可能性があり、(b)異なる親化合物農薬の効果は桁違いに異なる可能性があるからである。そのため、これらの尿中代謝物に基づく HBM データは、実際の農薬ばく露量を示す他のデータと組み合わせない限り、役に立たない可能性がある。

理想的には、内部ばく露量を示すバイオマーカーを使用して個人レベルでばく露量を定量化する必要がある。利用 可能なバイオマーカーのほとんどは短期間(数時間または数日)のばく露を対象としており、長期にわたって複数のサ ンプルを収集するコストと困難さを考えると、多くの研究では外部ばく露量として定量化されている。外部ばく露量の定 量的な推定には、ばく露の頻度と期間の両方を考慮する必要があり、グループレベルではなく個人レベルで行うことが 望ましい。多くの場合、外部ばく露は以下のような代理指標を用いて定量化される。

- 一般的な農薬への潜在的ばく露または実際の使用に関連する、対象者または親族が報告した仕事、職種、作業、 その他のライフスタイルの習慣。
- 特定の製品または製品群の取り扱いと、既存の農薬の記録や日誌を通じて文書化された、または栽培された作物 から推定された、これらへの潜在的なばく露。
- ・環境データ:環境農薬モニタリング、例えば水中、ばく露場所と考えられる特定の地理学上の地域からの距離及び /または居住期間。

多くの場合、これらの代理指標測定は、質問者によるものでも、自己申告に基づくものでもよいアンケート調査を用い て記録される。しかし、アンケートデータはしばしば個人の想起と知識に依存しており、想起バイアスや質問者や被験者 によってもたらされるバイアスの影響を受ける可能性がある。これらのバイアスの原因は、アンケートがバイオマーカーに 対して検証されていれば、ある程度定量化することができる(つまり、個々の質問が参加者のサンプルにおけるバイオマ ーカー濃度をどの程度予測しているか)。ばく露が後ろ向きに評価された場合、明らかな理由により、想起の精度が損 なわれ、検証が不可能になる可能性が高くなる。ばく露が記録に基づいて評価される場合も、例えば記録が不完全で あったり、不正確であったりすることで、同様の困難が生じる可能性がある。

これまでの多くの研究では、ばく露の持続時間が累積ばく露の代用として使用されることが多く、ばく露は時間的に 均一で連続的であると仮定している(例えば、雇用期間)が、農薬の場合はこの仮定に疑問を呈しなければならない。 一部の化学物質ではばく露パターンはかなり一定であるかもしれないが、市場に出回っている多数の農薬のばく露は、 季節や個人用保護具(PPE)、作業習慣によって異なり、多くの場合、使用の反復性は高くない。個人のレベルでは、 ばく露量は日ごと、時間ごとに異なることがあり、多くの場合、複数の農薬が関与している。この時間的変動性は、生物 学的半減期が短い農薬の全身ばく露において特に高い変動性をもたらし、長期にわたって個人のばく露に単一または 少数の測定値を外挿する際にかなりの不確実性をもたらす可能性がある。したがって、ばく露の推定値を改善するため には、時間をかけて多くの測定を繰り返すことが必要になるかもしれない。

#### 3.5. 不適切な、あるいは検証されていない健康影響のサロゲート

疫学研究では自己申告による健康影響が頻繁に用いられているが、その理由は、大規模なサンプルや限られた資金を用いた研究では、回答を検証することが困難であることなどが挙げられる。多くの研究では、自己報告された影響と医療記録との一致について調べられているが、このような指標の検証が不足しているために、特に大規模な集団ベースの研究では、誤分類につながる可能性があり、発見された関連性の信頼性を損なう可能性がある。

臨床的に明らかになった結果に依存すると、ばく露から疾患への毒力学的連続して進行したが、まだ明らかな臨床 的疾患状態に達していない人が、疾患を持っていないと誤分類される可能性が高くなる可能性がある(Nachman ら、 2011 年)。そのため、ばく露後の臨床症状の発現が遅れると、不適切な時期に臨床評価だけが用いられた場合、過小 申告の原因となることがある。

発がん性の場合、無症状であるが、腫瘍性状態に進行する可能性のある前腫瘍性病変として評価されている例があ る。これは、AHS における農薬ばく露と関連している有効性が未決定である単クローン性ガンマグロブリン血症 (MGUS)の例であり(Landgren ら、2009 年)、この状態は悪性多発性骨髄腫に進行するリスクが年平均 1%である (Zingone 及び Kuehl、2011)。しかし、MGUS が悪性多発性骨髄腫に進行するかどうか、いつ、どのように進行する かを予測することは困難である。動物実験では、農薬ばく露が前がん病変のリスクと関連している可能性があることを示 す研究があるので、前がん病変とがん病変の両方の転帰を組み合わせた疫学的解析を行うことで、そのような解析の 検出力を高めることができるかもしれない。

代替健康影響は、臨床的に関連する転帰に代わる注目される選択肢である。なぜならば、同じ疾患の様々な代替健 康影響が存在し、それらの健康影響はより早く発生したり、評価が容易であったりして、診断までの時間を短縮できるか らである。しかし、有効な代替エンドポイントは、因果関係を予測し、対象となる健康影響を正確に予測するものでなけ ればならない。さらに、これらの代替指標は農薬の作用機序に関連していなければならず、その予測性を裏付けるため に、確立された毒物学的エンドポイントに固定されていなければならない。代替指標は健康影響と相関があるかもしれ ないが、健康影響に対する要因の効果を捉えていないかもしれない。これは、代替指標が臨床健康影響の因果関係ま たは強く関連しているのではなく、付随する要因に過ぎないため、臨床健康影響の予測にはならない可能性があるから である。このように、代替健康影響の妥当性は、サロゲートを使用する上での大きな制限となりうる(la Cour ら、2010 年)。

しかし、有害健康影響を直接測定したのではなく、代わりに有効な代替指標を用いた疫学研究に基づいて、規制上の政策決定を下すことができるかどうかについては、懸念がある。代替エンドポイントとしての代替指標の使用は、臨床的に意味のある影響を予測する上での信頼性を立証する十分なエビデンスがある場合にのみ検討されるべきである。

#### 3.6. 統計解析と結果の解釈

農薬と健康影響との関係に関する疫学的文献にみられる統計解析と科学的知見の解釈は、他の分野で報告された 疫学研究と大きく異なるものではない。したがって、2.5節で示した疫学研究の利点と限界は、農薬に関する疫学研究 にも適用される。

農薬の疫学研究のいくつかの特徴は以下の通りである。(a) 農薬へのばく露を評価する際の測定誤差の存在下での適切な統計解析を行うことが少ないこと、(b) ばく露ー健康影響の関係に影響を及ぼす可能性のある他の重要な因子に関する情報が不十分なことである。これらの特徴については、次の段落で詳しく説明する。

a) 測定誤差のある統計解析

ばく露量を正確に測定するための困難さは、栄養疫学や環境疫学などの疫学研究の多くの分野で頻繁にみられる。 制御された実験室での実験環境の外で、短期及び長期のばく露を評価することが容易ではない。大規模集団では、個 人は様々な期間、様々な強度で様々な形で様々な薬剤にばく露されている。

しかし、栄養疫学や環境疫学とは異なり、農薬疫学では、測定誤差を適切に考慮した統計解析が広く利用可能であ り、このテーマに関する膨大な文献があるにもかかわらず、これまでのところほとんど利用されていない。その直接的な 結果として、これらの統計的手法が利用されていたとしても、推論的結論が正確で、精密なものではなかった (Bengtson ら、2016年; Dionisio ら、2016年; Spiegelman、2016年)。

b)その他の重要な関係因子に関する情報

対象となる結果に影響を与える可能性のある他の関連因子を特定して測定することは、科学のすべての分野で繰り 返し起こる重要な問題である。例えば、ある薬が平均的に病気を効果的に治すということを知っていても、その薬が子 供や妊婦に有害であるかもしれない。年齢、妊娠、その他の特性が薬の有効性に影響を与えるかどうかは、医師、患者、 製薬会社、医薬品承認機関にとって重要な情報である。

農薬疫学は、可能性のある関連因子を慎重に特定し、正確に測定し、徹底的に評価し、ばく露と健康結果の関係に おけるそれらの役割を評価する機会を提供している。最も多くの場合、関連因子は潜在的な交絡因子としてスクリーニ ングされている。交絡因子の影響が検出された場合には、統計解析で補正する必要があった。このことは、すでに収集 されたデータや今後の研究で収集される可能性のあるデータを再検討することで、この重要な問題を明らかにするため の更なる調査の余地を残している。農薬の文献における統計的手法は、主に二項確率やハザード回帰モデルなどの 基本的な回帰分析の標準的な応用に限定されてきた。潜在的に有用な解析アプローチ、例えば、傾向スコアマッチン グ、媒介分析、因果推論などは、農薬疫学に役立つであろう(Imbens 及び Rubin、2015 年)。

#### 4. 農薬リスク評価のための将来の疫学研究への再検討案

このセクションでは、利用可能な農薬の疫学的研究の評価方法と、規制目的に役立つようにそのような研究を改善 するための提案を取り上げることを目的としている。

疫学的データの潜在的な規制上の利用の可能性を検討する際に、農薬ばく露と健康影響に関する既存の疫学的 研究の多くは、個々の有効成分の評価においてその価値を制限する様々な方法論的限界や不完全性に悩まされてい る。農薬ばく露と健康影響に関する疫学研究は、半定量的なデータを生成するか、予測モデルのアウトプットに関して 定量的なリスク評価との関連性を高めることが理想的である。これにより、疫学的な結果は、農薬のリスク評価に一般的 に用いられる定量的なリスク評価に匹敵する言葉で表現できるようになる。このような疫学データを予測モデルと比較し てリスク評価を行う際に、どのように考えればよいのかという疑問が生じる。現行の農薬疫学研究の結果では、正確に測 定された定量的な用量反応関係はほとんど達成されていない。

農薬ばく露と健康影響に関連した疫学的証拠の質、信頼性、妥当性は、(a)個々の研究の質及び(b)利用可能な すべての研究から得られた複合的なエビデンスの評価を改善させることによって向上させることができる。

#### 4.1 疫学研究の質の評価と報告

リスク評価に使用するために文献から疫学研究を選択する際には、疫学研究の質と関連性を考慮する必要がある。 この研究の質は、次のようにして高めることができる(US-EPA、2012年;Hernándezら、2016年)。

- a) ばく露の適切な評価、特に個人レベルでのバイオマーカー濃度が用量反応評価を可能にする方法で報告されて いること。
- b)合理的に有効で信頼性の高い健康影響評価(よく観察された臨床症状または有効な代替指標)。
- c)交絡変数(複数の化学物質へのばく露を含む)を適切に考慮していること。
- d)サブグループ解析の実施と報告(例えば、性別、年齢、民族による層別化)。

生物医学的研究が多様な限界の対象となり、その限界に苦しむことは広く受け入れられている。農薬に関する疫学

研究の計画、実施、解析における弱点の評価は、誤解を招く可能性のある結果を特定し、信頼性の高いデータを特定 するために不可欠である。

ガイドラインやチェックリストは、特定の分野での最良の行動に向けて導く一連のルールや原則を提供することで、個 人が特定の基準を満たすのを助けるものである。疫学的証拠の評価を助けとなるためにいくつかのツールやガイドライ ンが開発されているが、農薬に関する研究を評価するための特定のツールは存在しない。これらの研究には、特定の 注意を必要とするばく露評価に関する特別な考慮事項があるが、既存の研究を批判的に評価するための標準的な疫 学的手法を適用できる可能性がある。現行の報告ガイドラインは通常、研究にバイアスをもたらした可能性のある側面 に焦点を当て、調査研究中に何が行われ、何が発見されたかを完全かつ明確に説明するために必要な最低限の情報 を規定している(Simera6、2010年)。

観察による疫学研究の品質評価のために、Newcastle-Ottawa スケール(NOS)や Research Triangle Institute (RTI)のアイテムバンクなど、多くのツールが特別に計画されている。後者は、化学物質ばく露の疫学研究のバイアスのリスクと精度を評価するための 29 の質問のチェックリストからなる実用的で検証済みのツールである。また、バイオモニタリング、環境疫学、短命化学物質(BEES-C)は、バイオモニタリングを用いて短命化学物質を評価する疫学研究の質を評価するために開発されたが(LaKind ら、2015 年)、主な要素が横断であり、より広範囲に適用可能であるため、難分解性化学物質や環境対策にも利用できる。評価スキームの開発に向けた 2 つの先行研究は、環境化学物質ばく露と神経発達に関する疫学研究に焦点を当てたものである(Amler ら、2006 年; Youngstrom ら、2011 年)。

報告の質に関しては、2008 年 6 月に発足した EQUATOR ネットワーク(Enhancing the QUAlity and Transparency Of health Research (EQUATOR) Network)は、健康調査研究の明白性と正確な報告を促進する 国際的な取り組みである。現在、90 以上の報告ガイドラインがリストアップされており、その中には観察による疫学研究 に特化したものもある(例:Strengthening the Reporting of OBservational studies in Epidemiology (STROBE))。STROBE ステートメントには、論文のタイトル、要約、緒言、方法、結果、考察のセクションに関連する 22 項目のチェックリストを使用して、横断、症例対照、コホート研究を含む観察による研究の正確で完全な報告に何を 含めるべきかについての勧告が含まれている(von Elm ら、2007 年)。STROBE ステートメントは、著者への指示書の 中で言及されている生物医学雑誌の数が増えてきており、支持されている。表 1 は、疫学研究の報告の質を評価する 際に STROBE が考慮すべき主な特徴をまとめたものである。STROBE の拡張機能として、STROBE Extension to Genetic Association studies(STREGA)や分子疫学研究の評価のための STROBE-ME ステートメントがある。 STROBE チェックリストではばく露と健康の影響について一般的にしか言及されていないため、PPR パネルは、農薬 ばく露と健康影響の分野に特化した EQUATOR ネットワークライブラリに含めるための STROBE ステートメントの拡張 版を開発することを推奨する。これは、研究者や規制機関が研究の質を批判的に評価する際に大いに役立つであろう。

STROBE ステート	STROBE ステートメントアイテム				
ファクター	項目	推奨			
タイトルと概要					
	1	a)要約のタイトルに一般的に使用される用語を用いて、研究の計画を示す。 b)何が行われ、何が発見されたかについて、有益でバランスのとれた要約を記載する。			
序章					
背景・根拠	2	報告された調査の科学的背景と根拠を説明する。			
目的	3	事前に決められた仮説を含めた具体的な目的を述べる。			
方法					
研究デザイン	4	研究デザインの重要な要素を論文の前の方で提示する。			
設定	5	募集期間、ばく露期間、追跡調査期間、データ収集期間を含めて、環境、場所、関連する 日付を記述する。			
参加者	6	a) コホート研究-参加資格の基準、参加者の選択の情報源と方法を示す。フォローアッ プの方法を記述する。 症例対照研究-適格性の基準及び症例の確認及び対照の選択の情報源と方法を示す。			

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		症例及び対照の選択の根拠を示す。
		横断研究-参加資格の基準及び参加者の選択の情報源と方法を示す。
		b) コホート研究-マッチさせた研究については、マッチさせた基準とばく露者と未ばく 露者の数を示す。
		」 露日の気を示す。 症例対照研究-マッチさせた研究の場合、マッチさせた基準と症例ごとの対照の数を
変数	7	すべての転帰、ばく露、予測因子、潜在的交絡因子及び効果修飾因子を明確に示す。該当
		する場合は診断基準を示す
データソース/測	8*	対象となる各変数について、データの出所と評価(測定)方法の詳細を述べる。複数のグ
定		ループがある場合は、評価方法の類似性を記述する。
バイアス	9	/ バイアスの原因となる可能性のあるものに対処するための対応を記述する。
研究サイズ	10	研究規模がどのようにして決定されたか説明する。
量的変数	11	<ul><li>     分析において量的変数がどのように扱われたかを説明する。該当する場合は、どのグル     <ul><li>         −プ分けが選択されたか、またその理由を説明する。         ■</li></ul></li></ul>
統計的手法	12	a) 交絡因子をコントロールするために使用したものを含め、すべての統計的方法を記述 する。
		b)サブグループと相互作用を調べるために使用された方法を記述する。
		c)不足しているデータにどのように対処したかを説明する。
		d)コホート研究-該当する場合、追跡調査までの期間の損失がどのように対処されたか
		を説明する。
		症例対照研究-該当する場合、症例と対照のマッチングがどのように対処されたかを 説明+2
		説明する。 横断研究-該当する場合は、サンプリング戦略を考慮した分析方法を記述する。
		<ul> <li>e) 感度分析を記述する</li> </ul>
結果		
参加者	13*	a)研究の各段階における個人の数を報告する一例えば、適格性の可能性がある、適格性 の審査を受けた、適格性があると判断された、研究に参加した、追跡調査を完了した、 分析をうけた、など。
		b)各段階で不参加の理由を述べる。
		c)フロー図の使用を検討する。
記述データ		
	14*	a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子
	14*	a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子 に関する情報を提供する。
	14*	a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子
アウトカムデータ	14*	<ul> <li>a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子</li> <li>に関する情報を提供する。</li> <li>b)対象となる各変数について、データが欠落している参加者の数を示す。</li> </ul>
		<ul> <li>a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子 に関する情報を提供する。</li> <li>b)対象となる各変数について、データが欠落している参加者の数を示す。</li> <li>c)コホート研究-追跡調査期間の要約(平均値や総量など)。</li> </ul>
		<ul> <li>a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子 に関する情報を提供する。</li> <li>b)対象となる各変数について、データが欠落している参加者の数を示す。</li> <li>c)コホート研究-追跡調査期間の要約(平均値や総量など)。</li> <li>コホート研究-時間経過に伴うアウトカムの数または要約評価尺度の報告。</li> </ul>
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アウトカムデータ	15*	<ul> <li>a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子 に関する情報を提供する。</li> <li>b)対象となる各変数について、データが欠落している参加者の数を示す。</li> <li>c)コホート研究-追跡調査期間の要約(平均値や総量など)。</li> <li>コホート研究-時間経過に伴うアウトカムの数または要約評価尺度の報告。</li> <li>症例対照研究-各ばく露カテゴリーの数値、またはばく露の要約評価尺度を報告する。</li> <li>横断研究-アウトカムの数または要約評価尺度の報告。</li> <li>a)未調整推定値及び該当する場合は交絡因子調整済み推定値とその精度を示す(例:95% 信頼区間)。どの交絡因子が調整されたか及びそれらが含まれている理由を明確にす る。</li> </ul>
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議論		
主要な結果	18	研究目的を参考にして、主要な結果を要約する。
限界事項	19	潜在的なバイアスや不正確さの原因を考慮に入れて、研究の限界について議論する。潜 在的なバイアスの方向性と大きさについて議論する。
解釈	20	目的、限界、分析の重複、類似研究の結果、関連エビデンスなどを考慮して、結果の全体 的な解釈を慎重に行う。
一般化可能性	21	研究結果の一般化可能性(外的妥当性)を考察する。
その他の情報		
資金調達	22	本研究の資金源と資金提供者の役割、また、該当する場合には、本論文の基礎となった 原著研究の資金源を述べる。

\*:症例対照研究では症例と対照について、また、コホート研究及び横断研究では、該当する場合には、ばく露群と非ばく露群について、別々に情報を提供する。

選択的報告は、有意性のない結果や注目されない有意な結果が公表されないことで起こる可能性がある。研究者は、 有意な結果やリスクの高い推定値の選択的報告を避けるべきである。この点では、疫学研究の報告の標準化は、選択 的な報告を減らすか回避するのに役立つであろう。STROBE 声明や同様の取り組みは、この目的のための有用なツ ールである。疫学研究の中には探索的でその場限りの性質を持つ研究もあるだろうが、これは出版物に明記されるべ きであり、選択的報告は最小限に抑えられるべきであり、そうすれば疫学研究の結果は最も適切な観点から解釈される (Kavvoura ら、2007 年)。

研究の事前登録とプロトコールの事前発表は、医薬品の臨床試験における報告バイアスと出版バイアスを減らすた めに、いくつかの雑誌の編集者と倫理委員会によって取られている措置である。観察による疫学研究についても、でき るだけ明白性をもって報告バイアスや出版バイアスを減らすことを実施するために、同様の提案がなされているが、疫 学者の間ではコンセンサスが得られていない(Pearce、2011年;Rushton、2011年)。これとは対照的に、優れた疫学 的実践を促進するための多くの取り組みが専門学会によって実施されてきた。例えば、国際疫学協会(IEA、2007年) やオランダ疫学協会の responsible epidemiologic Research Practice (DSE、2017年)などがその例である。

正式な疫学研究のデータの質の評価は、ばく露/影響の関連性についてのエビデンスを提供するか否かに関わらず、結果よりも個々の研究の方法論的特徴のみに基づいている。しかし、リスク評価においては、研究方法の質だけでなく、研究が提供する情報の質も評価することが重要である。実際、優れた研究であっても、正式な品質評価の際に、 情報の報告が不十分なために却下されてしまうことがある。

## 4.2. 研究デザイン

適切なばく露評価を行い、十分に実施された前向き研究は、最も信頼性の高い情報を提供し、バイアスがかかりにくい。前向き研究が利用可能な場合には、計画があまり強固でない研究の結果が追加的な裏付けとなることがある。前向き研究がない場合は、横断研究や症例対照研究の結果を考慮すべきであるが、慎重に解釈すべきである。しかし、 適切に計画された症例対照研究は、あまり適切に計画されていないコホート研究よりも優れている可能性があることは 認められている。解析的アプローチは研究デザインに合致したものでなければならず、必要とされる統計的手法の仮 定は慎重に評価されるべきである。

長期疾患の観察による研究は前向きであることが理想的であり、ばく露と健康影響との間の時間区分は、疾患の発症に要する時間に関して適切であるように計画されるべきである。がんや心血管疾患のように潜伏期間が長い(10年以上)ことが多い健康影響については、健康影響の評価に先立って複数回のばく露評価を行うべきである。免疫機能障害のような潜伏期間の短い他の健康影響では、適切な時間区分は数日から数週間の範囲であり、1回のばく露評価で十分であろう。要するに、研究の理想的な計画は、検討されている健康影響の潜伏期間に依存する。予測される潜伏期間は、追跡調査の長さとばく露量が定量化されなければならない頻度の両方を決定する。

## 4.3. 研究対象集団

EU の人口は 5 億人を超えており、かなり雑多であるため、低用量の農薬ばく露で影響を受ける可能性のある、より 感受性の高い個人が多数含まれていることが予想される。これに対処するために、層別サンプリングでは、いくつかの 主要な集団特性(性別、年齢、地理的分布、民族性、遺伝的変動など)に従って対象集団をサブグループに分割し、 各サブグループ内で無作為にサンプルを採取する。これにより、調査母集団の中でサブグループをバランスよく表現す ることができる。

次に、脆弱な集団は、小集団解析または感度分析のいずれかを用いて疫学研究で調査されるべきである。しかし、 そのような解析は事前に行う必要がある。事後的なサブグループ感度分析の場合、統計的閾値はそれに応じて調整さ れるべきで、結果の再現性はそれに従うべきである。脆弱な小集団のエビデンスは、理想的には、ばく露のバイオマー カー、前駆症状、経時的な疾患発生率の評価を含む前向き研究を必要とする。

多くの人が前臨床状態にあり、用量反応曲線の下端に敏感に反応してしまうため、集団における毒性による疾病の 増加の閾値を特定することは不可能かもしれない。このことを明らかにするためには、疫学データが、集団の広範な横 断面における化学物質ばく露と疾病リスクの関係を特徴づけ(あるいは前兆病変や重要事象を調べ)、低用量の傾きを しっかりと検討する必要がある。

脆弱な小集団に関連するエビデンスの程度に基づいて、用量反応評価が集団全体に焦点を当てるのか、一般集団 と影響を受けやすいサブグループに分けて評価するのかを検討すべきである。集団全体を対象とする場合、従来のア プローチでは、不確実性因子を用いて変動性に対処することになるが、毒性物質に反応して疾患のリスク分布がどのよ うに変化するかを評価することで、変動性のリスクへの影響を解析することも可能である。要するに、不顕性バイオマー カーに基づくリスク分布は、用量反応評価で捉えることができる毒力学的変動の表現である。

別のアプローチとしては、脆弱な小集団を一般集団とは別個のものとして扱い、その集団の実際の用量反応データ、 特定の薬物動態または毒物動態学的要因の調整、またはより一般的な補正や不確実性の要因に基づいた用量反応 モデルを用いて、その集団に固有の効果を割り当てることである。農薬については、特定の年齢層、疾患(または疾患 関連のエンドポイント)、遺伝的変異、または共ばく露が独特の脆弱性を生んでいることが分かっている場合には、一般 集団に対する効果の差を推定するよう努めるべきであり、それに基づいて、別個の効果を開発するか、または最も感受 性の高い集団または脆弱性のある集団に対する補正を加えた全体の集団に対する単一の効果をベースにすることを 検討すべきである。

## 4.4. ばく露評価の改善

疫学研究における農薬ばく露評価の困難さは、上記したように強調されている。農薬ばく露の記述(特に個々の農薬 へのばく露に関する定量的な情報)は、一般的に規制目的のためには十分な詳細が報告されておらず、特に潜伏期 間の長い疾患(多くのがんや神経変性疾患など)では、この限界を克服するのは困難である。

ばく露モニタリングを実施するために必要な方法は、申請者が申請書類の中で提出しなければならないことは注目 に値する。この規則の要求事項は、ばく露量の判定に使用できる有効な方法を要求している。欧州議会及び理事会の PPPの上市に関する規則(EC)No 1107/2009に基づき、有効成分のデータ要求を定めた欧州委員会規則(EU)No 283/2013 では、承認前の試験と承認後のモニタリングの両方をサポートするために必要な分析方法に関する情報が 記載されている。この文脈では、承認後の要求が最も有用性が高く、規則には実際に次の通りに記載されている。

'4.2. 承認後の管理及びモニタリング目的のための方法-方法は、以下の目的で提出されなければならない。

- a) 加盟国が確立された最大残留レベル(MRL) への準拠を決定できるようにするために、6.7.1 項の規定に従って 提出されたモニタリング残留物の定義に含まれるすべての成分の決定;これは、植物及び動物由来の食品及び飼料に含まれる、または上述の残留物を対象とする。
- b)7.4.2項の規定に従って提出された土壌及び水の残留基準をモニタリングする目的のための含有全成分の測定。
- c)申請者が、作業者、労働者、住民または居合わせただけの者(bystanders)のばく露が無視できる程度であること を示さない場合には、散布中または散布後に生成された有効成分及び関連する分解生成物の大気中の分析。

d) 体液中及び組織中の有効成分及び関連代謝物の分析。

これらの方法は、可能な限り、最も簡単な方法を採用し、最小限のコストで、一般的に利用可能な機器を必要とする ものとする。分析の特異性を確認し、報告されなければならない。それにより、モニタリング残留物の定義に含まれるす べての成分を測定できるようにしなければならない。必要に応じて、検証された測定法を提出しなければならない。方 法の直線性、回収率、精度(再現性)が測定され、報告されなければならない。

データは、LOQと残留可能性の高いレベル、またはLOQの10倍のいずれかで生成されなければならない。LOQ は、モニタリング残留物の定義に含まれる各成分について決定され、報告されなければならない。植物や動物由来の 食品や飼料中の残留物や飲料水中の残留物については、方法の再現性は、独立した試験施設のバリデーション(ILV) によって確認され、報告されなければならない。

このことから、要求事項は存在するが、食品や飼料中の残留物のモニタリングよりもとトのバイオモニタリングの方が やや厳しくないと結論づけることができる。

これらの既存の方法を使用しないと、特定の農薬の規制における疫学的証拠の使用の可能性が制限される。したが って、今後の研究を検討する際には、ばく露の誤分類を避けるために使用する手法を慎重に検討することが重要であ り、当該農薬について適切で詳細なばく露評価を行い、適切な用量反応分析を可能にし、使用された方法の妥当性を 証明することが重要である。

ばく露は、ばく露される生涯の期間に応じて、異なる健康影響を及ぼす可能性がある。ばく露評価がそのような重要 な時期に適切に対応していることを確実にすることで、疾患発症の可能性がある時期のばく露には、より大きな注意を 払う必要がある。これは、神経発達、肥満、アレルギー反応など、出生前または出生後早期に発生する複雑な多段階 の発達過程を伴う研究に特に関連していると考えられる。このため、単一の期間におけるばく露の測定では、環境因子 のすべての健康影響に対する関連ばく露を適切に特徴付けることができない可能性があり、したがって、環境因子に 対するいくつかの重要な生物学的に脆弱な期間におけるばく露を測定する必要がある可能性が生じてくる。化学物質 の範囲とばく露の強度及び研究範囲ではない他の物質とのばく露も含めた中で、現在のばく露とは異なる可能性があ る過去のばく露の評価を構築することは、特に困難である。

農薬ばく露を測定するすべての方法には長所と短所があり、特定の研究計画と目的は、特定の最適なアプローチを 通知するために慎重に考慮されるべきである。

観察による研究では、個人レベルのばく露評価は以下の方法を用いて改善することができる。

a)個人のばく露モニタリング:これは、測定装置を接触時での農薬濃度を記録するために使用することができる。個人用ばく露モニターは、研究参加者にとって高価で負担が大きいものであった。しかし、技術の進歩により、最近では大気中の浮遊物質ばく露のための個人ばく露モニターが安価で使いやすい装置になり、これらは集団研究に適している。農薬ばく露に特化した個人ばく露モニターには、空気中の濃度を測定するためのセンサー、経皮濃度を測定するための「皮膚」パッチ、他のばく露手段を測定するための塵埃を捕らえる屋内家庭用モニターなどがある。これらのモバイル技術の進歩は、詳細で強固なばく露評価がなされた観察による研究を提供するために利用することができる。このような機器は現在、大規模な集団研究や大規模なコホート研究からのデータを収集するために、ますます適応が増えている。これらは、ライフスタイルやその他の習慣を捉えるための携帯電話や携帯電話アプリケーションを介したリアルタイムのデータ転送などの他の技術的進歩と相まって、次世代の観察による研究では、現在のエビデンスと比較して、はるかに詳細で強固なばく露評価が可能になるだろう。しかし、膨大な量のデータを生成することは、組織的、統計的、技術的な課題、特に追跡調査時間の延長をもたらす可能性がある。倫理や個人データ保護の問題を考慮しなければならず、地域の規制により、このような技術の大規模な使用が妨げられる可能性がある。しかし、このような個人モニターの使用は、異なる潜在的なばく露経路のうちの1つの情報を提供するにすぎない。

b) ばく露のバイオマーカー(ヒト・バイオモニタリング(HBM))。代替的及び/または補完的なアプローチとして、異なる経路(経皮、吸入及び経口のばく露)を介したばく露の結果である内部ばく露量の確認がある。これらのバイオマーカーは、農薬への総体的なばく露を評価し、累積的なリスク評価に情報を提供する上で重要な役割を果たす可能性がある。バイオモニタリングには、検討対象の化学物質(親化合物または代謝物)またはその病態生理学的影響のマーカー(付加生成物など)の生体試料中の濃度を測定することが必要である。しかし、課題としては、生体試料中の濃度測

定値を関連する用量に外挿することに伴う不確実性が含まれる場合がある。

バイオモニタリングは異物の吸収量を確実に推定できる可能性があるが、現代の農薬とその代謝物は比較的迅速に 体内から排泄され、排泄半減期は通常数日で測定される(Oulhote 及び Bouchard、2013 年)。そのため、バイオマ ーカーの使用はそのため、バイオマーカーの使用は、資源を必要とし、かつ煩わしいものとなります。長期にわたるばく 露を監視するために、多数の人を対象に繰り返し実施しなければならない場合には、このプロセスはさらに煩わしいも のとなる。

とはいえ、吸収量の正確な積算値を提供できる可能性があるため、農薬とその代謝物の生物学的モニタリングは、ば く露評価の他のアプローチを調整するために有効に利用できる。このようなアプローチの例は、農業健康調査 (Agricultural Health Study)で使用されているものである(Thomas ら、2010 年;Coble ら、2011 年;Hines ら、 2011 年)。また、長いばく露履歴を構築するために、他の形態のばく露評価と組み合わせて HBM の手法を使用する こともできる。

バイオモニタリングは、ばく露の特性評価の精度を向上させ、環境に関連するばく露濃度で発生するばく露の変化を 調査することを可能にする。大規模なバイオモニタリング研究で収集されたデータは、今後の疫学研究におけるばく露 の分類を支援するための基準範囲を設定するのに有用である。また、バイオモニタリングデータは、リスク評価の改訂を 実施するための重要な情報を提供し、有害な健康影響に対して特別なリスクのある小集団を特定するのに役立つ。

生体試料他(Biobanks)は、生物試料の保管場所として、早期ばく露と遅発影響の関係を調査する目的で、ばく露のバイオマーカーを評価するために利用することができる。すなわち、生涯初期に発生したばく露が、生涯後期における疾患(神経行動障害、小児腫瘍、免疫毒性障害など)の発症に重要であるかどうかを調べ、現在の健康ガイドラインに沿って健康リスクを後ろ向きに評価することができる。

尿、血液、毛髪などのとトの試料中の代謝物レベルの測定結果だけは、実際に受けたばく露量を完全に把握できない。全身または組織・器官の総ばく露量を推定するためには、場合によっては生理学的毒物動態学(PBTK)アプロー チを用いた追加評価が必要となる。PBTK モデルは、化学物質の毒物動態を特徴づけるために使用される生理学的 なコンパートメントモデルであり、特にとトにおける化学物質の運命を予測するために使用される。各コンパートメント内 で化学物質が受ける血流速度、代謝及びその他のプロセスに関するデータは、PBTK モデルのマス-バランス・フレ ームワークを構築するために使用される。PBTK モデルは、外部ばく露を体内の内部(標的)ばく露量に変換するだけ でなく、バイオモニタリングデータから外部ばく露を推測するためにも使用することができる。さらに、PBTK モデルは検 証される必要がある。

毒物動態プロセス(ADME)は、標的に到達した有効成分の「内部濃度」を決定し、この濃度/用量を観察された毒 性効果と関連付けるのに役立つ。現行の規制でも試験実施が規定されているが、in vitro 試験、動物試験、ヒト試験な ど、有効成分の毒物動態に関するすべてのエビデンスを調査することは有益であろう。品質保証の問題や HBM 試験 に関連して考慮すべき要素についての更なる議論は、EFSA が委託したプロジェクトの報告書(Bevan ら、2017年)に 記載されている。

ばく露評価は、観察による研究における集団レベルでも、以下のような方法で改善することができる。

a)健康影響や農薬使用に関する登録データや大規模データベース(行政データベースを含む)から得られたデー タなど、新しい技術やビッグデータを利用した大規模な疫学研究は、より確かな知見が得られ、最終的には情報に基づ いた政策決定や規制に利用できる可能性がある。このようなデータは、維持管理が義務付けられている農家やその他 の専門的ユーザーなど、異なる集団による農薬使用の記録が含まれている可能性がある登録データの利用を中心に、 多くの努力が必要である%。このようなデータは、電子的健康記録(上記参照)にさらにリンクされ、これまでにないサンプ ルサイズ及びばく露とその後の病気に関する情報を持つ研究を提供し、最終的にはこれまで回答のなかった強固な問

<sup>9</sup> 規則 1107/2009 第 67 条は次のように述べている。記録の保持 1. 植物保護製剤(農薬)の生産者、供給者、流通業者、輸入業者及び輸出業者 は、自らが生産、輸入、輸出、保管又は市場に出した植物保護製剤(農薬)の記録を少なくとも5年間保管しなければならない。植物保護製剤(農 薬)の専門的使用者は、使用した植物防疫製品(農薬)の記録を少なくとも3年間、植物保護製剤(農薬)の名称、適用時間及び適用量、植物保 護製剤(農薬)が使用された地域及び作物を記載して保管しなければならない。これらの記録に含まれる関連情報は、要求があれば所轄官庁に 提供しなければならない。飲料水製造業界、小売業者又は住民などの第三者は、所轄官庁に連絡することにより、この情報へのアクセスを要求す ることができる。所轄官庁は、適用される国内法または共同体法に従って、当該情報へのアクセスを提供するものとする。

題に答えることができるようになるだろう。同時に、これらの登録や大規模なデータベースでは、有効成分に関する情報 をよりよく把握する必要がある。食品中の残留農薬ばく露は、監督下の残留試験と組み合わせて散布日誌データを使 用することで、より正確に推定することができる。この方法は、より包括的で強固な源データを含み、使用された農薬を より完全にカバーし、標準的な定量限界(LOQ)以下の残留物のより信頼性が高く正確な推定値を得ることができると いう利点がある(Larssonら、2017年)。

b) 地理的情報システム(GIS)や小地域調査への新しい高度なアプローチも、住居ばく露の推定値を提供する追加 的な方法として役立つかもしれない。GIS に基づくばく露指標(すなわち、農業用地への住居の近接性や住居周辺へ の影響のある農地面積)は、検証された場合、バイオモニタリングを補完する有用なツールとなる可能性があり、生物学 的半減期の短い農薬へのばく露を評価するために使用されてきた(Cornelis ら、2009 年)。このようなばく露の中には、 他の要因の中でも特に風向によって影響を受けるものがあるため、このアプローチを最大限に活用するためには、結 果の特別な分析を通じて、この点を考慮に入れる必要がある。また、これらの指標は、特定の指標ではないにせよ、生 物モニタリングデータよりも、長期にわたる非残留性農薬への累積ばく露のより代表的な指標となり得る(González-Alzaga ら、2015 年)。

すでに議論したように、個々の化合物の規制リスク評価に有用であるためには、疫学的ばく露評価は特定の農薬に 関する情報を提供すべきである。しかし、より一般的なばく露評価を含む疫学的研究は、一般的なリスク因子を特定し、 関連するとト集団における因果関係の推論を示唆する可能性もある。このような観察結果は、全体的な規制政策の情 報提供と、さらなる疫学的研究のための事項の特定の両方に重要であるかもしれない。

現代技術の最近の進歩により、新しい分析方法を用いて農薬ばく露を前例のない範囲で推定することが可能になった。

a)メタボロミクス(metabolomics)やアダクトミクス(adductomics)などのいわゆるオミクス技術の発展は、生物学的 マトリックス(血液、唾液、尿、毛髪、爪など)中に経時的に記録された異物や代謝物から、DNA やタンパク質との共有 結合体(アダクトミクス)や生物学的経路の理解に至るまで、幅広い分子の測定を通じてばく露評価を改善するための 魅力のある可能性を提示している。これらの方法論は、他のツールと組み合わせて使用することができる。また、このよ うな技術を規制毒性学に応用するには、さらなる研究が必要であるという認識と関心がある。エクスポソーム(exposome) (一生の間に個人が受けたばく露の全体)の利用は、「オミックス」技術とヒトのバイオモニタリングに適したバイオマーカ ーを使用することで、より良い結果を得ることができるかもしれない。それにもかかわらず、これらの方法論の検証が不 足していることと、大規模での使用を制限するコストのため、重要な制限が認められなければならない。

b)環境ばく露は従来「一回のばく露に一回の健康影響」というアプローチで評価されてきた。これに対して、エクスポ ソームは、受胎以降のヒト環境ばく露の全体を網羅しており、遺伝学の知識を補完することで、疾患の病因における環 境要因をよりよく特徴づけることができる。このように、エクスポソームには、生涯にわたる化学的ばく露だけでなく、感染 症、身体活動、食事、ストレス、内部生物学的因子(代謝因子、腸内フローラ、炎症、酸化ストレス)などの外部環境因 子や内部環境因子も含まれている。完全なエクスポソームを構築するためには、生涯にわたって継続的に異なる源か らの多くの外部ばく露と内部ばく露を統合しなければならない。しかし、真に完全なエクスポソームを測定することは不 可能である。エクスポソームのこれらすべての領域を従来のものとは異なるアプローチで捉える必要があるが、この目的 のためには単一のツールでは十分ではないと考えられている。

ばく露のより総合的なアプローチは、現在の疫学研究における従来の「一回のばく露-一回の健康影響」アプロー チに取って代わることを意図したものではない。しかし、それは複雑で多因子性の慢性疾患の予測因子、リスク因子、 保護因子についての理解を向上させるものである。エクスポソームは、生涯にわたる環境の影響やばく露を総合的に 記述し、統合する枠組みを提供している(Nieuwenhuijsen、2015年)。

これらの潜在的なバイオマーカーを検証し、最終的にはばく露評価の改善につなげるためには、共同研究や、大規 模なコンソーシアムを形成する疫学研究や探索的研究の統合が必要である。エクスポソームパラダイムを従来のバイオ モニタリング手法に組み込むことは、ばく露評価を改善する手段となる。エクスポソーム拡大関連研究(EWAS)は、健 康な人と病気の人の血液中の何千もの化学物質を測定し、病気との関連性を検査し、ばく露源を特定し、作用機序を 確立し、因果関係を明らかにするために、その後の調査で対象とすることができるばく露の有用なバイオマーカーを特 定することを可能にする(Rappaport、2012 年)。これらの主要な化学物質を特定し、症例と対照の独立したサンプル で疾患との関連性を検証した後、これらの化学物質は、大規模な集団からの血液を対象とした分析において、ばく露ま たは疾患進行のバイオマーカーとして使用することができる。

エクスポソームの概念に関連して、オミクス技術は複雑な化学物質の混合物への累積ばく露に対する生物学的反応 の特性やシグネチャーを測定する可能性を持っている。重要な進歩は、特定の生物学的試料中の個々のばく露を個 別に評価することなく、エクスポソームを特徴づけることができるユニークな生物学的マトリックスを特定することであろう。 オミクスデータの非標的特性は、ばく露に対する生物学的反応をより全体的な方法で捉え、ばく露に関連した健康影 響を裏付けるメカニズム論的な情報を提供することになる。重要なことは、オミクスツールは、多様なばく露がどのように して共通の経路で作用し、同じ健康影響を引き起こす仕組みを明らかにすることができるということである。

改良されたばく露評価は関連性を検出する力を高めるが、どのような個々の研究においても、各被験者のばく露評価を実施するために使用する供給源と被験者の総数のバランスを最適化することにより、研究の全体的な力を最大化することが必要である。

## 4.5. 健康影響

農薬については、これらの化学物質が単一の疾患領域に関連して特定の効果を示していないため、健康影響は広範囲にわたる。それぞれの健康影響について、文献には複数の定義が存在していて、程度の差こそあれ異なるデータベース間での再現性は不明であり、一般化できないという制限がある。健康影響の適切な定義は、観察による疫学研究の妥当性と再現性にとって非常に重要であり、これらの定義の一貫性と明確さは研究間で考慮する必要がある。前向きな観察研究では、明確な健康影響の定義、包含基準と除外基準、標準化されたデータ収集があるが、後ろ向き研究では通常、主にコード化されたデータに基づいた健康影響の同定に頼っており、疾患の分類とコード化は時間の経過とともに変化する可能性がある。主要な健康影響を定義するために使用された実際のコードの詳細な記述と検証作業の結果は、今後の研究活動にとって貴重なものである(Stang 6、2012 年; Reich 6、2013 年)。コード化された疾患の例としては、例えば ICD-10 があり、これは広範囲の悪性疾患を標準化するためのツールとして使用することができる。

いくつかのサーベイランス研究では、すべての潜在的な症例を特定するために感度の高いより広い定義を使用し、 その後、偽陽性の数を減らし、結果としてより正確な症例を得るために、高い陽性予測値のより狭く、より正確な定義を 適用することが望ましいとされている。対照的に、正式な疫学研究では、特定のイベントの定義が使用され、その精度 を決定するために検証される。しかしながら、「検証」では新たな定義をテストしないので、感度や特異度を測定できな いでだろう。

代替エンドポイントは、有効性が確認されていない限り避けるべきである。代替健康影響の妥当性を評価する基準に は、以下のようなものがある。

- ・代替指標が疾患の原因経路内にあることが示されていること。これは以下のエビデンスによって裏付けられる:バイオマーカーの反応が病理学と相関しており、他のバイオマーカーと比較して性能が向上していること;生物学的な理解と毒性との関連性(反応のメカニズム);メカニズム的に異なる化合物に対する一貫した反応と性、系統、種の違いによる類似した反応;用量反応の存在と反応の大きさと時間的関係;毒性に対する反応の特異性;すなわち、バイオマーカーは他の組織の毒性に対する反応や、標的臓器の毒性を伴わない生理学的効果を伴わない生理的効果を反映してはならない。
- ・代替健康影響と真の健康影響の両方を使用した少なくとも 1 つのよく実施された試験があること(Grimes 及び Schulz、2005年; la Cour ら、2010年)。これらの基準を評価するために、いくつかの統計的手法が使用されて、 それらが満たされていれば、代替指標の妥当性が高まる。しかし、多くの場合、不確実性が残っているため、疫学 研究に代替指標を適用することは困難である(la Cour ら、2010年)。

EU 全体の健康影響に関するデータは非常に広範囲に及ぶ可能性がある。これを効果的に管理することができれば、非常に大規模なサンプルサイズを用いて悪影響を評価する疫学研究において、より大きな統計力を発揮できる可能性がある。これらの研究に必要な前提条件は、新たな軽微な影響、慢性的な影響、または層別化した場合の小集団

への影響を検出する可能性があるが、リスク評価の範囲を超えている。これらの研究には、調和のとれた診断、データ 保存及び社会的利益のための匿名化された個人データへの法的に承認されたアクセスと相まって、健康情報学への 国境を越えたアプローチが含まれている。健康記録には、適切な中毒症候群の分類が含まれていなければならない。 後者は、入力データの品質を保証するために、医療と医療補助のトレーニングの改善を必要とするかもしれない。

生物学的モニタリングが採用されるもう一つの機会は、調査がいわゆる影響のバイオマーカーを含む場合である。こ れは、定量化可能な生化学的、生理学的、またはその他の変化であり、その大きさに応じて、確立された、または可能 性のある健康障害や病気に関連している。影響のバイオマーカーは、機能的または構造的損傷に先行する初期の生 化学的変化を反映している必要がある。このように、最終的に毒性につながるメカニズムの知識は、特定の有用なバイ オマーカーを開発するために必要であり、その逆もまた然りで、影響のバイオマーカーは、疾患の発生のメカニズムの 経路を説明するのに役立つかもしれない。このようなバイオマーカーは、生物学的システムにおける初期の可逆的な事 象を特定するものであり、後の反応を予測するものでなければならず、その性質上、前臨床的なものと考えられる。実 験的ーオミクス技術の進歩は有望であり、リスク評価戦略、すなわち作用機序、反応バイオマーカー、内部ばく露量の 推定、用量一反応関係に関する確かな情報を提供するだろう(DeBord ら、2015 年)。これらの技術は、その妥当性と 信頼性を評価するために検証されなければならない。妥当性が確認されれば、それらの技術は規制目的で利用できる ようになる。

## 5. 農薬リスク評価への警戒データの貢献

第2・4節で議論した正式な疫学調査に加えて、その他のヒトの健康データは、その場限りの報告書から、あるいは計画的なプロセスとして、すなわち公衆衛生当局や認可者によって国家レベルで実施されているモニタリングシステムを通じて、生成することができる。第2・4節に沿って、本節ではまず、このようなモニタリングシステムがどのように運用されるべきか、農薬のモニタリングに関する現状はどうなっているのか、そして改善のためにどのような勧告ができるのかをレビューする。

## 5.1. ケースインシデント研究の一般的な枠組み

有害事象の収集、報告、評価を継続的に行うことは、同じ有害事象が後から別の場所で発生する可能性を減らすことで、利用者やその他の人々の健康と安全の保護を向上させ、また、そのような事象の結果を緩和する可能性がある。 そのためには、当然ながら、収集した情報をタイムリーに発信する必要がある。このようなプロセスを警戒(vigilance)と呼んでいる<sup>10</sup>。

例えば、EU では、医薬品の安全性監視は医薬品安全性監視 (pharmacovigilance)して知られており、医薬品安 全性監視システムは、加盟国の規制当局、欧州委員会、欧州医薬品庁 (EMA)の間で運営されている。一部の加盟国 では、国内の管轄当局の調整の下に地域センターが設置されている。製造業者や医療従事者は、国レベルの管轄当 局に事件を報告する。これにより、有害事象に関するあらゆる情報が記録され、一元的に評価され、その後の対応につ いて他の当局に通知することができる。記録は EMA によって一元化され、欧州の医薬品安全性監視システムの調整 をサポートし、医薬品の安全で効果的な使用に関するアドバイスを提供する。

## 5.2. ケースインシデント報告の現在の枠組みの主な限界

いくつかの EU の規制では、ヒトに農薬が原因で発生した有害事象(職業環境での急性または慢性ばく露後に発生 したもの、偶発的または故意の中毒など)の通知及び/または収集及び/または報告を義務付けている。これらには 以下のものが含まれる。

・ EC 規則 1107/2009 の第 56 条は、「植物保護製剤(農薬)の認可を受けた者は、直ちに加盟国に通知しなけれ

<sup>10</sup> 調査という概念は、何かを測定し記録するための単一の努力を意味し、サーベイランスとは、疾病の不在を証明したり、疾病の存在や分布を特定して情報を適時に発信できるようにするために、集団の傾向を検出するために、標準化された調査を繰り返すことを意味する。モニタリングとは、集団の環境や健康状態の変化を検出するために、日常的な測定や観察を断続的に分析することを意味するが、反応を引き出すことはない。監視は、綿密かつ継続的に注意を払うプロセスを意味するため、監視や単なるモニタリングとは異なり、この背景では特に化学物質の使用に関連した販売後の事象を扱う。

ばならない」と規定している。この目的のために、認可保有者は、植物保護製剤(農薬)の使用に関連して、ヒト、動物及び環境におけるすべての疑われる有害な反応を記録し、報告しなければならない。通知義務には、国際機関や第三国の植物保護製剤(農薬)や有効成分を認可する公的機関による決定や評価に関する関連情報も含まれていなければならない。

・農薬の持続可能な使用を達成するための共同体行動の枠組みを確立した EC 指令 128/2009の第7条は、次のように要求している。加盟国は、作業者、農業労働者、農薬散布地域の近くに住む人など、定期的に農薬にばく露される可能性のある集団の間で、農薬による急性中毒事故や慢性中毒の発生状況に関する情報を収集するシステムを設置しなければならない。3.3.情報の類似性を高めるために、欧州委員会は加盟国と協力して、2012年12月14日までに「農薬使用がとトの健康と環境に与える影響のモニタリングと調査に関する戦略的ガイダンス文書」を作成する。しかし、この意見書を発表した時点では、この文書はまだ公表されていない。

間接的ではあるが、農薬と報告に適用される追加の規制が3つある。

- ・ 農薬の統計に関する EC 規則 1185/2009 は、加盟国が調和のとれたフォーマットに従って農薬の販売と使用に 関するデータを収集することを要求している。上市に関する統計は毎年欧州委員会に、農業利用に関する統計は 5年ごとに送信されなければならない。
- ・ 食品法の一般原則と要件を定めた規則(EC)178/2002 の第 50 条では、食品と飼料を対象とした改良・拡大され た迅速警報システム(RASFF)が設定されている。このシステムは欧州委員会によって管理されており、ネットワー ク加盟国、欧州委員会、当局が加盟している。それは残留農薬の認可されていない事例や食中毒の事例を報告 する。
- ・ EC 規則 1272/2008 (CLP 規則)第45条(4): EU 加盟国の市場に危険な化学物質の混合物を市場に出す輸入 業者と顧客ユーザーは、その加盟国の任命機関/毒物センターに通知書を提出しなければならない。通知書に は、化学成分や毒物学的情報、混合物が属する製品カテゴリーなど、混合物に関する特定の情報を記載する必 要がある。通知書に製品分類に関する情報を含めることで、指定団体/毒物取締センターは、同等の統計解析 (例えば、リスク管理措置の実施)を行い、報告義務を果たし、MS 間で情報交換を行うことができる。したがって、 製品カテゴリーは実際の緊急時の医療対応には使用されないが、ばく露や中毒の傾向を特定し、将来の中毒事 例を防ぐための対策をとることができる。正式に採択された場合、新規則は2020年1月1日から適用される。

実質的な立法規定がある一方で、今日までのところ、医薬品安全性監視システムに類似した単一の EU「植物薬理 監視」<sup>11</sup>システムは PPP には存在しない。むしろ、加盟国の公衆衛生にリスクをもたらす可能性のある化学物質のハザ ードについて警告、通知、報告、情報共有を行うための多くの警告システムが EU 内で開発されている。これらのシステ ムは、医薬品、食品、消費者製品、労働災害、国際保健規則(IHR)に基づく通知、EU 毒物センターや公衆衛生当局 によって検知した事象など、さまざまな分野をカバーしている。これらのシステムのそれぞれは、管轄官庁、公的機関、 政府、規制当局、公衆衛生当局にタイムリーな警告を通知し、配布して、公衆衛生へのリスクを最小限に抑え、管理す るための効果的な行動をとることを可能にしている(Orford ら、2014 年)。

EU では、急性農薬ばく露・事故に関する情報は、主に毒物管理センター(PCC)によって収集・報告されたデータに 基づいている。PCC は、一般集団や職業環境において、自分たちが知っている急性と慢性のばく露/中毒の両方の 事例を収集している。通常、症例は十分に文書化されており、情報にはばく露・事故の状況、原因物質と疑われるもの の説明、ばく露のレベルと期間、臨床経過と治療、因果関係の評価が含まれている。重症の場合は、通常、血液や尿 中の毒素や代謝物の測定が行われる。しかし、センターに報告された症例の追跡調査は、長期化する可能性のある影 響を特定するために、さらに注意を払う必要がある。

毒物センターのデータを使用するには、2 つの重要な障害がある:各国の毒物センターからの報告書は常に公表さ

<sup>&</sup>lt;sup>11</sup>「フィトビジランス(phytovigilance)」は植物に対する警戒システムを意味し、農薬は作物の「薬」であることを意図しているため、ここでは「フィト ファーマコビジランス(phytopharmacovigilance)」という用語がより適切であると考えられている。さらに、フランスでは、土壌、水、大気、環 境、動物のデータなどをカバーする広い用語として使われている。

れているわけではなく、公表されている場合でも、データ収集の形式やコーディング、因果関係の評価には大きな不均 一性がある。実際、各加盟国は独自の収集活動のためのツールを開発しており、ばく露データの比較や交換には困難 が伴う。2012 年、欧州委員会は、新たな化学物質事象への欧州の対応を支援するための共同研究開発プロジェクト 「化学物質健康脅威のための警告・報告システムフェーズ III(ASHTIII)プロジェクト」に資金を提供した。検討された 様々なツールや方法論の中で、欧州の PCC からのばく露データを交換・比較する方法が開発された。実現可能な研 究として、ワークパッケージ 5 には、加盟国が農薬ばく露データを比較できるようにするための、調和のとれた強固なコ ード化システムの開発が含まれていた。しかし、PCC コミュニティとの協議の結果、データのコーディングと収集活動の さらなる調整が必要であることが示された。その結果、ばく露データを加盟国間で比較できるようにするためには、EUと 加盟国レベルでの更なる支援と調整が必要であると結論づけられた(Orford ら、2015 年)。

PCC が収集したデータに加えて、いくつかの加盟国は、労働衛生監視に特化したプログラムを立ち上げた<sup>12</sup>。これら は、業務上の農薬による傷害や病気、中毒が疑われる症例について、医師による自発的なイベント通知(使用者による 自己申告の場合もある)に基づいている。医療データに加えて、収集された情報には、作物の種類、散布方法、温度、 風速、個人用保護具の着用状況などに関するデータが含まれる。一度収集されたこれらのデータは調査され、定期的 に報告書が発行され、再登録中の製品の安全性を評価するための有用な情報となる。これらのデータはまた、新たな 問題を浮き彫りにし、政策立案者のためのエビデンスに基づく予防措置を策定することを可能にする。EU レベルでは、 欧州労働安全衛生庁(EU-OSHA)<sup>13</sup>は、職業上の農薬関連疾病データのモニタリング方法をほとんど持っていない。 米国では、国立労働安全衛生研究所(NIOSH)が資金を提供し、農薬に特化したプログラムがいくつかの州で実施さ れている<sup>14</sup>。

要約すると、現在、ヒトのデータは、症例報告書や症例集積、毒物センターの情報、検視官の裁判結果、労働衛生 監視プログラムや市販後の監視プログラムの形で収集されている。しかし、申請者が提出した医療データには、このよう な情報がすべて含まれているわけではない。これは、さまざまな情報源が多様で異質な性質を持っているため、アクセ スできないものもあるためである。

- ・工場生産労働者の労働衛生監視を通じて収集されたデータ、あるいはそれが行われたとしても、医療データは非常に限られており、一般的には基本的な臨床血液測定、身体検査、潜在的にはどこでどのようにばく露されたかという単純な指標であり、通常は長期的なフォローアップは行われていない。さらに、最新の工場(特に EU)での労働者のばく露は一般的に非常に低く、多くの場合、潜在的なばく露は(特定の化学物質に特化した施設でない限り)様々な農薬へのばく露である。
- ・さらに、製造中の有効成分への職業上ばく露からのデータの報告は、しばしば調合された植物保護製剤(農薬)との接触から生じる観察結果と組み合わされる。実際、植物保護製剤(農薬)中の共配合剤の存在は、急性毒性学的プロフィルを変更することができる。したがって、適切な評価を容易にするために、ヒトで収集した結果を報告する際には、それ自体が有効成分なのか PPP なのかを明確に特定しなければならない。

EC 規則 283/2013 の第 5.9 章の一部でもある有効成分や配合された植物保護製剤(農薬)や提案された治療法に よる中毒の診断に関する特定のデータの要求に関しては、情報が欠落していたり、毒性作用のモードがとトで起こること が知られていて特定の解毒剤が特定されている場合に限定されていたりすることがよくある。

<sup>&</sup>lt;sup>12</sup> 例えばフランスの Phyt'attitude は、Sociale Agricole, Mutualite, Sociale Agricole によって開発された警戒プログラムである: http://www.msa。

<sup>&</sup>lt;sup>13</sup> https://osha.europa.eu/en/about-eu-osha

 $<sup>^{14}\</sup> SENSOR\ \mathcal{T}{\it ud}\mathcal{T}{\it d}\mathcal{T}{\it ud}\mathcal{T}{\it d}\mathcal{T}{\it ud}\mathcal{T}{\it d}\mathcal{T}{\it d}\mathcal$ 

Epidemiological studies and pesticides

### 5.3. ケースインシデント報告の現行枠組みの改善提案

重複と努力の無駄を避けるために、論理的な次のステップは、すべての関係する公的部門と民間部門の関係者と一 緒に、医薬品のために実施されているものと同様の化学物質のための EU の「植物薬理監視」システムを開発すること であろう。このネットワークは、献身的で特別な訓練を受けた地方の産業保健医や開業医を基盤とすることができ、シス テムを確立し、成功裏に維持するために加盟国が供給源を配分すべきである。実際、このようなネットワークは急性の 影響を検出するのに有用であろう。また、特定の健康影響(喘息、感作など)や新たな職業関連疾患の検出のためのセ ンチネルサーベイランスネットワークとしても機能するであろう。実際、このようなシステムを段階的に構築する方法につ いては、すでに多くの経験が得られているが、それにもかかわらず、これが実施されるまでには何年もかかることが想定 されている。収集されるデータの特性(情報源は多様である可能性がある)、収集された情報の質と完全性(特に状況)、 観察された効果の重症度と説明責任(観察された効果と製品との間のリンク)など、いくつかの困難が生じる。ルールは、 ある「評価者」から別の「評価者」まで同一であるように定義されなければならない。植物薬理監視システムが目的に完 全に適合していることを保証するために、ネットワークは長期的に安定していなければならない(例えば、関与する国の 組織の継続性、採用された一貫した方法論など)。植物薬剤モニタリングデータの使用は、リスク評価の目的に限定さ れることはなく、リスク管理上の政策決定(例えば、製品認可の条件の改定や最終的には製品の取り下げなど)に影響 を及ぼす可能性があるが、これは最初からすべての利害関係者に明確でなければならない。

このようなシステムは、(主に)農薬として使用されている化学物質のみを対象としたものでは意味がないかもしれない。しかし、すでに農薬に関する法律の規定があることを考えると、このシステムの開発は農薬に優先して行われる必要があるかもしれない。

結論として、欧州委員会は加盟国とともに、EU 全体の農薬の警戒枠組みの開発に着手すべきである。これには以下が含まれるべきである。

- ・EUレベルでのヒトでの健康影響データ収集活動の調和
- ・ EU 全体のデータベースの編集の調整
- ・各加盟国で発生したすべての PPP 中毒を収集するために、各国レベルでのポイズンセンターと規制当局との連携を改善すること
- ・因果関係のデータ評価の整合化を伴う農薬使用がとトの健康に及ぼす影響のモニタリングに関するガイダンス文書
- ・ EU 全体を対象とした定期的な報告書

## 6. 農薬のリスク評価を支援するための疫学研究と監視データの利用の提案

本節では、実験的研究に基づくリスク評価プロセス(第 6.1 節)を概説し、そのプロセスに疫学的研究がどのような情報を付加しうるかを論じる。次に、第 6.2 節では、疫学研究の信頼性の評価について述べる。6.3 節では、信頼性があると認められた1つ以上の研究の関連性を評価する。

#### 6.1. リスク評価プロセス

リスクアセスメントとは、健康に悪影響を及ぼす可能性のある化学物質やその他の汚染物質、薬剤によるヒトや環境 へのリスクを評価するプロセスである。規制目的のために、リスク管理者に情報を提供するために使用されるプロセスは、 4 つのステップで構成されている(EFSA、2012 年 a)。一方では、毒性影響の特性(ハザード同定)と、農薬と毒性影 響の間に考えられる用量反応関係(ハザード特性評価)に関する情報が収集される。一方で、ヒト(消費者、散布者、労 働者、居合わせただけの者、住民)と環境へばく露可能性についての情報が求められる(ばく露評価)。これら2つの要 素は、集団が基準ばく露量を超える量にばく露される可能性があることを推定するために、リスク特性評価の中で考慮 される。通常、これは規制目的のためのリスク管理者への情報提供に用いられる。

#### a) ステップ 1. ハザードの同定

疫学的研究と監視データは、農薬ばく露と健康影響とが関連する可能性を示すことができるため、ハザードの特定 に関連している。この背景では、疫学的データは、実験モデルでは検出されなかった影響を「ホライズン・スキャニング」 する上で、非常に貴重な情報を提供することができる。重要なことは、これらの研究はまた、脆弱な集団のサブグループ、生涯における感受性の高い時期、性別による選択的影響など、リスクが高まる可能性についての情報を提供することである。

b) ステップ 2. ハザードの特性評価(用量反応評価)

前述したように、通常、疫学的データを使用する場合には、ばく露量が割り当てられることはほとんどないため、古典 的な用量反応の枠組みは考慮されない。質の高い疫学研究が利用可能な場合の課題は、それらを数値入力としてス キームに統合するのが最善かどうかを見極めることである。農薬のリスク評価に疫学的データを使用する場合、用量反 応フレームワークが考慮されることはほとんどない。しかし、EFSA CONTAMパネルのこれまでの科学的見解では、基 準ばく露量を設定するための基礎として疫学を使用してきた、特にカドミウム、鉛、ヒ素、水銀の場合は、最もよく知られ ていてデータが豊富である(EFSA、2009年 a,b、2010年 b、2012年 b)。これらが用量反応評価の基礎とならない場 合でも、監視と疫学的データは、実験動物を用いた用量反応研究の妥当性を検証したり、無効にしたりするための裏 付けとなるエビデンスを提供することがある。化学物質の様々な用量とばく露された集団における有害な影響の発生率 との間の関係を特性評価するためには、ばく露または用量の特性評価、反応の評価、無影響量を特定するために観察 されたデータが適合する用量反応モデルの選択が必要である。2 つの課題が提起される。すなわち、無影響量を特定 するために、疫学的データから用量反応を導き出すことができるのか、ということである。もしそうでない場合、疫学的情 報はハザードの特性評価に貢献できるのか、ということである。

用量反応関係を理解することは、EU の優れた植物保護対策が予想されるよりも高いばく露量の使用による有害な 健康影響が関連していることが証明されるが、低いばく露量の使用では関連性が観察されない。この背景では、RR ま たは OR を明らかにした疫学研究の統計的要約は、研究デザインが必要な基準を満たしている場合には、ハザード特 性評価プロセスに投入するための有用な定量的情報となる可能性があることは明らかである。

c) ステップ 3. ばく露評価

ばく露の評価に関するデータは、制御されていない様々な「実社会」の要因が解析を混乱させる複雑な状況では、 推定が困難なことが多い。前述したように、現代の生物学的モニタリングは、コストの高さ、実施可能性、ロジスティック スなどの実際的な理由から、一般のヒト集団ではほとんど実施されていない。しかし、近い将来、農薬への定量的ばく 露に関する生物学的モニタリング研究やデータが増加することが予想されている。

ステップ 4. リスクの特性評価。この最後のステップでは、ばく露に関するデータを健康ベースの基準値と比較し、ば く露された集団における健康障害のリスクを推定する。ヒトのデータは、標的臓器、用量反応関係、毒性影響の可逆性 に関する完全な毒性学的データベースからの外挿に基づいて行われた推定の妥当性を検証するのに役立ち、基準値 の定義に直接影響を与えずに外挿のプロセスを再確認するのに役立つ(London ら、2010 年)。

疫学的データは、不確実性因子(UF)との関連で考慮されることもある。一般的に動物データでは、影響の種間変 動を考慮するために 10 の UF が使用され、これにさらに 10 の係数を加えてヒト集団の異なる部分の感受性の変動を 考慮する。しかし、ヒトのデータのみを考慮する場合(動物のデータよりも重要な場合)もあり、種族間のばらつきを考慮 した 10 の係数が適用される。現時点では、規則(EC)No 1107/2009 の第4条(6)が次のように規定していることに留 意する。「ヒトの健康に関連して、ヒトから収集したデータがない場合、動物試験に由来する安全マージンを低下させて はならない」と規定している。このことの意味するところは、リスク評価において疫学的データはリスク評価で使用される 警戒レベルを高めるためにのみ使用され、関連するヒトのデータが入手可能であっても UF を低下させるために使用さ れてはならないということである。

#### 6.2. 個々の疫学研究の信頼性の評価

WOE 評価のために疫学をどのように考慮すべきかを決定する際に考慮すべき因子は以下に記載されており、観察 疫学的研究のためのバイアスのリスクツールで広く概説されている<sup>15</sup>。以下の例は、網羅的なリストではないが注目すべ

<sup>&</sup>lt;sup>15</sup> 介入またはばく露の観察研究におけるバイアスと交絡因子のリスクの評価。RTI アイテムバンクのさらなる発展 (https://www.ncbi.nlm.nih.gov/books/NBK154464/)とコクランハンドブック。

き因子を示している。

- ・研究デザインと実施。研究デザインは、ばく露と健康影響及びリスクのある集団の予想される分布を考慮して適切 なものであったか?その研究は主に仮説生成モードまたは仮説検証モードで実施されたか?
- ・集団。研究は、十分に定義された集団から目的の個人をサンプリングしたか?研究は、ばく露群と非ばく露群の健 康影響について有意な差を検出するのに十分な統計力と精度を有していたか。
- ・ ばく露の評価。ばく露の評価に使用された方法は有効で、信頼性があり、適切であったか? 広範囲のばく露が調 査されたか? ばく露は定量的レベルで評価されたのか、それともカテゴリカルまたは二分法(例:経験対未経験)で 評価されたのか? ばく露は前向きに評価されたか、あるいは後ろ向きに評価されたか?
- ・健康影響の評価。健康影響の評価に使用された方法は有効で、信頼性が高く、適切であったか?健康影響に関 するデータ収集には標準化された手順が用いられていたか。情報の偏りを避けるために、健康影響はばく露状態 とは独立して把握されていたか?
- ・交絡因子の管理:潜在的な交絡因子が適切に特定され、考慮されていたか?それらはどのように管理されていた か?これらの因子を記録するために使用された方法は有効で、信頼性があり、適切であったか?
- ・統計解析。研究は、対象となる健康影響に対するばく露の独立した影響を定量的に推定したか?データの解析に おいて交絡因子が適切に管理されていたか。

・研究の報告は適切であり、透明性の原則とSTROBE 声明(または同様のツール)のガイドラインに従っているか。

研究の評価は、それぞれの研究が持つ可能性のある潜在的な限界の特性と、疫学的データベースの全体的な整合 性の評価を示すものでなければならない。

さらに、他の既知のリスク因子に関する健康影響の特性と特異性は、リスク評価目的のためのヒトデータの評価に影響を与える可能性があり、特に誘発期間や潜伏期間の長い慢性的な影響のような複雑な健康エンドポイントの場合には、その評価に影響を与える可能性がある。

表 2 は、単一の疫学研究で評価すべき主なパラメータと、各パラメータの関連する程度(低、中、高)を示している。 特定の科学的考察はケースバイケースで適用されるべきであるが、これらの基準を厳格かつ明確な方法で実施するこ とは非現実的である。

パラメータ	高	中	低
試験のデザ インと実施	前向き研究 特定の仮説(化合物と 健康影響の特定)	症例対照研究。ばく露または 健康影響評価を十分にカバー していない前向き研究	横断、生態学的研究 症例対照研究では、ばく露や健康 影響評価が十分にカバーされて いない
集団	ランダムサンプリング。十分な検出 力を保証するのに十分な大きさの サンプルサイズ	疑わしい研究検出力、詳細に 正当化されていない	研究集団の選定方法についての 詳細な情報がない
	母集団の特性が十分に把握されて いる(脆弱なサブグループを含む)	標的集団の代表的なサンプル ではない 母集団の特性が十分に解明さ れていない	母集団の特徴が十分に解明され ていない
ばく露評価	検証された方法を用いた正確かつ 精密な定量的ばく露評価(ヒトのバ イオモニタリングまたは外部ばく 露) 被験者が回答した化学物質ばく露 に関する有効なアンケート及び/ま たはインタビュー	特定のマトリックス中の非有 効なサロゲートまたはバイオ マーカーと外部ばく露 被験者または代理人が回答し た化学物質ばく露に関するア ンケート及び/またはインタ ビュー	乏しいサロゲート 質の低いアンケート及び/また はインタビュー;化学物質のグル ープについて収集された情報 化学物質に特化したばく露情報 は収集されていない;農薬の使用 の有無の一般的な評価
健康影響評 価	有効で信頼性の高い健康影響評価 研究集団において標準化され、妥当 性が確認されていること カルテまたは診断結果が記載され	標準化された健康影響、母集 団で有効性が確認されていな い、またはスクリーニングツ ール、またはカルテが不明確	標準化されていない、検証されて いない健康影響 不適切な健康影響、または自己報 告された健康影響

表 2:疫学的観察研究の重み付けのための研究の質に関する考察<sup>(a)、(b)</sup>

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	ていること	な場合	
交絡因子コ ントロール	科学的な課題に関連する重要な交 絡因子と標準的な交絡因子の適切 なコントロール 明らかに示された交絡因子を慎重 に考慮	交絡因子は部分的にコントロ ール 交絡因子と標準変数を中程度 にコントロール 科学的な課題に関連するすべ ての変数が考慮されているわ けではない	研究のデザイン及び解析段階で 潜在的な交絡因子及び効果修飾 因子をコントロールしていない
統計解析	研究デザインが適切であること、適 切なサンプルサイズに支えられて いること、データを最大限に利用し ていること、よく報告されているこ と(選択的ではない) 交絡因子をコントロールするため の統計的手法が用いられており、調 整済み及び未調整の推定値が提示 されている サブグループと相互作用解析が実 施されている	受け入れ可能な方法、情報を 失う分析的な選択、明確に報 告されていない 事後分析を実施したが、明確 に示された	記述的統計、または二変量分析の 疑わしいものだけが作られてい る 比較が行われていない、または明 確に記載されていない 分析の不備(多変量解析など)
報告	材料と方法の主要な要素と結果は、 十分に詳細に報告されている 研究の各段階における参加者数が 報告されている 調査中の関連性のについて信憑性 のあるメカニズムが示されている	材料と方法のいくつかの要素 や結果は、十分な詳細が報告 されていない 結果の解釈は中程度に対応	報告の不備(効果推定値の解釈、 交絡因子コントロール) 選択的報告 ばく露と健康の関係に影響を及 ぼす可能性のある関連因子に関 する情報の不足 推論目的の焦点がずれている 正当化された結論ではない

(a):パラメータ全体の総合的な評価に基づく総合的な研究品質ランキング。

(b): Muñoz-Quezadaら(2013)とLaKindら(2014)を順に引用したUS-EPA(2016)からの引用。

上記の評価が、疫学研究が評価され定量的にまとめられているエビデンス総合演習の一部である場合、農薬ばく露 に関連する絶対的リスクをより正確に推定し、さらに定量的なリスク評価を行うことが可能となる。

農薬疫学データの場合には、バイアスのリスクと信頼性に関してヒトデータを整理するための第一段階として、3 つの 基本的なカテゴリーが提案されている<sup>16</sup>。(a)バイアスのリスクが低く、信頼性が高い(上記の品質要因のすべて、また は大部分が軽微な方法論的限界で対処されている);(b)バイアスのリスクが中程度で信頼性が中程度(上記の品質要 因の多くが中程度の方法論的限界で対処されている);(c)バイアスのリスクが高く、信頼性が低い(結果の妥当性を低 下させる、または潜在的な因果関係をほとんど解釈できない、といった重大な方法論的限界や欠陥があるため)。後者 の研究は、主にばく露評価の不備、ばく露及び/または健康影響の誤分類、または関連する交絡因子の統計的調整 の欠如により、リスク評価には受け入れられないと考えられている。リスク評価は、十分に定義されたデータ品質基準を 満たしていない疫学研究の結果に基づくべきではない。さらに、予備的研究の結果は、リスク評価に使用する前に、将 来の研究で確認する必要がある。

### 6.3. 疫学研究のエビデンスの強さの評価

このセクションでは、農薬ととト健康影響との関連性に関する様々な疫学研究から得られた結果を組み合わせて要約 することに関連したいくつかの重要な問題について簡潔に論じている。

疫学研究の重み付けのアプローチは、主に修正された Bradford Hill 基準に基づいている。これは、事象と起こりえる結果(強さ、一貫性、特異性、時間性、生物学的勾配、妥当性、統一性、実験と類推)との間の潜在的な因果関係を示すエビデンスを提供する条件のグループである(表 3)。明らかに、これらの基準を満たせば満たすほど、意味のある

<sup>16</sup> これらのカテゴリーは、現在 EFSA が農薬有効成分のピアレビューに使用している、許容可能、補助的、非許容のカテゴリーに準拠している。www.efsa.europa.eu/efsajournaEFSA Journal 2017;15(10):5007

関連性の証拠としてその関連性を提起する根拠が強くなる。しかし、Bradford Hill は、因果関係とは何かを明確にすることを目的とせず、基準を十分に、あるいは絶対的に必要なものとしてかんがえず、単に常識的な評価の中で考慮することが重要であると考えている。

表 3:エビデンス統合のための修正された Bradford Hill 基準に基づく WOE	解析の考察
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カテゴリー	考察事項
関連性の強さ	関連性の強さ(関連性の大きさだけでなく、統計的有意性も)の評価には、基礎となる方法の 検討、文献の WOE との比較及びここで議論されている他の基準を含む他の背景的要因の考慮 が必要である。
関連性の一貫性	関連性は、複数の独立した研究、特に異なる計画で、異なる状況下で異なる集団で実施された 研究において一貫性があるべきである。この基準は、現代のデータ統合に照らして、すべての エビデンス系統(疫学、動物実験、in vitro システムなど)にまたがって一貫性のある結果に も適用される。
特異性	特定の結果をばく露に結びつけるエビデンスの独自の基準は、因果関係についての強力な議 論を提供できるようになり、データ統合の背景の中で新たな興味深い意味合いを持つように なったかもしれない。データの統合は、複雑なばく露に関連した様々な結果の中から、いくつ かのメカニズム論的な特定性を明らかにするかもしれない。特異性の欠如は、疾患に関連する 特異的な薬剤を絞り込むのに役立つかもしれない。
時間性	薬剤へのばく露と適切な時間枠内での影響の出現との間の時間的順序のエビデンスは、因果 関係を支持する最良の論拠の一つを構成する。このように、2つの尺度間の時間的進行を確実 にする研究デザインは、因果関係の推論においてより説得力がある。
生物学的勾配(用量反 応)	より大きなばく露またはばく露の持続時間に関連した影響の増加は、因果関係を強く示唆し ている。しかし、その不在は因果関係を排除するものではない。
生物学的妥当性	実験的エビデンスに基づいた生物学的に信憑性のあるメカニズムによって説明され、支持されたデータは、関連性が因果関係にある可能性を強化する。しかし、メカニズム論的データの 欠如は因果関係に反するエビデンスとして捉えられるべきではない。
統一性	エビデンスの解釈は理にかなったものでなければならず、ばく露-疾病パラダイムの下で問題となっている健康影響の生物学について知られていることと矛盾するものであってはならない。もしそうであれば、ヒトに最も近い種の方がヒトとの関連性が高いと考えるべきである。
実験的エビデンス	無作為化実験の結果は、他の研究デザインに基づく結果よりも因果関係の強いエビデンスを 提供する。あるいは、非実験的研究からの関連は、関連から導き出された無作為化予防が結論 を導く場合には、因果関係があると考えられる。
重要事象の結果	特定の健康影響について確立された MOA/AOP の基礎となる重要事象(すなわち、in vitro、 in vivo、またはヒトのデータ源を組み合わせた測定可能なパラメータ)をそれぞれ明確に説明 する。完全に解明された MOA/AOP は、ヒト健康リスク評価に疫学研究を使用するための要 件ではない。

Höfler (2005)、Fedak ら (2015) 及び US-EPA (2016) からの引用。

予測的因果関係については、「事象 Y が事象 X の後に続いたので、事象 Y は事象 X によって引き起こされたに違いない」という論理的誤謬を避けるために注意を払わなければならない。Höfler (2005 年) は、より正確な「反事実」の定義を次のように引用している:「しかし、E があれば、D は発生しないか、発生しなかっただろうが、E があれば発生するだろう/しただろう」。しかし、記号論理を用いたより詳細な記述もある (Maldonado 及び Greenland、2002 年)。Rothman 及び Greenland (2008 年) は、「反事実効果の唯一の必須条件は、原因が効果に先行しなければならないという状態である」と述べている。結果または「影響」として提案された事象がその原因に先行している場合、事象間の関連性はあるかもしれないが、因果関係は確かにない」と述べている。

## 6.3.1. 疫学的証拠の統合

観察による研究のシステマティックレビューとメタアナリシスは、農薬の潜在的なハザード、ばく露反応の特徴、ばく露 シナリオとばく露評価の方法、そして最終的にはリスクの特性評価の理解を強化する情報を提供することができる(van den Brandt、2002 年)。システマティックレビューは、特定のトピックに関するすべての関連研究を特定し、評価し、統 合することでバイアスを低減することを目的とした、詳細で包括的な計画と事前に定められた検索戦略を伴う。システマ ティックレビューの主なステップは以下の通りである:研究課題の策定、包含基準と除外基準の定義、異なるデータベ ースにまたがる研究の検索戦略、事前に定めた戦略に従った研究の選択、データ抽出とエビデンス表の作成、選択した研究の方法論的品質の評価、バイアスのリスクを含めた評価、データの統合(研究が許せばメタアナリシスを行うこともできる)、結果の解釈と結論の導き出し(EFSA、2010 年 a)。しかし、農薬疫学の分野では、標準化と調和が困難であるため、エビデンスの統合は困難である。それにもかかわらず、疫学研究の強固性と妥当性を評価する上で、エビデンス統合は極めて重要な役割を果たすべきである。

このエビデンスの評価に役立つ統計ツールが開発されている。ほぼ同一のばく露と転帰に関する複数の研究が利 用可能な場合、これらの研究は重要な科学的証拠を提供することができる。ばく露と転帰が研究間で定量化され、調 和されている場合には、類似した計画の個々の疫学研究からのデータを組み合わせることで、より正確なリスク推定値 を得るのに十分な検出力を得ることができ、不均一性の評価を容易にすることができる。適切なシステマティックレビュ ー及びエビデンスの定量的な統合を定期的に行う必要がある(例えば、世界がん研究基金のがんリスク因子のメタアナ リシスの継続的な更新のためのアプローチ<sup>17</sup>)。研究は、以前に発表された観察による研究の基準に従って評価され、 可能性のある選択バイアス、測定誤差、サンプリング誤差、異質性、研究デザイン及び結果の報告と提示について慎 重に検討されるべきである。

メタアナリシスとは、一般的に、異なる研究で報告された結果を組み合わせて比較するための統計的手法の集積を 示すために使用される用語である(Greenland 及び O'Rourke、2008 年)。メタアナリシスの技術は、小さな研究効果 や過剰な有意性バイアスなど、研究分野における多様なバイアスの存在を調べるために使用されることがある。しかし、 メタアナリシスは、各研究デザインに関連している可能性のある根本的なバイアスを克服するものではない(すなわち、 交絡、想起バイアス、または他のバイアスの原因が排除されない)。システマティックレビューやメタアナリシスが農薬の 影響について結論を導き出すことができる範囲は、含まれた研究から得られたデータや結果が有効かどうか、つまり検 討された研究の質に大きく依存する。特に、一貫したバイアスの結果として、オリジナルの研究間で一貫した結論が得 られれば、システマティックレビューでは偏った結論が得られることになる。同様に、無効な研究のメタアナリシスでは、 誤った影響推定値に狭い信頼性間隔が生じるなど、誤解を招く結果になる可能性がある。

レビューされた文献の基本的な研究の特徴を要約することに加えて、典型的なメタアナリシスには以下の要素が含まれるべきである。(a)対象となる各健康影響の平均影響量と影響量分布及び影響量分布の不均一性の検討(b)影響 量分布に存在する変動性を系統的に解析し、効果量の大小に関連する研究の特徴を特定するサブグループ解析(c) 引き出された結論の妥当性を評価するための出版バイアス解析及びその他の感度分析(Wilson 及び Tanner-Smith、 2014 年)。

メタアナリシスでは、基礎となる研究集団の影響量分布を適切に記述するモデルを指定することが重要である。意味のある影響量分布を用いたメタアナリシスは、定量的リスクをリスク評価モデルに統合するのに役立つ。従来の正規の固定影響モデル及びランダム影響モデルは、パラメータと共変量に条件付きで正規の影響量母集団分布を仮定している。このようなモデルは、全体的な影響量を推定するのには適切かもしれないが、影響量分布が非正規の形状を示す場合には予測には確実に適していない(Karabatsos 6、2015 年)。

## 6.3.2. 研究間の異質性を探索するツールとしてのメタアナリシス

異なる研究の結果を評価する際には、多くの側面を慎重に評価すべきである。メタアナリシスを行う研究者は、調査 の範囲を、考慮した研究を平均した関連性の大きさの結果に限定する傾向があります。その動機は、多くの場合、考慮 した研究を平均した関連性の強さの決定に限定する傾向がある。影響の個々の推定値は偶然性によって変化するた め、ある程度のばらつきは予想される。しかし、推定値は意味のある場合にのみ要約されなければならない。見落とさ れがちな重要な側面として、サブグループを超えた個人間の関連の強さの不均一性がある。研究間の不均一性は評 価され、存在する場合には定量化される必要がある(Higgins、2008 年)。メタアナリシスでは、異なる研究からの結果 の間の不均一性は、同質性と同じくらい有益であるかもしれない。観察された結果の矛盾の根底にある理由を探ること

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<sup>&</sup>lt;sup>17</sup>世界がん研究基金インターナショナル。継続的更新プロジェクト(CUP) http://www.wcrf.org/int/research-we-fund/ continuous-updateproject-cup

www.efsa.europa.eu/efsajourna

#### は、一般的に大きな理解につながる。

図1は、3つの農薬(A、B、C)のそれぞれが2つの研究のメタアナリシスで評価されている仮想例の3つのフォレストプロットを示している。各農薬の両方の研究が、最高の品質と科学的な厳密さを持っていると仮定している。バイアスが疑われない。

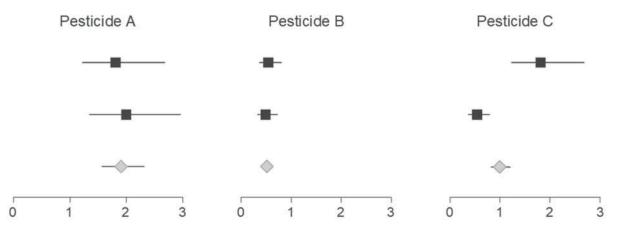


図 1:3 つの農薬(A、B、C)のそれぞれが 2 つの研究のメタアナリシスで評価されている仮想例のフォレストプロット。 各プロットの x 軸は、ばく露した個体とばく露していない個体を比較した、対象疾患の推定リスク比を表している。 四角は各研究における推定リスク比を示し、灰色のダイヤモンドは要約されたリスク比を示している。横線は 95%信頼区間を示す。

以下の文章には、図1の結果の解釈について、農薬を1つずつ、短くコメントしている。

- ・ 農薬 A へのばく露は、病気のリスクを2倍にするようである。結果は2つの研究の間で一致しており、信頼区間には帰無値である1が含まれていない。しかし、これらの結果は、(a)同じばく露と疾患について実施された他の研究では、リスク比が約2であること、または(b)個人のどのグループ(例えば、男性か女性か、若年者か高齢者)でもリスク比が2であることを暗示するものではない。
- ・ 農薬 B へのばく露は、病気のリスクを半減させるようである。結果は2つの研究の間で一致しており、信頼区間 には帰無値である1は含まれていない。しかし、これらの結果は、(a)同じばく露と疾患について実施された他 の研究では、(a)リスク比が約半分になること、または(b)個人のどのグループ(例えば、男性か女性か、若年者 か高齢者か)でもリスク比が約半分になることを暗示するものではない。
- ・ 農薬 C へのばく露は、一方の研究では病気のリスクが2 倍になり、他方の研究ではリスクが2 分の1 になるよう である。結果は2 つの研究の間で矛盾しており、また、信頼区間には帰無値である1 が含まれていない。しか し、これらの結果は、(a)同じばく露と疾患について実施された他の研究では、リスク比が約1 であること、また は(b) 個人のどのグループ(例えば、男性か女性か、若年者か高齢者か)でもリスク比が約1 であることを暗示 するものではない。

図1に示された結果は、どのようなエビデンスを提供できるか?

どのような研究で報告されたリスク比も、すべての関連因子がコントロールされている場合にのみ、他の集団に一般 化することができる(Bottai、2014年;Santacatterina及びBottai、2015年)。この背景では、関連因子とは、対象と なる健康影響に確率的に依存する変数のことである。例えば、心血管疾患は、若年者よりも高齢者の方が多い。したが って、年齢は心血管疾患の関連因子である。図1に示された結果から得られるエビデンスは、検討した各研究でこのス テップを踏んだ場合にのみ有効となる可能性がある。もしそうであれば、2つの研究のそれぞれで考慮された個人の特 定のグループでは、農薬Aへのばく露がリスクを2倍にするというエビデンスがある。リスク比がそれぞれの研究集団の 要約測定値であるならば、どの結論も一般化されるべきではない。しかし、農薬Aのリスク比がいかなる因子でも調整さ れておらず、基礎となる母集団が2つの研究で大きく異なっていた場合、関連因子が存在せず、農薬Aはどのサブグ ループの個人においてもリスクを2倍にするというエビデンスが残っている。 農薬 B はリスクを半減させるようであり、推定された信頼区間は農薬 A よりも農薬 B の方が狭い。しかしながら、農薬 A についての上記の条件のもとでは結果を一般化できる可能性は農薬 B についても維持されている。 農薬 C に関しては、フォレストプロットから、この農薬へのばく露が、一方の研究では個人のグループで病気のリスクを上げ、他方の研究ではリスクを下げるというエビデンスが得られた。 繰り返しになるが、リスク比がそれぞれの研究集団の要約測定値であるならば、どの結果も一般化されるべきではない。 農薬 C に関する2 つの研究間の矛盾の背後にある理由を調査することは、 農薬 A または農薬 B に関する研究間の類似性の背後にある理由を調査するのと同じくらい多くの科学的予測を提供することができる。

一般的に、図1の3つのパネルのそれぞれにある銀色のダイヤモンドのようなフォレストプロットによって提供される 全体的な要約評価尺度は、ほとんど科学的な関係を持たない。異なる研究の結果を評価する際には、多くの側面を慎 重に評価しなければならない。見落とされがちな重要な側面は、サブグループを超えた個人間の関連の強さの不均一 性である。研究を記述した出版物でサブグループ解析に関する情報が提供されている場合、これは慎重に評価される べきである。感度分析は、異なる研究で得られた結果を補完すべきである。これらの解析は、異質性と、情報とサンプリ ング誤差とともに、関連する因子を制御していない場合の影響を評価することを目的とすべきである。図2 に総観図を 示す。

バイアス	
測定誤差などの情報誤差、効果の大きさの倍率	
関連する要因	
どれが検討され、どれが検討されなかったか 各研究ではどのように分布していたか についての推論の結果がどの母集団であるか	
サンプリング誤差	
関心のあるパラメータの推定値のp値ではなく標準誤差	

#### 図 2:複数の研究を評価・比較する際に考慮すべき項目

## 6.3.3. ハザード同定のためのメタアナリシスの有用性

ヒトのデータはリスク評価の多くの段階で利用できる。単一の疫学研究(同じ農薬に関する追加研究が入手できない 場合)は、質の高い研究(表 2 に示す基準による)でない限り、単独のハザード同定のための情報源として使用すべき ではない。代わりに、システマティックレビューやメタアナリシス(必要に応じて)など、多くの研究をまとめたエビデンス統 合技術を利用すべきである。慢性疾患に関連するデータの定量的な統合のために多くのメタアナリシスが実施されて いるが、リスク評価モデリングへの応用はまだ限られている。

重要なことは、エビデンス統合は、現在のエビデンスの方法論的評価とバイアスのリスク評価を提供し、不確実性の 領域を強調し、強固で信頼性の高いエビデンスとの関連を特定することである。

図 3 は、疫学研究をリスク評価に適用するために提案された簡単な方法論を示している。最初の考慮事項は、同じ 健康影響を扱う異なる疫学研究を組み合わせる必要性である。これは、EFSA のシステマティックレビューのためのガ イダンス(EFSA、2010 年 a)で提案されている基準に従って行うことができる。次に、研究デザインと実施、母集団、ば く露評価、健康影響評価、交絡因子の管理、統計解析、結果の報告など、WOE 評価のための 6.2 節に記載されてい

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る要素に基づいてバイアスのリスクを評価する。信頼性が低いと分類された研究は、リスク評価のために受け入れられないと考えられる。残りの研究は重み付けを行い、ハザードの特定に使用する。

定量的データが利用可能な場合、メタアナリシスを実施して要約データを作成し、利用可能な、あるいは選択基準を 満たすすべての個々の研究の結果を組み合わせることで、統計的な検出力とリスク推定値(OR、RR)の精度を向上さ せることができる。メタアナリシスは、関連の大きさが検討した研究の平均値に決定するので、ハザード同定のためのよ り強力な基盤を提供する。さらに、特定の状況下では、健康影響におけるこれらの測定された差(OR、RR)を用量反応 関係に変換できるため、リスク特性の測定基準に移行する可能性がある(Nachman ら、2011 年)。実際には非常に珍 しいことではあるが、これにより、動物からの外挿法を使用することなく、ヒトにおける重大影響の同定や基準値の設定 が可能になる。

メタアナリシスでは不均一性が一般的であるため、どの研究を定量的に組み合わせることができるかを評価する必要 がある。異質性は、異なるサブグループにおける多様な影響を表す真正なものである場合もあれば、バイアスの存在を 表す場合もある。異質性が高い場合(I<sup>2</sup>が50%を超える場合)は、異なる情報源からのバイアスを集約するリスクが高い ため、要約尺度を得るために個々の研究を組み合わせるべきではない。感度分析及び/またはメタ回帰によって異質 性の原因を探るべきである。さらに、メタアナリシスにおける多様なバイアスの存在、例えば、小規模な研究効果、出版 バイアス、過剰な有意性バイアスなどを調べるべきである。基礎となる研究集団の影響量分布を適切に記述するモデ ルをみつけることが重要である。

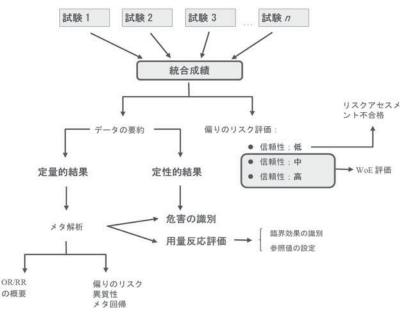


図3:疫学研究をリスク評価に活用するための方法論

#### 6.3.4. 潜在的な用量反応モデル化のための類似の疫学研究からのデータの蓄積

他の研究と同様に、単一の疫学研究から得られた結果は、複製によって検証する価値がある。複製の数が豊富な場合には、メタアナリシスによって複製された疫学研究の全セットを評価し、主要な健康影響事象について、研究間で結果が一貫しているかどうかを確認することについては価値があるかもしれない。このようなアプローチにより、因果関係の存在についてより強固な結論が得られるであろう。

ハザードが同定されると、リスク評価の次のステップは、異なるばく露レベルでの有害な影響のリスクを推定するための用量反応評価を実施することであり、特定の集団に対して健康への有害な影響が認められない濃度以下のばく露レベルを推定する。しかし、このステップでは、個人レベルでの完全に定量的な(または少なくとも半定量的な)ばく露データが必要である。定量的統合から得られた要約推定値は、研究間の相対比較が可能となるようなばく露の連続変数の変化(またはばく露のある一定割合の変化)に対する OR を示すものであれば、リスク評価にとってより有益であり、研

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究間の相対比較が可能となり、健康に基づく基準値を導き出すのに役立つ。このような枠組みの中でのみ、類似した計画のヒト研究からのデータを統合して、適切な用量反応曲線をモデル化するのに十分な力を得ることができる (Greenland 及び Longnecker、1992年; Orsini ら、2012年)。

逆に、メタアナリシス的アプローチは、すべての研究で同じ割合で含まれる有効成分へのばく露が必ずしも必要では ないため、ばく露を「はい」または「いいえ」(これまでにあり、または決してない)と解釈するメタアナリシスに基づいて複 合 OR が計算された場合には、限られた価値しかないかもしれない。これらのケースでは、メタアナリシスは一貫して農 薬ばく露に関連したリスクの増加を示しているが、リスク評価のためには、ばく露は特定の農薬クラスの影響を特性評価 する必要があり、同じクラス内でも効力が異なる可能性があるため、個々の農薬の影響を特性評価する必要がある (Hernándezら、2016 年)。

このアプローチは、Point of Departure を特定することを可能にし(例えば、ベンチマーク用量(BMD))、疫学研究 を定量的リスク評価に統合することに関連するであろう。現在、BMD モデリングは実験研究からの用量反応データの 解析に使用されているが、観察による疫学研究からのデータにも同様のアプローチを適用することが可能である (Budtz-Jørgensonら、2004年)。EFSA 科学委員会は、BMD アプローチは、実験研究や疫学研究からの用量反応 データを利用して潜在的なリスクをよりよく特徴付け、定量化するために、Reference Point を得るための NOAEL(no observed-adverse-effect level)アプローチと比較して、より科学的に進んだ方法であると結論づけている。このアプロ ーチは、原則としてヒトのデータにも適用可能である(EFSA Scientific Committee、2017年 b)が、対応するガイドラ インはまだ作成されていない。

観察による疫学研究からの用量反応データは、いくつかの点で典型的な動物試験の毒性データとは異なる可能性 があり、これらの違いは BMD の計算に関連する。ばく露データは、多くの場合、少数の十分に定義された用量群に当 てはまらないことが多い。ほとんどの実験研究とは異なり、観察による研究には完全に未ばく露の対照群が含まれてい ない場合がある。この場合、用量反応曲線を作成することは必ずしもばく露量ゼロでの観察を必要としないため、BMD アプローチが適用される。しかし、ばく露量ゼロでの反応は低用量外挿法で推定する必要がある。したがって、疫学デ ータから得られる BMD はモデル依存性が強い(Budtz-Jørgensen 6、2001 年)。

疫学データは、BMD アプローチを適用するためには、特に特定の農薬とそのばく露に影響を与えるという点で、十 分な品質のものでなければならない。このような BMD アプローチについては、明確なルールとガイダンス、モデルパラ メータの定義を考慮する必要があり、制御された実験環境からの BMD アプローチとは異なる可能性がある。BMD モ デリングアプローチは重金属やアルコールに関する疫学データに適用されているが(Lachenmeier ら、2011 年)、現 在のところ、農薬に関する個別の研究は、用量反応モデリングに使用するのに適しているものはほとんどなく、他の研 究と組み合わせて使用することはあまりない。しかし、今後も研究は実施され、同様の報告がなされ、それらの研究を蓄 積して、より強固な評価を行うことができるようにすべきである。

## 7. 多様なエビデンスの統合:ヒト(疫学データと警戒データ)と実験の情報

本節では、まず第 7.1 節で、実験研究や疫学研究に由来する主なエビデンスの特性の違いについて考察する。使用したアプローチは、EFSA Scientific Committee Guidance on WOE(EFSA Scientific Committee、2017 年 b) で推奨されているもので、これらの異なる情報を評価し統合するために、信頼性、関連性、一貫性の 3 つの段階を区別している。最初の段階では、疫学的研究(セクション 6 で述べている)や実験的研究(この意見書の範囲を超えている) である個々の研究の信頼性の評価で構成されている。次に、信頼性があると判断された 1 件以上の研究の関連性(エビデンスの確実性)を疫学(第 6 節に記載)と毒物学の原則を用いて評価する。次に、第 7.2 節では、WOE アプローチで検討された疫学的及び実験的研究からの様々な関連情報を、ヒトに対する一貫性と生物学的妥当性を評価するために、どのようにしてまとめるかを検討する。

## 7.1. 異なるエビデンスの起源と特性 実験的アプローチと疫学的アプローチの比較

農薬の規制リスク評価では、毒性影響に関する情報は、規則(EC)283/2013 及び 284/2013 で要求され、OECD ガイドラインに従って実施された一連の実験結果に基づいている。これらの実験は in vivo または in vitro で実施され ているため、規則(EC)1107/2009 に基づき申請者が提供することが要求されているように、農薬については常に質の 高い実験データが存在している。EFSA の有効成分のピアレビューによると、各エビデンスの信頼性を評価するために、 許容範囲、補足範囲、不許容範囲といういくつかのカテゴリーが設定されている。 in vivo または in vitro 毒性試験の データの質及び信頼性は、ハザード及びリスク評価のための試験の妥当性を判断するためのより構造的な裏付けをよ り良く提供する評価方法を用いて評価されるべきである。農薬の健康リスク評価に使用できるように、実験的研究の実 施と報告のための基準が提案されている(Kaltenhäuser 6、2017 年)。

標準化された試験ガイドラインや優良試験所規範(GLP、例えば OECD 試験ガイドライン)に従って実施された農薬 有効成分の動物(in vivo)試験は、通常、他の研究よりも信頼性が高いとされている。しかし、このような枠組みの下で 実施された研究の方がバイアスのリスクが低いというエビデンスはないため(Vandenbergら、2016年)、GLPと非GLP の両方を問わず、関連するすべての研究から得られたエビデンスも考慮し、重み付けを行うべきである。このように、査 読された科学文献からのデータは、方法論的信頼性を評価した後に十分な品質のものであれば、農薬有効成分の規 制リスク評価のために考慮されるべきである。WOE 全体への貢献は、試験系、試験計画、統計的方法、試験項目の特 定、結果の文書化、報告などの要因によって左右される(Kaltenhäuser ら、2017 年)。

ハザード及びリスク評価のための試験の妥当性を判断するためのより良い補助とするために、in vitro 毒性試験の 内部妥当性も評価されるべきである。in silico モデルは、構造活性相関(SAR)を導出し、ヒトにおける有効成分の作 用様式や作用機序の同定や特性評価のための現行の毒性試験を補完するために使用することができる。これらの代 替毒性試験(及び非試験)アプローチは、動物データがない場合、例えば農薬の潜在的な神経発達や内分泌かく乱 作用をスクリーニングし、動物試験の信頼性を高めるのに役立つ可能性がある。規制目的のために動物試験の数を最 小限にすることが求められていることを考慮すると、動物試験以外の情報はWOE評価に使用できる適切な独立したエ ビデンスを提供することができる。

化学物質のビト健康への影響から特定の化学物質へのばく露に伴うリスク、化学物質の混合物の毒性、毒性反応の バイオマーカーの関連性、あるいは新しい毒性試験法の評価まで、多くの毒性学的問題がシステマティックレビューの 対象となっている(Hoffmann ら、2017 年)。例えば、以前の Scientific Opinion では、EFSA は AOP アプローチの 枠内で毒性学的メカニズムの決定にシステマティックレビューを使用した(Choi ら、2016 年; EFSA Scientific Committee、2017 年 c)。

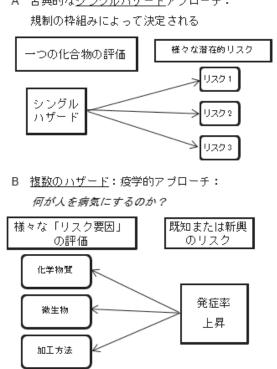
有効成分の毒性データの他に、食事や飲料水を通じてヒトにばく露された場合には、代謝物や残留物についてもデ ータが必要となる場合がある。これらの研究から得られた結果は、食品消費やその他のばく露源から推定される予想さ れるヒトばく露量との関連で検討される。このアプローチの長所は、動物の代謝経路が必ずしもヒトの代謝経路と類似し ているとは限らないにもかかわらず、in vivo 試験では潜在的な毒性代謝物を考慮していることである。

実験動物を用いた実験研究は、交絡因子が排除されるように計画された研究であるが、疫学研究では必ずしもそうと は限らない。しかし、規制研究に使用される動物は、一般的には近親交配されて遺伝的に同一であり、制御された環 境のために、定量的及び定性的な化学物質感受性のすべての特性を欠いている。それにもかかわらず、ヒト疾患の動 物代替は、その科学的妥当性とヒトへの外挿性が問われており、動物データとヒトの結果との間にしばしば見られる相 関性の欠如は、疾患経路や遺伝子発現プロフィルにおける疾患誘発性の変化における実質的な種間差に起因してい ると考えられている(Esch ら、2015 年)。そのため、多くの実験モデルは複雑な多因子疾患を捉えていないため、動物 からヒトへの外挿はかなりの不確実性を伴うものとなっている。したがって、現在のリスク評価はその特性上、予測的なも のであり、化学物質に特化したものであり、ヒトは環境、食事、職業上の源からの多数の化学物質にばく露されているた め、あるいは異なる毒物動態の違いのために、十分ではないかもしれない。動物からヒトへの外挿の不確実性を考慮し て、規制当局のリスク評価のアドバイスは、単に安全性が確認されている関連の Point of Departure (NOAEL、 LOAEL、または BMDL)を考慮するのではなく、不確実性因子(UF)を用いてこれらの値を下げ、急性毒性または慢性毒性の安全な参照用量値を提案するものである。

実験動物を用いた研究の限界を考えると、たとえそれ自体に限界があるとしても、「実社会」での疫学研究が必要で ある。疫学的研究では、集団のばく露の真の範囲(または推定された範囲)を組み入れているが、これは通常、一貫し た速度と用量の大きさで発生するのではなく、断続的で一貫性のない用量で発生する(Nachman ら、2011 年)。疫学 的研究は実社会のばく露に基づいているため、実際のヒトのばく露についての予測を提供し、それを疾病に結びつけ ることができ、種を超えた外挿に関連する不確実性を回避することができる。したがって、リスク評価は、優れた植物保 護対策 (Good Plant Protection Practice)と現実的な使用条件に基づいて行われるべきであると規定した規制 1107/2009の第4条の要件に対応していると言える。このように、疫学的研究は、高用量外挿の必要性を回避しつつ、 問題の定式化とハザード/リスクの特性評価を支援する(US-EPA、2010 年)。

したがって、疫学的研究は、(a)動物モデルでは検出が困難な特定のとトの健康影響との関連性を特定する。(b)動 物モデルで特定された影響のとトへの関連性を明らかにする(c)動物モデルが利用できない、または限定された、健康 影響を評価する能力を提供する(Raffaele ら、2011 年)。疫学的証拠は、十分に強固な農薬の疫学的研究が利用可 能な場合にのみ、動物実験によるエビデンスよりも考慮される。しかし、疫学研究では、健康影響に影響を与え、結果 を混乱させる様々な要因が常に存在する。例えば、疫学的データが農薬製剤へのばく露が有害であることを示唆して いても、通常、農薬へのとトばく露を正確に評価することの複雑さから、どの成分が原因であるかを特定することはでき ない。一部の製剤補助剤は本質的には毒性がないが、有効成分の毒物動態を変化させる場合には、毒性学的に関連 性がある可能性がある。さらに、ばく露に関連した測定されていない因子による交絡を完全に排除することはできない が、仮説的な交絡因子(まだ認識されていない)は実際の交絡因子ではない可能性があり、リスク(または効果量)の推 定値に意味のある影響を与えるためには、疾患やばく露と強く関連していなければならないが、必ずしもそうとは限らな い。

多くの病気は複数のリスク因子と関連していることが知られているが、脆弱なシステムに対する個々の農薬のハザードの影響を評価するためには、通常、ハザードごとのアプローチが考慮される(図 4A)。特に、単一リスク解析では、特定の条件下で発生する特定のハザードやプロセスに起因する個々のリスクを決定することができるが、異なる環境ストレス要因(自然現象または人為的要因のいずれか)によって引き起こされる複数のリスクを統合的に評価することはできない(図 4B)。リスク評価は、複数の有害な健康影響の同時発生のエビデンスを評価するための手順を開発することが有用である(Nachman ら、2011 年)が、これはよりヒトの環境で起こることと一致している。これらの理由から、適切に実施されれば、疫学研究はリスク評価プロセスとの関連性が高い。



# A 古典的な<u>シングルハザード</u>アブローチ:

#### 図 4:古典的な毒性学的研究と比較した場合の疫学的研究の役割

疫学的データと並行して、特に急性毒性については、警戒データが追加のエビデンスの流れを提供することができ る。通常、症例は十分に文書化されており、リスク評価のさまざまな段階で情報を利用することができる。これらの情報 には、ばく露のレベルと期間、臨床経過、因果関係の評価などが含まれる。重度のケースでは、毒素及び/または代 謝物が通常、血液または尿中で測定され、動物データとの比較が可能であり、場合によっては毒性値を設定することが できる。

要約すると、実験的研究、疫学的研究、及び警戒データは、エビデンスを収集し評価するための 2 つの異なるアプ ローチを表している。すなわち、実験的な研究計画と比較的均質な代理母集団を用いた(通常は単一物質に対する) 管理されたばく露から得られるものと、非実験的な研究デザインを用いた混合ばく露条件(及び変化する)から不均質 な対象集団で観察される変化を反映したものである(ECETOC、2009 年)。 疫学と毒物学は、それぞれがヒトへのハザ ードの特定に重要かつ異なる貢献をしている。このことは、両方のエビデンスの流れを補完的なものにしており、それら を組み合わせることで強力なアプローチが可能になる。動物実験は常に疫学的研究の解釈に情報を与えるものである べきであり、その逆もまた然りであるため、これらを独立して研究・解釈すべきではない。

## 7.2. ヒトの観察データと実験動物の実験データの重み付けの原則

信頼性の高いとト(疫学的または警戒研究)研究の特定し、プールされたとト研究の関連性の評価した後、関連性が あると判断された別個のエビデンスを、同様に関連性があると判断された他のエビデンスと統合する必要がある。

したがって、最初の検討事項は、検討対象の健康影響がどれだけ毒性学的及び疫学的研究でカバーされているか ということである。特定の健康影響/エンドポイントについて動物試験とヒト試験の両方が利用可能であると考えられる 場合、これは、様々なエビデンス源の重み付けに先立って、個々の試験が信頼性とエビデンスの強固さを評価されるこ とになる(疫学的研究については、それぞれ 6.2 項と 6.3 項)。異なるデータセットは補完的で確実なものであるが、個 別には不十分な場合があり、ヒトの健康リスクを適切に特定するための課題となる可能性がある。良好な観察データが 不足している場合は、実験データを使用しなければならない。逆に、実験データが利用できない場合や、既存の実験 データがヒトに関連していないことが判明した場合には、リスク評価は利用可能で適切な観察による研究に頼らなけれ ばならないかもしれない。

リスク評価のために、複数のエビデンス(特にヒト研究と実験研究)から得られたデータを体系的に統合するためのフレームワークが提案されている(図 5)。このような統合は、修正された Bradford Hill 基準(表 3)を用いて、関連性、一 貫性、生物学的妥当性を考慮した WOE 解析に基づいている。ヒトと動物のデータを比較解釈するためには、この枠組 みは以下の原則に依存すべきである(ECETOC、2009 年; Lavelle ら、2012 年)。

- エビデンスの全体を評価すべきであるが、信頼性があると判断された研究(許容可能なエビデンスまたは補足的エ ビデンスに分類された研究)のみがさらに検討される。とト研究または実験研究から得られたデータの信頼性が低い(許容できないと分類される)と考えられる場合は、リスク評価を行うことはできない。
- ・複数のエビデンスが関連していることが判明した場合には、WOE アプローチに従うべきである。農薬有効成分については、OECDの試験ガイドラインに従った実験研究は、それに反するエビデンスがない限り、信頼性が高いとみなされる。動物実験からのエビデンスの強固さは、代替的な農薬毒性試験または非試験方法(例えば、それぞれ in vitro 試験と in silico 試験)に高い信頼性がある場合に向上させることができる。疫学的証拠については、メタアナリシスを実施することで、個々の研究よりも効果の大きさをより正確に推定でき、また、研究間のばらつきを調べることができる(第6.3節参照)。
- 次に、評価されるステージに関連性が高いと判断された研究は、データがヒトまたは動物の研究からのものである かどうかに関わらず、より重要視されるべきである。ヒトのデータが最も関連性が高く、作用機序的な科学的根拠に 支えられている場合には、リスク評価の各段階で優先されるべきである。ヒトデータと実験データの関連性が同等ま たは類似している場合には、どのデータセットが優先されるかを判断するために、それらのデータの一致性(エビ デンス系統間の一貫性)を評価することが重要である。
  - ーヒトデータと実験データが一致している場合、リスク評価では、ハザードの特定(例:両方とも同じハザードを示す)またはハザードの特性評価(例:両方とも同じような安全用量を示唆する)のいずれにおいても類似した結果が得られるため、すべてのデータを使用すべきである。このように、両者は互いに補強し合うことができ、両方のケースで同様のメカニズムを想定することができる。
  - -不一致の場合、フレームワークはこの不確実性を考慮する必要がある。ハザードの特定については、一般的 にハザードの存在を示唆するデータが優先されるべきである。用量反応については、低い許容量のデータが 優先されるべきである。不一致が生じた場合には、この相違の理由を検討すべきである。その理由が基礎と なる生物学的メカニズム、またはヒトと動物モデル間の毒物動態学的差異に関連している場合は、リスク評価 の信頼性が高まる。逆に、その理由が理解できない、あるいは説明できない場合は、リスク評価の信頼性が 低下するかもしれない。このような場合には、生物学的エビデンスにおける矛盾に対する考察を行うべきであ る。

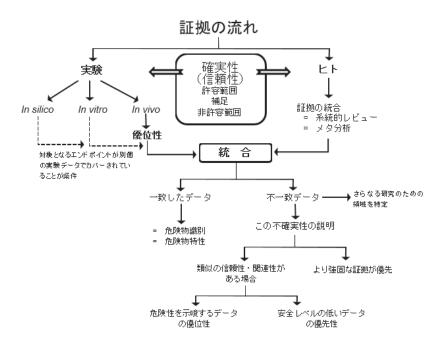


図 5:リスク評価のためのヒトと動物のデータ統合の方法論

疫学研究はリスクを解析するための補完的なデータを提供するものであり、十分に計画された毒物学的 in vivo 研 究やメカニズム研究と併せて文脈を考慮する必要がある。複数のエビデンスを統合して得られたエビデンスの総合的な 強度は、少なくとも単一のエビデンスで得られた最高のエビデンスと同程度になる。この統合的アプローチは、毒性学 的証拠と疫学的証拠をどのように重み付けして統合するかについての明確な指針を提供するものである。これは複雑 な作業であり、疫学的データが多因子性、多発性、慢性の疾患を対象としている場合には、毒物学的モデルや疾患特 異的動物モデルが限られているため、さらに困難な作業となる。

## 7.3. 異なる起源のエビデンスの重み付け

WHO/IPCS は、WOE アプローチをリスク評価に関連すると考えられるすべてのエビデンスが評価され、重み付けされるプロセスと定義している(WHO/IPCS、2009 年)。WOE アプローチは、化学物質のリスク評価を例にとると、異なる一連のエビデンス(in vivo、in vitro、in silico、母集団研究、モデル化されたばく露データや測定されたばく露データなど)の評価を必要とする。課題は、体系的で一貫性がある明確な方法で、これらのタイプのエビデンスを重み付けすることである(SCENIHR、2012 年)。重み付けは、形式的には定量的なものであってもよいし、リスクの基準を参照した分類に依存するものであってもよい。

EFSA のパネルと科学技術委員会による科学技術データの評価に WOE アプローチを使用するための明確な基準 を提供するために EFSA ワーキンググループが設立された(EFSA、2015 年 b)。このワーキンググループの目的は、 個々の研究がどのように選択され、どのように重み付けされるべきか、結論に到達するためにどのように知見を統合し、 結論に関する不確実性をどのように特定するかについて、利害関係者に支援を提供することであった。

WOE アプローチは、DAR や RAR のピアレビュープロセスにおける農薬のリスク評価において一貫して考慮されて いない。構造化された WOE アプローチを用いずに専門家の判断だけで行うことがより一般的になっている。いくつか の例を挙げると、グリホサートのピアレビュー(EFSA、2015 年 c)では、報告者である加盟国(RMS)は、疫学的データ を含む産業界または公的文献からのすべてのデータを考慮し、確立された事後基準と、探索された毒性の各エンドポ イントについて「全体的な」NOAELを提案するために利用可能なすべてのデータを考慮した特定の WOE アプローチ を採用している。

US-EPA は最近、「健康リスク評価にヒトの疫学的データと事象データを組み込むためのフレームワーク」に従って、

農薬のクロルピリホスのピアレビューに WOE アプローチのための特定の基準を適用した。この特定のケースでは、実験的毒性研究、疫学研究、生理学的ベースの薬物動態・薬力学(PBPK-PD)モデリングを含む多くのエビデンスを横断して、定量的・定性的な知見を統合するために WOE 解析が実施された。クロルピリホスは、規則(EC)No 1107/2009に基づく文献検索に関する EFSA ガイダンスの例としても使用されている。さらに、EFSA の結論(EFSA、2014年a)は、2011年に出された最初の結論を修正するために US-EPA のレビュー(2011年)を考慮に入れている。

まとめると、根本的な因果関係の可能性を高めるための組織的なツールとして、修正された Bradford Hill 基準を使用して、利用可能な科学的データを評価するために、より広範なWOEアプローチを適用することができる(表 3)。疫学は因果関係の立証にますます貢献しているが、この目的のための重要なステップは、生物学的妥当性の立証である(US-EPA、2010年; Adami ら、2011; Buonsante ら、2014年)。

## 7.4. 健康影響の基礎となる生物学的メカニズム

生物学的メカニズムとは、農薬とその生物学的標的との相互作用に続く健康影響につながる主要なステップを記述 したものである。毒性のメカニズムは、健康への悪影響につながる主要なステップとして記述されている。影響につなが るすべてのステップを理解する必要はないが、化学的相互作用に続く重要なイベントを特定することは、メカニズム(健 康に悪影響を及ぼす場合の毒性)を記述するために必要である。多くの疫学研究で農薬ばく露と慢性疾患との関連性 が示されているが、ヒトの疫学的観察にメカニズム的な裏付けと生物学的な妥当性を与えるためには、補完的な実験研 究が必要である。実験でのばく露は、実験動物の生物学的メカニズムがヒトで発生することを条件に、ヒトの集団に関連 するものでなければならない。

疫学研究の解釈の一部として生物学的妥当性を確立することは重要であり、最新の技術とアプローチを活用すべき である(7.6節)。この意味では、AOPの枠組みは、毒性結果の基礎となる生物学的メカニズムを調査し、実験研究と観 察による研究の両方で観察された関連性の因果関係を知るために、異なる情報源からの複雑な情報を体系的に整理 し、統合するためのツールとして利用できる(7.5節)。

農薬の潜在的な毒性作用の基礎となる生物学的メカニズムや経路について特定の情報を提供するためのデータの 利用は、特定の健康影響に関連した生物学的機能について調査された農薬化学物質のみであるため、限られたもの である。特に、同等の動物実験の結果の間に一致点がある場合や、異なる化学物質が同じ毒性パターンを示す場合 には、作用機序(MOA)仮説を立てることが可能な場合がある。毒物と標的臓器を特定することは、考えられる影響の 用量反応曲線とその時間的関係と同様に不可欠である。毒性につながるさまざまな主要事象と MOA 仮説を特定でき れば、ヒトに対するこれらの事象の妥当性を評価することが可能になることもある(ECETOC、2009年)。

農薬のスルホキサフロル (Sulfoxaflor) は、MOA が広範囲に研究されてきた例であり、2014 年 11 月に開催された ECHA/EFSA MOA/HRF ワークショップでも広く取り上げられている。スルホキサフロルはラットとマウスの両方で肝発 がん性を誘発した。これらの肝腫瘍に対する MOA を決定するための試験は、発がん性試験が終了する前または終了 するまでに MOA データが入手できるように、標準的な毒性試験のバッテリーの一部として、統合的かつ前向きな方法 で実施された。WOE アプローチで評価された MOA データは、スルホキサフロルの齧歯類での肝腫瘍の MOA がヒト では発生しないことを示している。この理由から、スルホキサフロルはヒトの潜在的な肝臓発がん性物質ではないと考え られている。

さらに、MOA データが影響の可能性がないことを示す場合もある。仮に、ヒトでは有害な影響が発生する可能性が ないことを示す生物学的データがある場合は、疫学研究の解釈に反映させる必要がある。それにもかかわらず、害虫と ヒトの間の一次標的部位選択性は農薬の安全性において重要な役割を果たしているが、哺乳類における二次標的も 考慮しなければならない。

複数の農薬にばく露した場合、リスクを組み合わせるかどうかの判断は、農薬が共通の毒性メカニズム(同じ標的組織で同じ分子標的に作用し、同じ生化学的作用機序で作用し、共通の毒性中間体を共有する)を共有しており、同じ重大影響を引き起こす可能性がある場合、あるいは単に同じ標的臓器を共有しているという観察に基づいて判断することができる(EFSA、2013年 a,b)。しかし、累積リスク評価は本意見書の範囲を超えている。

# 7.5. 有害転帰経路(AOPs)

AOP の解析法は、リスク評価に有用な方法で、関連する化学的、生物学的、毒物学的な情報を収集し、評価するた めの枠組みを提供している(OECD、2013 年)。AOP は、化学物質と生物学的標的との相互作用(標的分子への作用 (Molecular initiating event:MIE))から、ヒトの健康に関連する in vivo での有害な事象に至るまでの一連の重要 事象として定義することができる。これらの重要事象はすべて MOA の必要な要素であり、経験的に観察可能であるか、 またはそのような事象の生物学的ベースのマーカーを構成するものでなければならない。したがって、AOP は、リスク 評価に関連する生物学的組織のレベルで、1 つの MIE から 1 つの有害な事象に至る直線的な経路である。AOP の 目的は、因果関係の連鎖において、1 つの MIE から 1 つの有害な事象へとつながる重要事象の段階を記述するため の、柔軟なフレームワークを提供することである(EFSA PPR パネル、2017 年)。重要な事象は実験的に測定可能で なければならず、有害な事象は通常、in vivo での OECD 試験ガイドラインに関連している。しかし、場合によっては、 有害な事象が試験ガイドラインに記載されている先端エンドポイントよりも低いレベルの生物学的組織のレベルにあるこ ともある(OECD、2013 年)。

特定の MIE はいくつかの毒性影響につながる可能性があり、逆に、複数の MIE は同じ毒性影響に収束する可能 性がある。しかし、各 AOP は 1 つの MIE と 1 つの毒性影響象のみであるが、無制限の数の中間ステップを伴うことも ある(Vinken、2013 年)。生物学的組織の異なるレベルでの重要事象は、同じレベルの組織での複数の事象よりも大 きな WOE をもたらすことに留意すべきである(OECD、2013 年)。

毒性反応に関与する重要な生化学的ステップは、関連する科学文献の詳細な調査や実験研究から同定され、検索 される。構造データ、「オミクスベース」データ、in vitro、in vivo、あるいは in silico のデータなど、あらゆるタイプの情 報を AOP に組み込むことができる。しかし、in vitro データよりも in vivo データの方が優先され、対象となるエンドポ イントは代替エンドポイントよりも優先される(Vinken、2013 年)。特定された AOP は、生物学的に妥当性をもつ必要 があるため、正常な生物学的プロセスと両立しないものであってはならない。

定性的 AOP (AOP 開発のための OECD ガイダンスに従った WOE の組み立てと評価を含む AOP として意図され ている)は、経路に悪影響を与える農薬へのばく露と有害な影響との間の関連性の生物学的妥当性を裏付ける(また は裏付けがないことを特定する)ことによって、疫学研究をリスク評価に統合するプロセスの出発点であり、標準的なア プローチであるべきである。したがって、定性的な AOP は、メカニズム的知見に基づく疫学研究の生物学的妥当性を 裏付けるために、ハザード同定の目的のためだけに開発される可能性がある(EFSA PPR パネル、2017 年)。

AOP フレームワークは、異なる情報源から収集された複雑な情報のレビュー、整理、解釈を行うための柔軟で透明 性の高いツールである。このアプローチには、因果関係の推論に関連する不確実性を定性的に特徴づけ、不確実性 を低減するためには、追加のメカニズム的研究や疫学研究がより効果的であるかどうかを特定するという付加的な利点 がある。したがって、AOP フレームワークは、有害な影響が生物学的にもっともらしいかどうかを探るためのリスク評価に 有用なツールである。生物学的に妥当性を解析する目的では、AOP は重要なツールとなり得る。特に、規制上の動物 を用いた毒性試験が陰性であっても、疫学研究で観察された先端エンドポイント(または関連するバイオマーカー)の 評価が AOP に基づいて不十分であると考えられる場合には、AOP は重要なツールとなり得る。先端エンドポイントをメ カニズム的に記述することにより、AOP はリスク評価におけるハザードの特定と特性評価のステップに貢献する。AOP フレームワークは化学的には不明確な点があるため、MOA 及び/または試験・評価に関する統合的アプローチ (IATA)フレームワークで補完されれば化学物質特有のリスク評価をサポートすることになる(EFSA PPR パネル、 2017 年)。

AOPとMOAのデータは、疫学研究の結果を評価し、その結論に重み付けをするために使用できる。それらの結論 が生物学的メカニズムの深い理解と矛盾するものであろうと、あるいは単に経験的なものであろうと、一度確立された AOPや MOAの枠組みと一致する他の結論よりも、それらの結論の重要性は低く設定されるべきである。しかし、十分 に文書化された AOPの例は比較的少なく、完全な AOP/MOA の枠組みはリスク評価に疫学的研究を用いるための 要件ではない。

したがって、AOP は、動物実験で観察された末端の影響(apical effects)に大きく依存する現在の試験パラダイム

ではなく、メカニズムに基づくリスク評価への移行を促進するための重要な要素である。リスク評価のパラダイムをメカニズム的な理解へと移行させることで、単一の農薬のヒトへの健康影響を予測する上での動物データの限界を下げることになり、また、農薬ばく露の累積リスク評価に関する現在の取り組みを支援することにもなる(EFSA PPR パネル、2017年)。

#### 7.6. 毒性の基礎となる生物学的経路とメカニズムを特定するための新しいツール

毒性経路の解明は、特にバイオモニタリング、オミクス技術、システムバイオロジー(毒性学)の進歩から、顕在化した 疾患への毒性力学的進行における初期の生物学的摂動の新規バイオマーカーを特定する機会をもたらしている。疫 学におけるオミクスの革命は、早期効果の新しいバイオマーカーの可能性を秘めており、関連のメカニズム、生化学的 経路、因果関係を調査する機会を提供している。

疫学調査におけるバイオモニタリングデータの価値が認識されつつあることは、ばく露と転帰の客観的な尺度を提供 することで、誤分類を減らすのに役立つかもしれない。ばく露、転帰、感受性に関するバイオマーカーデータが増加し ている限り、疫学は農薬ばく露の関数としての毒性力学的進行の理解と最終的にはリスク評価に大きな影響を与えるこ とになるであろう。リスク評価者にとっての課題は、毒性力学的経路に沿った軽微で初期の変化が、下流への影響の可 能性の増大を示唆していることを認識することである(Nachman ら、2011 年)。オミクスデータは、農薬の影響を受ける 経路を特定することで MOA への理解を深め、リスク評価の第一段階であるハザードの特定を支援することができる。

生物学的サンプルのトランスクリプトーム(Transcriptomic,)、メタボローム(metabolomic)、エピゲノム (epigenomic)、プロテオミクス(proteomic)のプロファイリングは、環境化学物質の影響下での細胞の進化状態の詳 細な画像を、時には個々の分子レベルで提供し潜在的な健康影響との早期のメカニズム的な関連性を明らかにするこ とができる。今日では、オミクス技術の進歩が規制毒性学にもたらす課題と長所については、まだ研究が進められてい る(Marx-Stoelting ら、2015 年)。これらのバイオマーカーの特異性を評価するための明確なルールが必要である。

毒物学の文脈において、最も有用であり進歩しているオミクス技術は、MOAの解析とAOPの誘導、バイオマーカーの同定であり、これらはすべて疫学にも役立つ可能性がある。例えば、(a)トランスクリプトミクス:遺伝子発現(mRNA) プロファイルの比較は、バイオマーカーの発見、発現遺伝子の機能グループ(Gene Ontology カテゴリー)へのグループ化、または遺伝子セット分析に使用することができる。これらの手法により、生物学的メカニズムに関する様々な情報が得られる可能性がある。(b)プロテオミクス:試料のタンパク質プロファイリングを調べ、タンパク質の量や転写後の修飾を高度に分析し、ばく露後の生物学的経路の変化や疾患の発症に関連している可能性がある。(c)メタボロミクスは、核磁気共鳴分光法や質量分析法をベースにした技術を用いてデータを作成し、ソフトウェアやデータベースを介して分析することで、ばく露や疾患と相関のあるマーカー(分子シグネチャーや経路)を特定するものである。(d)エクスポソーム(個人が生活の中で受けたばく露の総量)の利用は、ヒトのバイオモニタリングに適したオミクス技術とバイオマーカーを使用することができるかもしれない。それにもかかわらず、これらの方法論の検証不足とそのコストの問題から、大規模な使用には限界がある。

オミクス技術を環境衛生研究に応用するには、研究デザイン、バリデーション、再現性、時間的分散、メタデータ分析 に特別な配慮が必要である(Vlaanderen ら、2010年)。大規模な研究では、生体サンプルで測定された分子プロファ イルの個人内変動は、時間の経過とともに大きく変動する遺伝子発現、タンパク質レベル、または代謝物のプロファイ ルの個人間変動よりも少ない変動を示す。これらの個人間変動がばく露変化に関連した変動よりも大きくならないことが 重要であるが、これが実現するかどうかは定かではない。

生物学的に意味のあるオミクスのシグネチャーは、オミクスーばく露及びオミクスー健康の関連性の研究を行うことに よって同定され、高度なリスク評価に有用なデータを提供する。このアプローチは、末端の毒性エンドポイントから、化 学物質によって誘発された分子/細胞応答の摂動に起因する毒性経路の初期の主要イベントへと移行することを支 持するものである(NRC、2007年)。

## 7.7. 疫学における新たなデータの機会

現在の技術的状況では、スマートフォン、テキストメッセージ、クレジットカードでの購入、オンラインでの行動、電子カ

ルテ、全地球測位システム(GPS)、スーパーマーケットの購買データなど、多くの情報源からの前例のない量のデータ のデジタル化と保存が可能になっている。これらのデータ源の中には、リスク評価のための貴重な情報を提供するもの もあるが、それらの多くには法的枠組みを超えて、科学的または規制上の目的のために使用することの倫理性につい て疑問が生じることもある。具体的な例としては、電子カルテ、職業や環境に関するアンケート、地理的な位置、健康や 社会保障番号など、機密性が高い、または特に保護されていると考えられる健康に関連する個人情報を含むデータが 挙げられる。これらの様々な形態の健康情報が容易に作成、保存、アクセスされている。ビッグデータは、研究者に多く のデータ源をまたいで記録を照合したり、リンクしたりする能力を提供する。健康情報と遺伝性情報のビッグデータ源の リンクは、病気の予測因子を理解するための大きな可能性を期待されている(Salerno ら、2017 年)。しかし、現在の方 法を用いてデータを体系的かつ効率的に処理、解析、解釈すること、あるいは大量データの中から関連するシグナル を特定することには課題がある(全米科学・工学・医学の環境研究・毒物学委員会が 2017 年の報告書で指摘している) <sup>18</sup>。

さらに、国民健康保険や退院データベースから抽出された薬剤費などの医療行政データは、農業人口調査や地理 的マッピングから抽出された農業活動に関するデータと相互にリンクさせることができる。このような情報が集団レベル でしか得られない場合もあることは認識されているが、個人レベル及び/または個人の習慣に関するデータを得ること が重要な課題である。

バイオバンクはまた、健康な集団や病気にかかった集団からの新たなデータ源を構成するものである。バイオバンク は、多様な研究目的のために保存されているとトの生物学的標本と関連情報の整理されたコレクションで構成されてい る。これらのバイオサンプルは、ばく露評価やばく露の再構成に有用なデータを生成する可能性のある新技術の応用 に利用可能である。研究の計画と実施が調和されていれば、データとサンプルはバイオバンク間で共有され、強力な 蓄積分析や反復研究を促進することができる(Burton ら、2010 年)。

深い表現型(deep phenotyping)を用いた大規模な疫学研究は、優れた表現型を持つ研究参加者と前述のデータ を結びつける前例のない機会を提供する。例えば、英国のバイオバンクでは、アンケート、病歴、身体測定データだけ でなく、50万人以上の参加者全員のゲノムワイドな関連データを持つ血液と尿のサンプルを保存し、病院の事例統計、 国の登録データ、プライマリーケアの記録とリンクさせている。大気汚染や騒音レベルに関する情報を得るために、参 加者の郵便番号を大気汚染や騒音の推定値にリンクさせている。さらに、これらのばく露に関する個人レベルのデータ を収集するために、個人ばく露モニタリングの試験的実施が行われる予定である。これらのアプローチは、地理的リンク、 購買・職業登録とのリンク、個人のばく露モニタリングのいずれかを通じて、農薬ばく露に関する情報を得るために拡張 される可能性がある。同様のバイオバンクは、他の多くのEU諸国にも存在する(http://www.bbmri-eric.eu/BBMRI-ERICは、ほとんどのEUの研究を収集している)。

# 8. 全体的な推奨事項

## 8.1. 単一の疫学的研究に関する勧告

疫学的研究を改善するための以下の勧告は、規制(EU)No 1107/2009 で言及されている「認知された基準」に準拠し、農薬のリスク評価に特に価値のあるものとすることを目的としている(「利用可能で、ばく露レベルとばく露期間に関するデータを裏付けとし、認識された基準に従って実施された場合、疫学的研究は特に価値があり、提出しなければならない」としている)。したがって、これらの勧告は、このような研究をどのように実施するかについての研究者のための実践的な指針としてではなく、農薬リスク評価にさらに活用するための研究を計画している研究者のためのものと考えることができる。

## **a)研究デザイン**(交絡を含む)

1)前向き疫学的デザインは因果関係推論のためのより強力なエビデンスを提供するので、農薬リスク評価のための 他の計画よりもこれらの研究が奨励される。

<sup>18</sup> 全米科学・工学・医学アカデミー、地球・生命研究部門、環境研究・毒物学委員会、リスクベースの評価に21世紀の科学を組み込む委員会。ワシントン(DC)。全米アカデミープレス(米国);2017年1月

- 2) 今後の疫学研究は、調査対象の課題に適切に答えるために、適切な標本数を用いて実施されるべきである。その ためには、研究デザインの段階で検出力解析を行う必要がある。
- 3)今後の研究では、異質性、小集団、ばく露方法、感受性の時期や条件(妊娠、発育、疾患など)を考慮する必要がある。
- 4)幅広い潜在的交絡変数(他の化学物質への共ばく露、ライフスタイル、社会経済的要因など)を研究の計画段階 (例:マッチング)で測定または考慮すべきである。
- 5) 毒性に影響を与え、効果を調節する宿主因子を考慮する。これらには、遺伝的多型データ(例:パラオキソナーゼ -1 遺伝子型)や栄養因子(例:ヨウ素の状態)などが含まれる。
- 6)研究者間の共同研究は、個々のコホートの有効性を高めるコンソーシアムを構築するために奨励される。

将来のばく露評価のために、新規技術の利用を含め、関連する生物学的試料の収集と適切な保管を行うべきである。 b) ばく露(測定、報告のためのデータ変換、統計解析)

- 1) ばく露に関する特定の情報の収集は、可能な限り、ばく露の広範な定義、非特定の農薬の記述及び「一度もない」 対「今までにあり」のような広範なばく露の分類を避けるべきである。それにもかかわらず、これらのカテゴリーは、 特定の状況下では、例えばクラス効果を予測するために価値があるかもしれない。
- 2) 農薬の幅広いクラス(無関係な物質の一般的なグループ)、または「殺虫剤」、「除草剤」など、あるいは一般的な 「農薬」だけを対象とした研究は、リスク評価にはあまり役に立たない(あるとすれば)。特定の名前のついた農薬や 共調合製剤を調査した研究の方がリスク評価には有用である。
- 3)同じ化学クラスに属する農薬、または同じ毒性作用モードや毒性学的効果をもたらす農薬は、同じカテゴリーにグ ループ化されている可能性がある。ばく露の頻度、持続時間、ばく露の強度などの情報を追加することで、ばく露 パターンの推定に役立てることができるかもしれない。
- 4) 職域疫学研究では、作業者や労働者の行動や PPE の適切な使用は、これらのばく露修飾がばく露を大幅に変 化させ、それによって潜在的な関連性を変化させる可能性があるため、適切に報告されなければならない。
- 5) ばく露測定の精度を向上させることは、特にコホート研究において、ますます重要になってきている。病因学的に 関連する期間をカバーする長期のコホート研究では、繰り返しの生物学的測定や自己報告されたばく露の繰り返 しの更新を使用することにより、ばく露の測定の精度を向上させるべきである。
- 6) 農薬の使用記録、登録データ、GIS、地理的マッピングなどを含むより広い集団の環境ばく露の間接的な尺度及び大規模なデータベース(行政データベースを含む)から得られたデータは、探索的研究には貴重であるかもしれない。これらのデータが利用できない場合は、記録・登録を開始すべきである。同様に、食品消費データベースからの農薬への食事ばく露の推定や、モニタリングプログラムからの残留農薬レベルの推定も利用できる。直接的なばく露評価と同様に、間接測定の各方法は、偏りや誤分類のリスクを検討し、適切な重み付けを行うべきである。
- 7)可能な限り、ばく露評価は、異なるばく露レベルを確立するために、指定された農薬へのばく露の直接測定を使用すべきである(例えば、個人的なばく露測定/生物学的モニタリング)。新しい研究では、個人ばく露モニタリングの新しい方法を探求すべきである。結果は、集団間のばく露を標準化するために、標準化された単位を用いて表現されるべきである。
- 8)長期にわたるばく露評価の特性評価は、より包括的なばく露モニタリング戦略を実施し、アンケートやバイオモニタリングデータに裏付けられた作業ばく露マトリックスから収集された長期にわたるばく露決定要因に関する情報と相まって、利益を得ることができる。ばく露評価モデルは、重要なばく露パラメータを特定することを可能にする HBM の研究によって包括的にサポートされることができる。そのような場合には、モデル内のパラメータの仮定を 調整することで、より現実的なばく露の評価につなげることができる。
- 9) エクスポソームの概念とメタボロミクスの使用は、特に、より良いばく露測定(ばく露のバイオマーカー)、脆弱な小 集団の特定、毒性経路の生物学的解釈(疾患のバイオマーカー)のための次世代の疫学研究に大きな可能性を 秘めている。

10) 農薬混合物へのばく露(及び毒性)に関する知識の向上は、包括的なリスク評価に有益である。共通の標的に作

用する複数の農薬への複合ばく露の共同作用を考慮すること、または類似の有害作用を誘発することは、累積リ スク評価に関連している。そのためには、混合物の全成分を把握し、MOA、用量反応特性、成分間の潜在的な 相互作用を理解しておく必要がある。ばく露の特性を把握することは、ばく露のパターンや大きさが時間の経過と ともに変化する複数の農薬への複合ばく露において重要な要素である。

- c) 有害な健康影響(測定、報告のためのデータ変換、統計解析)。
  - 1)自己申告による健康上の影響は、研究に指定された医療専門家による病状の独立した盲検評価により、回避するか、または結論を出すべきである。
  - 2)研究の対象となる健康影響は十分に定義されているべきであり、妥当性が確認されている場合を除き、代替エンド ポイントは避けるべきである。疾患や疾患のサブクラスの定義が時間の経過とともに変化する場合には注意が必要 である(がん、神経変性疾患など)。
  - 3) 早期の生物学的効果を示す生物学的マーカーを利用して、疾患の病態の理解を深めるべきである。これらの定量的な生物学的パラメータは、実験動物を用いた研究から得られた結果と比較して、研究の感度を向上させ、誤分類を減らし、ヒトへの関連性を高めることができるため、疫学の有用性を高めることができる。これらの再定義されたエンドポイントは、毒性力学的経路における初期のイベントであり、連続的なスケールで測定されることが多いため、よりあからさまな従来の健康影響よりも好ましいかもしれない。
  - 4)効果のバイオマーカーの使用は、農薬への総体的ばく露を評価し、累積的なリスク評価に役立てることができるか もしれない。
  - 5)健康影響を疫学研究を用いて特定し、急性・慢性の事故記録と実験結果を結びつけることを可能にするリードアク ロス手法(read across methods)を開発する。
- d)統計(記述的統計、ばく露と影響の関係のモデル化)。
  - 1) 統計解析は、事前に定められた解析(統計)プロトコールに基づき、探索的研究のための事後的な解析を避け、統計的に有意であるかどうかにかかわらず、すべての結果を報告すべきである。
  - 2) データは、適切な場合には、直接または間接的な測定法が使用されているかどうかにかかわらず、個人/集団の ばく露と用量反応評価を推定するための数学的モデル化を可能にするような方法で報告されなければならない。
  - 3)報告書には、関連因子やばく露の構造が異なる基礎となる集団に基づいた研究において、未調整と調整の両方の割合と、対象となる結果の割合と率を含めるべきである。
  - 4)考えられる関連因子及びばく露と健康転帰の関係におけるそれらの役割は、慎重に同定され、正確に測定され、 徹底的に評価されるべきである。ほとんどの場合、関連因子は潜在的な交絡因子としてスクリーニングされている。 交絡因子が検出された場合には、感度分析を含む適切な統計的手法を用いて、交絡因子を調整する必要がある。
  - 5) プロペンシティスコアマッチング (propensity score matching)、メディエーション解析 (mediation analyses)、因 果推論などの潜在的に有用な解析手法を農薬疫学に適用することが奨励されている。
  - 6)対象とした農薬ばく露と疾患との間の関連が統計的に有意であることが判明した場合、特に(推定される)検出力の低い研究では、統計的に有意な効果の大きさの推定値(例えば、オッズ比 OR または相対リスク RR)が人為的に増大するか、または拡大するかの程度を決定するために、検出力解析/設計計算を実行することが一般的に良い対応とされている<sup>19</sup>。

<sup>19</sup> 検出力とサンプルサイズの推奨事項及び効果量の算出とデザインの計算を含む関連問題に関する追加情報は、本報告書の附属書 D に記載されている。特に、検出力の計算では、疫学研究で明確に報告されるべき3つの値が必要である。(i)非ばく露群の被験者数(対象となる疾患の有無を含む)、(iii)非ばく露群の被験者数(対象となる疾患の有無を含む)、(iii)非ばく露群の福患者数。

#### e)結果の報告

- 1)結果報告は、STROBE 声明及び統計報告に関する EFSA ガイドライン(EFSA、2014 年 b)に概説されている疫 学研究の良好な報告の慣行に従うべきであり、効果量の推定値を含む本意見書で指摘されている更なる提案を 含むべきである。
- 2)いくつかの疫学研究は探索的で事後的な特性を持つものもあるがが、そのことを認識し、適切な統計解析によっ て裏付けられるべきである。
- 3) 疫学研究は、さらなる調査のために生データへのアクセスを提供し、その全結果と解析に使用したスクリプトやソフ トウェアパッケージを提供することが奨励されている。
- 4) 結果の再現性を検証するために使用したスクリプトや統計ツールとともに、すべての結果を報告するか、またはオ ンライン源を利用して寄託する。
- 5) すべての資金源を報告し、財務上の問題やその他の潜在的な利害関係者を適切に報告する。

一般的な勧告として、PPR パネルは、リスク評価における疫学研究の価値、透明性、説明責任を高めるために、疫 学研究のためのガイダンスの開発を奨励している<sup>20</sup>。疫学研究の質の向上は、責任ある研究の実施と科学的誠実さと ともに、リスク評価にこれらの研究を組み入れることに利益をもたらすであろう。

## 8.2. サーベイランス

- 1) EU 指令 2009/128 の第 7 条で要求されている市販後サーベイランスプログラム(産業用及び一般集団)を設定 することにより、急性及び慢性の事故の報告を増加させる。これは、産業保健医とのサーベイランスネットワークを 構築し、PPPを扱う国の当局と毒物管理情報センターとの連携を強化することにより達成すべきである。
- 2)急性・慢性事故の因果関係の程度/強度(「推定可能性」)を評価するための有効な方法を開発し、EU 加盟国間の整合化された報告を支援するための用語集と類義語集を開発する。
- 3) EU 加盟国からの整合化されたデータを EU レベルで収集し、欧州委員会/EFSA が定期的に検討し、最も関 連性の高い結果に焦点を当てた報告書を発表すべきである。
- 4) 農薬に関する EU 全体の警戒体制を構築する。
- 5)診断決定、データ入力、管理を担当する医療・救急スタッフのための毒物学コースにおいて、農薬のトキシドロームに関する研修を改善する余地がある。

## 8.3. 複数の疫学研究のメタアナリシス

- 1) 個々の研究の方法とバイアスの徹底的な評価、研究間の異質性の程度の評価、異質性の根底にある説明の展開、 エビデンスの定量的な要約(一貫性があれば)を考慮に入れて、疫学研究からのエビデンスを蓄積することができ る。
- 2) すべてのエビデンス統合作業において、関連するバイアスのリスクツールを用いて研究をレビューすべきである。 計画が異なる研究や計画の特徴が異なる研究では、バイアスのリスク評価のために(いくつかの)異なる課題が必要になるかもしれない。
- 3)エビデンス統合は、特定の期間に限定されるべきではなく、エビデンス全体を含めるべきである。これらの努力は、 特定の健康影響または疾患カテゴリーに焦点を当てれば、より適切である。
- 4) エビデンス統合の取り組みでは、効果量の定量的な統合に加えて、計算された予測間隔、小試験効果と非対称 性バイアス、対象となる対角線、交絡、過剰な有意性バイアス<sup>21</sup>及び不均一性の推定値についても考慮すべきで ある。

<sup>20</sup> 例として、オランダ疫学協会が責任ある疫学的研究実践に関するガイドラインを作成した(2017年)

<sup>&</sup>lt;sup>21</sup> 過剰シグニフィカンスバイアスとは、特定のアウトカムに関する公表されている文献の中に、統計的に有意な結果が得られた研究が多すぎる状況 を指す。このパターンは、出版バイアスを伴う文献の強いバイアスを示唆している。 選択的な結果報告、選択的な分析報告、または捏造されたデータが説明の対象となる可能性がある(Ioannidis and Trikalinos、2007 年)。

- 5) 不均質性が存在する場合、高度に選択された母集団を用いた研究は、それぞれの母集団を代表するものではないが、統計的な不均質性ではなく真の不均質性を示すものである可能性があるため、価値があることが証明され、 検討に値する。
- 6)年齢、人種、性別など、研究間での一貫性のある報告があれば、メタアナリシスがより充実すると思われる。
- 7) 個々の農薬の定量的データが疫学研究から得られる場合には、それらのデータを組み合わせたり、プールして用 量反応モデリングを行うことで、定量的なリスク推定値や Point of Departure(BMDL、NOAEL)の開発が可能 となる。
- 8) 個々のコホートでは十分な統計的力がない疾病ばく露関連を研究するために、コホート研究の国際的なコンソー シアムがデータプールを支援するよう奨励されるべきである(例:AGRICOH)。

#### 8.4. 疫学的証拠と他の情報源との統合

- 1) すべてのエビデンス(疫学、動物、試験管内データ)は、バイアスがなく、平等に精査されるべきである。
- 2)リスク評価のために、観察による研究、動物/基礎科学研究、その他のエビデンス源を組み合わせるために、有 効かつ調和のとれた方法を開発すべきである。
- 3)実験データとヒトデータの両方が、ハザードの特定と用量反応評価に寄与するべきである。
- 4) 複数のエビデンスから得られたデータを体系的に統合することは、Bradford Hill 基準を修正したものを用いて、 関連性、一貫性、生物学的妥当性を考慮した WOE 解析に基づくべきである。この枠組みの基礎となる原則は 7.2 節に記載され、図 5 に要約されている。
- 5) WOE アプローチを用いて、疫学的知見を他の情報源(実験毒性学からのデータ、作用機序/AOP)と統合すべ きである。統合された調和のとれたアプローチは、体系的かつ一貫した方法で WOE の全体的な枠組みの中で動 物、メカニズム、ヒトのデータをまとめることによって開発されなければならない。
- 6) AOP フレームワークは、様々な種類の研究成果を統合するための構造化されたプラットフォームを提供する。
- 7)動物データ、in vitro データ、ヒトデータは、それぞれのエンドポイントについて全体として評価されるべきである。 実験から得られた結果が各エンドポイントのヒトのデータと一致しているかどうかの結論を導き出すことができ、これ を RARs に含めることができる。

### 9. 結論

本意見書は、既存の疫学研究を含む科学的に査読された公表文献の検索を要求する規制 1107/2009 の下で、農 薬の認可更新時(可能であれば認可プロセス時)の査読プロセスを支援することを目的としている。これらは有効成分 の更新プロセスに適しており、更新のために提出される書類には有効成分に関連する新しいデータを含める必要があ ることを示す規則 1141/2010 にも準拠している。

以下では、参照条件の4つの重要な要素を繰り返し、個々の用語に対応する部分を順番に示している。それぞれの ToRs でグループ化された文章から導かれるように、それぞれのToR に関連する推奨事項も以下のように示す。

「PPR パネルは、外部科学報告書(Ntzani ら、2013 年)で観察された農薬ばく露ととトの健康影響との関連性と、これらの知見が規制上の農薬リスク評価の文脈でどのように解釈され得るかを検討する。したがって、PPR パネルは、報告書で収集された疫学研究を体系的に評価し、研究の主要なデータギャップと限界に対処し、その提言を行う」。

PPR パネルは特に以下を行う。

- 1)利用可能な疫学研究の質と妥当性に関して外部科学報告書で明らかにされたものに基づいて(必ずしもこれに限 定されないが)ギャップと限界のすべての情報源を収集し、レビューする。セクション 3、20・24 頁、セクション 5.2 33・35 頁:勧告は適切ではない。
- 2) ポイント1 で明らかになったギャップと限界に基づき、調査結果の質、妥当性、信頼性を向上させ、それが農薬リス ク評価にどのように影響を与えるかについて、将来の疫学的研究のための潜在的な改善点を提案する。これには、 研究デザイン、ばく露評価、データの質とアクセス、健康影響の診断分類、統計解析が含まれる。(セクション 4 の

回答 24-33 頁: セクション 8.1、8.2 及び 8.3 の推奨事項 54-58 頁。54-58)

- 3) 情報及び/または基準が不十分または不足している分野を特定し、リスク評価における適用を改善し最適化する ために、農薬疫学的研究をどのように実施するかについての提言を行う。これらの推奨事項には、第1項で特定 されたギャップと限界に基づいて、ばく露評価(バイオモニタリングデータの使用を含む)、脆弱な集団のサブグル ープ及び/または対象となる健康影響(生化学的、機能的、形態学的、臨床的レベルでの)の調和を含むべきで ある。(セクション 4.2-4.5 の回答 27-33 頁、セクション 5.3 36 頁:セクション 8.1 c) 1-4、56 頁の推奨事項)
- 4) WOE などの評価報告書草案のピアレビュープロセスにおいて、疫学的情報を実験毒性学、AOP、作用機序などのデータと統合しながら、農薬のリスク評価に疫学的知見を適切に活用する方法について議論する。(6.2 及び 6.3 節の回答 37-45 頁、7 節 45-54 頁:8.4 節の回答 58 頁)

上記で説明したように、適切な疫学的データと承認後のサーベイランスは、ハザードの特定や、方法論の改善により ハザードの特徴を明らかにすることで、リスク評価の枠組みに有用に貢献することができる。WOE 解析、不確実性分析、 バイアスの同定と推定により改善することができる。利用可能な関連文献を収集し、システマティックレビューを含む関 連する EFSA 基準を用いてその妥当性と質を検討し、EU 法で規定されている DAR、RAR、承認後の枠組みの中で 結果の議論を導入することは申請者の責任である。

適切な品質の定義には、サンプルサイズの解析、統計的手続き、効果の大きさの推定値の浮動、バイアスの評価、 導き出された結論への寄与が必要である。研究の特性は、リスク評価プロセスのすべての関連ポイントで考慮する必要 があり、例えば、生殖に関する疫学的データは、生殖影響を明らかにするために計画された実験動物研究と一緒に考 慮され、生殖毒性(ECHAの場合)のための表示勧告の背景で考慮される。

EU 域外の国での使用実績がない限り、関連する疫学的研究は、DAR への影響が制限されるが、RAR とサーベイ ランスの枠組みは、最初の承認後の時間が経つにつれて、また他の法域での原薬の先行使用がある場合には、段階 的に疫学から恩恵を受けることができる可能性がある。RAR とサーベイランスのプロトコールはこの違いを反映させるこ とが推奨される。

上記の提言は、リスク評価における疫学的データの利用に関連した現在及び将来の強み・弱み・機会・脅威の解析 に基づく詳細な論拠に基づくものである。大まかには以下の通りである。

#### 強み:

- ・その証拠は人間の特定のリスクに関するものであること。
- ・健康影響は、毒素へのすべてのばく露の影響を総合的に測定するものであること。
- ・影響を受ける可能性のある人々から主観的な経験を引き出すことができること。

#### 弱み:

- ・ 農薬へのばく露は通常複雑である;特定の有効成分の寄与は容易に解読されない。
- ・ばく露は、正確に管理された条件が不足している様々な設定で発生する。
- ほとんどのデータは混合集団の反応を再現している。
- 多くのデータは低レベルの関連性を示しており、再現性がなく、高度な解析を必要とする。

機会:本意見書に記載されている限界は、公表されている多くの疫学研究に適用されるが、農薬のリスク評価に利益 をもたらす機会がある。これには以下が含まれる。

- ・軽微な健康影響を明らかにし、敏感な小集団の経験を明らかにする可能性のある研究のために、非常に多くの潜 在的にばく露された個人にアクセスできること。
- ・ 潜在的な毒素とその残留物の組織負荷を確立するためのバイオモニタリングと新しい分子アプローチを用いたば く露推定の改善の見通し。
- ・実験動物の反応に基づく従来のリスク評価にとトのデータを完全に統合する可能性。
- WOE, AOP, Expert judgement, Expert Knowledge Elicitation (EEKE) 及び Uncertainty Analysis を利 用して、潜在的に関連性のあるデータの質の違いを評価する。
- ・ 専門の疫学者や統計学者と協力して、疫学的結果の解釈を再検討し、慢性ばく露リスクや複合ばく露リスク、用量

反応データなどの困難な分野に取り組むための改善案を提案する機会がある。

・ 様々な国の情報源からのデータを蓄積することには、大きな情報技術の機会が存在する。関連する法的、方法論 的、倫理的な問題が克服されれば、より多くの価値あるデータを収集することができる。このデータが社会的利益 のために「ビッグデータ」の設定で使用できる形で利用できるようになれば、疫学研究を大幅に改善できる可能性 がある。しかし、第一に、個人のプライバシーと本質的な商業的な機密性を守る必要がある。これらの障害が克服 されれば、疫学研究の統計的な力を向上させ、ハザードをより良く特定し、場合によっては特徴づけるために応用 することができる。これらの目的は、EU の高いレベルで合意された行動によって効果的に実現することができる。 データとインタラクティブなプラットフォームを提供するための相互間承認は、集団の健康情報、食品消費データ、 有効成分と共調合製剤の空間的・時間的な適用データの調和によって裏付けられる必要がある。このような豊富 なデータは、因果関係と信頼性のエビデンスを強化する基準である一貫性の向上を支援することが期待できる。そ れは、農薬毒性からの特別な保護を必要とするかもしれない脆弱なグループをよりよく特定できるようになる疫学研 究のためのより大きなサンプルサイズを約束している。

#### 脅威:

- ・ 非現実的であり、社会に否定的な結果をもたらすとト集団や野生生物、環境に対するリスクレベルの認識が広まっ ていること。
- 他の有効な情報源からのデータを損なうような、誤った陽性または誤った陰性の結論をもたらす不十分な実験計 画。
- 効果的なサーベイランスが行われていなかったり、匿名化された適切なデータを社会的利益のために利用できる。 ようにする気がなかったりした結果、新たなリスクに対応できなかったこと。
- ・登録(がんや先天性異常)やサーベイランスプログラムによるばく露(特に職業上ばく露)に関する適切な情報収集 の失敗によるデータの浪費。
- 診断基準の調和の失敗、統合解析のための十分に詳細な組み合わせ可能な形でデータを記録しなかったことに よるデータの浪費、健康統計データベースに入力されるデータの最適な品質を可能にするための関連するトキシ ドロームについての医療や救急医療スタッフのトレーニング不足。

## 参考文献

- Adami HO, Berry SC, Breckenridge CB, Smith LL, Swenberg JA, Trichopoulos D, Weiss NS and Pastoor TP, 2011. Toxicology and epidemiology: improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. Toxicology Sciences, 122, 223-234.
- Amler RW, Barone Jr S, Belger A, Berlin Jr CM, Cox C, Frank H, Goodman M, Harry J, Hooper SR, Ladda R, LaKind JS, Lipkin PH, Lipsitt LP, Lorber MN, Myers G, Mason AM, Needham LL, Sonawane B, Wachs TD and Yager JW, 2006. Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical
- exposure. Neurotoxicology, 27, 861–874.
  Bengtson AM, Westreich D, Musonda P, Pettifor A, Chibwesha C, Chi BH, Vwalika B, Pence BW, Stringer JS and Miller WC, 2016. Multiple overimputation to address missing data and measurement error:
- application to HIV treatment during pregnancy and pregnancy outcomes. Epidemiology, 27, 642–650. Bevan R, Brown T, Matthies F, Sams C, Jones K, Hanlon J and La Vedrine M, 2017. Human Biomonitoring data collection from occupational exposure to pesticides. EFSA supporting publication 2017: EN-1185, 207 pp
- Bottai M, 2014. Lessons in biostatistics: inferences and conjectures about average and conditional treatment effects in randomized trials and observational studies. Journal of Internal Medicine, 276, 229-237.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2001. Benchmark dose calculation from epidemiological data. Biometrics, 57, 698-706.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2004. Effects of exposure imprecision on estimation of the benchmark dose. Risk Analysis, 24, 1689-1696.
- Buonsante VA, Muilerman H, Santos T, Robinson C and Tweedale AC, 2014. Risk assessment's insensitive toxicity testing may cause it to fail. Environmental Research, 135, 139–147.
  Burton PR, Fortier I and Knoppers BM, 2010. The global emergence of epidemiological biobanks: opportunities and challenges. In: Khoury M, Bedrosian S, Gwinn M, Higgins J, Ioannidis J and Little J (eds.). Human Genome Epidemiology. Building the evidence for using genetic information to improve

health and prevent disease. 2nd Edition, Oxford University Press, Oxford. pp. 77–99.

- Choi J, Polcher A and Joas A, 2016. Systematic literature review on Parkinson's disease and Childhood Leukaemia and mode of actions for pesticides. EFSA supporting publication 2016:EN-955, 256 pp. Available online: http:// www.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-955/pdf
- Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Beane Freeman LE, Blair A, Sandler DP and Alavanja MC, 2011. An updated algorithm for estimation of pesticide exposure intensity in the agricultural health study. International Journal of Environmental Research and Public Health, 8, 4608-4622.
- Coggon D, 1995. Questionnaire based exposure assessment methods. Science of the Total Environment, 168, 175-178.
- Cornelis C, Schoeters G, Kellen E, Buntinx F and Zeegers M, 2009. Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case control study on bladder cancer. International Journal of Hygiene and Environmental Health, 212, 172-185.
- la Cour JL, Brok J and Gøtzsche PC, 2010. Inconsistent reporting of surrogate outcomes in randomised clinical trials: cohort study. BMJ, 341, c3653. DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS and Yucesoy B, 2015.
- Systems biology and biomarkers of early effects for occupational exposure limit setting. The Journal of
- Occupational and Environmental Hygiene, 12(Suppl 1), S41–S54. Dionisio KL, Chang HH and Baxter LK, 2016. A simulation study to quantify the impacts of exposure measurement error on air pollution health risk estimates in copollutant time-series models. Environmental Health, 15, 114.
- DSE (Dutch Society for Epidemiology), 2017. Responsible Epidemiologic Research Practice (RERP). A guideline developed by the RERP working group of the Dutch Society for Epidemiology, 2017 (available at https:// www.epidemiologie.nl/home.html, https://epidemiologie.nl/fileadmin/Media/docs/ Onderzoek/ Responsible\_Epide miologic\_Research\_Practice.2017.pdf)
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2009. Framework for the Integration of Human and Animal Data in Chemical Risk Assessment. Technical Report No. 104. Brussels. Available online: http://www.ecetoc.org/uploads/Publications/documents/TR%20104.pdf
- ECHA/EFSA, 2014. Workshop on Mode of action and Human relevance framework in the context of classification and labelling (CLH) and regulatory assessment of biocides and pesticides. November 2014. Available online: https://echa.europa.eu/documents/10162/22816050/moaws\_workshop\_proceedings\_en.pdf/ a656803e-4d97-438f-87ff-fc984cfe4836
- EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids. EFSA Journal 2004;81, 1-49 pp. https://doi. org/10.2903/j.efsa.2004.81
- EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;980, 1–139 pp. https:// doi.org/10.2903/j.efsa.2009.980
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2009b. Opinion 2009;7(10):1351,Scientific on arsenic in food. EFSA Journal 199pp. https://doi.org/10.2903/j.efsa.2009.1351
- EFSA (European Food Safety Authority), 2010a. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637
- EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM), 2010b. Scientific Opinion Food. EFSA Journal 2010;8(4):1570,on Lead in 151pp. https://doi.org/10.2903/j.efsa.2010.1570
- EFSA (European Food Safety Authority), 2011a. Submission of scientific peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal
- 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092 EFSA (European Food Safety Authority), 2011b. Statistical significance and biological relevance. EFSA Journal 2011;9(9):2372, 17 pp. https://doi.org/10.2903/j.efsa.2011.2372
- EFSA (European Food Safety Authority), 2012a. Scientific Opinion on risk assessment terminology. EFSA Journal 2012;10(5):2664, 43 pp. https://www.efsa.europa.eu/en/efsajournal/pub/2664
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2012b. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in
- food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985 EFSA (European Food Safety Authority), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293
- EFSA (European Food Safety Authority), 2013b. Scientific Opinion on the relevance of dissimilar mode of
- action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12):3472, 40 pp. https://doi.org/10.2903/j.efsa.2013.3472 EFSA (European Food Safety Authority), 2014a. Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos. EFSA Journal 2014;12(4):3640, 34 pp. https://doi.org/ 10.2903/j.efsa.2014.3640
- EFSA (European Food Safety Authority), 2014b. Guidance on statistical reporting. EFSA Journal 2014;12(12): 3908, 18 pp. https://doi.org/10.2903/j.efsa.2014.3908 EFSA (European Food Safety Authority), 2015a. Stakeholder Workshop on the use of epidemiological data

in pesticide risk assessment. EFSA supporting publication 2015:EN-798, 8 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/798e

EFSA (European Food Safety Authority), 2015b. Increasing robustness, transparency and openness of scientific assessments - Report of the Workshop held on 29-30 June 2015 in Brussels. EFSA supporting publication 2015: EN-913. 29 pp. Available online: http://www.efsa.europa.eu/sites/default/ files/corporate

publications/ files/913e.pdf

- EFSA (European Food Safety Authority), 2015c. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302, https://doi.org/10.2903/j.efsa.2015.4302 107pp.
- EFSA PPR Panel (European Food Safety Authority Panel on Plant Protection Products and their Residues), 2017. Scientific Opinion on the investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 2017;15(3):4691, 325 pp. https://doi.org/10.2903/j.efsa.2017.4691
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi. org/10.2903/j.efsa.2017.4971.
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017c. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1): 4658,
- 41 pp. https://doi. org/10.2903/j.efsa.2017.4658
   von Elm E, Altman DG, Egger M, Pocock SJ and Gøtzsche PC, Vandenbroucke JP and STROBE Initiative, 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ, 335, 806-808.
- Esch EW, Bahinski A and Huh D, 2015. Organs-on-chips at the frontiers of drug discovery. Nature Reviews.
- Drug Discovery, 14, 248–260. Fedak KM, Bernal A, Capshaw ZA and Gross S, 2015. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerging Themes in Epidemiology, 30, 14
- Gibson SB, Downie JM, Tsetsou S, Feusier JE, Figueroa KP, Bromberg MB, Jorde LB and Pulst SM, 2017.
   The evolving genetic risk for sporadic ALS. Neurology, 89, 226–233.
   Gómez-Martín A, Hernández AF, Martínez-González LJ, González-Alzaga B, Rodríguez-Barranco M,
- Lopez-Flores I, Aguilar-Garduno C and Lacasana M, 2015. Polymorphisms of pesticide metabolizing genes in children living in intensive farming communities. Chemosphere, 139, 534–540.
- González-Alzaga B, Hernández AF, Rodríguez-Barranco M, Gómez I, Aguilar-Garduño C, López-Flores I, Parrón T and Lacasaña M, 2015. Pre- and postnatal exposures to pesticides and neurodevelopmental effects in children living in agricultural communities from South-Eastern Spain. Environment International, 85, 229-237.
- Greenland S and Longnecker MP, 1992. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. American Journal of Epidemiology, 135, 1301–1309. Greenland S and O Rourke K, 2008. Meta-analysis. In: Rothman K, Greenland S and Lash T (eds). *Modern*
- *Epidemiology. 3.* Lippincott Williams & and Wilkins, Philadelphia. pp. 652–682. Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN and Altman DG, 2016. Statistical
- tests, P values, confidence intervals, and power a guide to misinterpretations. European Journal of Epidemiology, 31, 337-350.
- Grimes DA and Schulz KF, 2005. Surrogate end points in clinical research: hazardous to your health. Obstetrics and Gynecology, 105, 1114-1118.
- Gustafson P and McCandless LC, 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. International Journal of Environmental Research and Public Health, 7, 1520-1539.
- Hernández AF, González-Alzaga B, López-Flores I and Lacasaña M, 2016. Systematic reviews on neurodevelopmental and neurodegenerative disorders linked to pesticide exposure: methodological features and impact on risk assessment. Environment International, 92–93, 657–679.
- Higgins JP, 2008. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. International Journal of Epidemiology, 37, 1158-1160.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295-300.
- Hines CJ, Deddens JA, Coble J, Kamel F and Alavanja MC, 2011. Determinants of captan air and dermal exposures among orchard pesticide applicators in the Agricultural Health Study. Annals of Occupational Hygiene, 55, 620-633.
- Hoffmann S, de Vries RBM, Stephens ML, Beck NB, Dirven HAAM, Fowle JR 3rd, Goodman JE, Hartung T, Kimber I, Lalu MM, Thayer K, Whaley P, Wikoff D and Tsaioun K, 2017. A primer on systematic reviews in toxicology. Archives of Toxicology, 91, 2551-2575.
- Höfler M, 2005. The Bradford Hill considerations on causality: a counterfactual perspective. Emerging Themes in Epidemiology, 2, 11.

IEA (International Epidemiological Association), 2007. Good Epidemiological Practice (GEP) 2007. Available online: http://ieaweb.org/good-epidemiological-practice-gep/

Imbens G and Rubin D, 2015. Causal Inference for Statistics, Social, and Biomedical Sciences: An

Introduction. Cambridge University Press, New York, NY.

- INSERM, 2013. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.
- Ioannidis JP and Trikalinos TA, 2007. An exploratory test for an excess of significant findings. Clinical Trials, 4, 245–253.
- Jurek AM, Greenland S, Maldonado G and Church TR, 2005. Proper interpretation of non-differential misclassification effects: expectations vs observations. International Journal of Epidemiology, 34, 680-687
- Kaltenhäuser J, Kneuer C, Marx-Stoelting P, Niemann L, Schubert J, Stein B and Solecki R, 2017. Relevance and reliability of experimental data in human health risk assessment of pesticides. Regulatory Toxicology and Pharmacology, 88, 227-237.
- Karabatsos G, Talbott E and Walker SG, 2015. A Bayesian nonparametric meta-analysis model. Research Synthesis Methods, 6, 28-44.
- Kavvoura FK, Liberopoulos G and Ioannidis JP, 2007. Selection in reported epidemiological risks: an empirical assessment. PLoS Medicine, 4, e79.
- Lachenmeier DW, Kanteres F and Rehm J, 2011. Epidemiology-based risk assessment using the benchmark dose/margin of exposure approach: the example of ethanol and liver cirrhosis. International Journal of Epidemiology, 40, 210-218.
- LaKind JS, Sobus JR, Goodman M, Barr DB, Furst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teequarden J, Tornero-Velez R and Weisel CP, 2014. A proposal for assessing study quality: biomonitoring, environmental epidemiology, and short-lived chemicals (BEES-C) instrument. Environmental International, 73, 195–207.
- LaKind JS, Goodman M, Barr DB, Weisel CP and Schoeters G, 2015. Lessons learned from the application of BEES C: systematic assessment of study quality of epidemiologic research on BPA, neurodevelopment, and respiratory health. Environment International, 80, 41-71. Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV and
- Alavanja MC, 2009. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. Blood, 113, 6386–6391. Larsson MO, Nielsen VS, Brandt CØ, Bjerre N, Laporte F and Cedergreen N, 2017. Quantifying dietary
- exposure to pesticide residues using spraying journal data. Food and Chemical Toxicology, 105, 407-428. Lash TL, Fox MP and Fink AK, 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer,
  - New York.
- Lavelle KS, Robert Schnatter A, Travis KZ, Swaen GM, Pallapies D, Money C, Priem P and Vrijhof H, 2012. Framework for integrating human and animal data in chemical risk assessment. Regulatory Toxicology and Pharmacology, 2012; 62, 302–312.
- London L, Coggon D, Moretto A, Westerholm P, Wilks MF and Colosio C, 2010. The ethics of human volunteer studies involving experimental exposure to pesticides: unanswered dilemmas. Environmental Health, 18, 50.
- Maldonado G and Greenland S, 2002. Estimating causal effects. International Journal of Epidemiology, 31, 422 - 429
- Marx-Stoelting P, Braeuning A, Buhrke T, Lampen A, Niemann L, Oelgeschlaeger M, Rieke S, Schmidt F, Heise T, Pfeil R and Solecki R, 2015. Application of omics data in regulatory toxicology: report of an international BfR expert workshop. Archives of Toxicology, 89, 2177-2184.
- McNamee R, 2003. Confounding and confounders. Occupational and Environmental Medicine, 60, 227-234.
- Monson R, 1990. *Occupational Epidemiology*, 2nd Edition. CRC Press, Boca Ration, FL. Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E and Vega C, 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology, 39, 158–168.
- Nachman KE, Fox MA, Sheehan MC, Burke TA, Rodricks JV and Woodruff TJ, 2011. Leveraging epidemiology to improve risk assessment. Open Epidemiology Journal, 4, 3-29.
- Nieuwenhuijsen MJ, 2015. Exposure assessment in environmental epidemiology. In: Vrijheid M (ed.). The Exposome-Concept and Implementation in Birth Cohorts Chapter 14. Oxford University Press.
- NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press.
- NRC (National Research Council), 2009. Science and Decisions: Advancing Risk Assessment. The National Academies Press, Washington, DC.
- Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E and Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013: EN-497, 159 pp.
- OECD (Organisation for Economic Co-operation and Development), 2013. Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment, No. 184. Paris. Avilable online: http:// search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282013% 296&doclanguage=en
- Orford R, Crabbe H, Hague C, Schaper A and Duarte-Davidson R, 2014. EU alerting and reporting systems for potential chemical public health threats and hazards. Environment International, 72, 15-25.
- Orford R, Hague C, Duarte-Davidson R, Settimi L, Davanzo F, Desel H, Pelclova D, Dragelyte G, Mathieu-Nolf M, Jackson G and Adams R, 2015. Detecting, alerting and monitoring emerging chemical health threats: ASHTIII. European Journal of Public Health, 25(supp 3), 218.
- Orsini N, Li R, Wolk A, Khudyakov P and Spiegelman D, 2012. Meta-analysis for linear and nonlinear doseresponse relations: examples, an evaluation of approximations, and software. American Journal of

Epidemiology, 175, 66–73.

Oulhote Y and Bouchard MF, 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environmental Health Perspectives, 121, 1378–1384.

Pearce N, 2011. Registration of protocols for observational research is unnecessary and would do more harm than good. Occupational and Environmental Medicine, 68, 86-88.

Pearce N, 2012. Classification of epidemiological study designs. International Journal of Epidemiology, 41, 393–397. Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA, Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Hemminki K, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, Lebailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, t Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Sanjose S, Schernhammer E, Costantini AS, Seixas N, Shy C, Siemiatycki J, 2015. Silverman DT, Simonato L, Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stayner LT, Steenland K, Stenzel M, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. LARC monographs: 40 years of evaluatingcarcinogenic hazards to humans. Environmental Health Perspectives, 123, 507–514.

Raffaele KC, Vulimiri SV and Bateson TF, 2011. Benefits and barriers to using epidemiology data in environmental risk. The Journal of Epidemiology, 4, 99-105.

Raphael K, 1987. Recall bias: a proposal for assessment and control. International Journal of Epidemiology, 16, 167–170.

Rappaport SM, 2012. Biomarkers intersect with the exposome. Biomarkers, 17, 483-489.

Reich CG, Ryan PB and Schuemie MJ, 2013. Alternative outcome definitions and their effect on the performance of methods for observational outcome studies. Drug Safety, 36(Suppl 1), S181–S193.

Rothman KJ, 2002. *Epidemiology – An Introduction*. Oxford University Press, Oxford.

- Rothman KJ and Greenland S, 1998. *Modern Epidemiology. 2.* Philadelphia: Lippincott Williams & Wilkins, 27 pp.
- Rothman KJ, Greenland S and Lash TL, 2008. *Modern Epidemiology, 3rd Edition*. Lippincott Williams & Wilkins, Philadelphia, PA, USA.
- Rushton L, 2011. Should protocols for observational research be registered? Occupational and Environmental Medicine, 68, 84–86.
- Salerno J, Knoppers BM, Lee LM, Hlaing WW and Goodman KW, 2017. Ethics, big data and computing in epidemiology and public health. Annals of Epidemiology, 27, 297–301. https://doi.org/10.1016/ j. annepidem. 2017.05.002
- Santacatterina M and Bottai M, 2015. Inferences and conjectures in clinical trials: a systematic review of generalizability of study findings. Journal of Internal Medicine, 279, 123–126. https://doi.org/10.1111/joim.12389
- SČENIHR, 2012. Memorandum on the use of the scientific literature for human health risk assessment purposes –weighing of evidence and expression of uncertainty.

Simera I, Moher D, Hoey J, Schulz KF and Altman DG, 2010. A catalogue of reporting guidelines for health research. European Journal of Clinical Investigation, 40, 35–53.

- Skelly AC, 2011. Probability, proof, and clinical significance. Evidence-Based Spine-Care Journal, 2, 9–11.
- Spiegelman D, 2016. Evaluating Public Health Interventions: 4. the nurses' health study and methods for eliminating bias attributable to measurement error and misclassification. American Journal of Public Health, 106, 1563–1566.
  Stang PE, Ryan PB, Dusetzina SB, Hartzema AG, Reich C, Overhage JM and Racoosin JA, 2012. Health
- Stang PE, Ryan PB, Dusetzina SB, Hartzema AG, Reich C, Overhage JM and Racoosin JA, 2012. Health outcomes of interest in observational data: issues in identifying definitions in the literature. Health Outcomes Research in Medicine, 3, e37–e44.
- Thomas DC, 2009. *Statistical Methods in Environmental Epidemiology.* Oxford University Press, Oxford, UK.
- Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G, Croghan CW, Jones PA, Knott CE, Lynch CF, Sandler DP, Blair AE and Alavanja MC, 2010. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. Journal of Exposure Science & Environmental Epidemiology, 20, 559–569.
  Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod
- Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod MR and Ioannidis JP, 2013. Evaluation of excess significance bias in animal studies of neurological diseases. PLoS Biology, 11, e1001609.
- Turner MC, Wigle DT and Krewski D, 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis.
- US EPA (United States Environmental Protection Agency), 2011. Chlorpyrifos: preliminary human health risk assessment for registration review, 30 June 2011, 159 pp. US-EPA (U.S. Environmental Protection Agency), 2010a. Framework for incorporating human
- US-EPA (U.S. Environmental Protection Agency), 2010a. Framework for incorporating human epidemiologic & incident data in health risk assessment (draft). Office of Pesticide Programs. Washington, DC, 2010.

Epidemiological studies and pesticides

- US-EPA (U.S. Environmental Protection Agency), 2010b. Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment. Arlington, USA, April 22, Health Virginia, 2010b. Available online https://archive.epa.gov/scipoly/sap/meetings/web/pdf/020210minutes.pdf
- US-EPA (U.S. Environmental Protection Agency), 2012. Guidance for considering and using open literature toxicity studies to support human health risk assessment. Office of Pesticide Programs. Washington, DC, 2012. Available online: http://www.epa.gov/pesticides/science/lit-studies.pdf
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides December 28, 2016. Avilable online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf
- Vandenberg LN, Ågerstrand M, Beronius A, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS, Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ and Rude, n C, 2016. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environmental Health, 15, 74.
- van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A and Goldbohm A, 2002. The contribution of epidemiology. Food and Chemical Toxicology, 40, 387–424. Vinken M, 2013. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology, 312,
- 158 165.
- Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, Rothman N and Vermeulen R, 2010. Application of OMICS technologies in occupational and environmental health research: current status and projections. Occupational and Environmental Medicine, 67, 136-43.
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. EHC 240: principles and methods for the risk assessment of chemicals in food.
- Wilson SJ and Tanner-Smith EE, 2014. Meta analysis in prevention science. In: Sloboda Z and Petras H (eds.). *Defining prevention science*. Advances in Prevention Science (vol. 1): Defining Prevention Science Springer, New York. pp. 431–452.
- Youngstrom E, Kenworthy L, Lipkin PH, Goodman M, Squibb K, Mattison DR, Anthony LG, Makris SL, Bale AS, Raffaele KC and LaKind JS, 2011. A proposal to facilitate weight-of-evidence assessments: Neurodevelopmental of Environmental Epidemiology harmonization Studies (HONEES). Neurotoxicology and Teratology, 33, 354–359. Zingone A and Kuehl WM, 2011. Pathogenesis of monoclonal gammopathy of undetermined significance
- and progression to multiple myeloma. Seminars in Hematology, 48, 4–12.

## 用語集と略語

ADI	一日の許容摂取量。食品または飲料水に含まれる農薬の量の尺度で、相当な健康リスクを伴わず
	に生涯にわたって日常的に(経口的に)摂取することができる。
ADME	薬理学(及び毒物学)で使用される略語(体内動態)で、化学物質の吸収、分布、代謝及び排泄
	のために使用され、生物体内でのその処理を示す。
AOP Adverse O	utcome Pathway(有害性転帰経路)。リスク評価に関連する有害な影響につながる生物学的事象
	を構造的に表現したもの。
ARfD	急性参照用量(Acute Reference Dose)。食品または飲料水に含まれる農薬の量(通常は体重べ
	ースで表される)の推定値で、評価時に知られているすべての事実に基づいて、24時間以内に消
	費者が健康上のリスクを認めずに摂取できる量。
バイオマーカー	「生物学的マーカー」とも呼ばれる。正常な生物学的プロセス、病原性プロセス、または治療的介
	入に対する薬理学的反応の指標として客観的に測定され、評価される特性。
BMD	ベンチマークドーズ。バックグラウンドと比較して有害な影響の反応率(ベンチマーク反応または
	BMR)に所定の変化をもたらす閾値の用量または濃度。95%の下限値(BMDL)が計算され、健
	康に基づいた参照値を導き出すための出発点としてさらに使用される。
HBM	ヒューマンバイオモニタリング(Human biomonitoring)。ヒトの生物学的体液または組織におけ
	る化学物質及び/またはその代謝物の測定。また、すべてのばく露経路からの総合的なばく露か
	ら得られる化学物質の内部ばく露量とも呼ばれる。
ヒトデータ	研究者が研究参加者に働きかけることなく、要因と健康影響との間の自然な関係を観察する観察

による研究(疫学研究とも呼ばれる)が含まれる。警戒データもこの概念に該当する。対照的に、研 究者が研究デザインの一部として介入する介入研究(実験研究または無作為化臨床試験とも呼 ばれる)は、本意見書の範囲外である。

- IARC International Agency for Research on Cancer(国際がん研究機関)。世界のがんの原因と発生に関する 研究を実施し、調整することを役割とする世界保健機関(WHO)の機関。
- LOAEL(LOAEL) 最小中毒量(Lowestobserved-adverse-effect level)。毒性試験で評価され、有害な影響(対象生物の形態、生化学、機能、または生涯への有害な変化など)を示す化学的ストレス因子の最低濃度または用量。

NOAEL 無毒性量。毒性または毒性影響が観察されなかった最高用量。

- OR オッズ比。ばく露と結果との関連性を示す尺度。OR は、特定のばく露を受けた場合に転帰が起こ る確率を、ばく露がなかった場合に転帰が起こる確率と比較して表している。
- PBTK-TD Physiologically based toxicokinetic/toxicodynamic modelling (PBTK-TD) Physiologically based toxicokinetic/toxicodynamic modelling とは、生理学的プロセスに関する先端的な知識 を他の既知/観察された情報と統合して、ヒト、前臨床試験動物種及び/または他の生物の体内で の化合物の転帰と影響を模倣することを目的とした数学的モデル化手法である。
- PPP 植物防疫製品(農薬)。用語「pesticide(殺虫剤)」はしばしば「plant protection product(植物 防疫製品)」と互換的に使用されるが、pesticide(殺虫剤)は植物/作物以外の用途、例えば biocide(殺生物剤)などもカバーするより広い用語(農薬)である。
- RR 相対リスク(Relative risk)。ある事象(病気の発生など)がばく露したグループで発生する確率と、 比較対照の非ばく露グループで発生する確率との比。

RMS Rapporteur(ラポーター)の加盟国。農薬有効成分の毒性評価に関する書類の評価及び評価を 最初に担当する欧州連合の加盟国。

感度 ある検査で個人を正しく「疾病」と同定する能力。疾患が存在する場合に検査が陽性となる可能性。 特異性 個人を疾患無しと正しく同定する検査の能力。疾患がない場合に検査が陰性である可能性。

代替エンドポイント(surrogate endpoint)臨床エンドポイントの代わりとなることを目的としたバイオマーカー。

AHS 農業健康調査

- ASHTIII 化学物質による健康影響の脅威に対する警告と報告システム、フェーズ III
- BEES-C バイオモニタリング、環境疫学、短命化学物質
- DAR 評価報告書草案
- DDE ジクロロジフェニルジクロロエチレン
- DDT ジクロロジフェニルトリクロロエタン
- EMA 欧州医薬品庁
- EPA 米国環境保護庁
- EQUATOR 健康研究の質と明白性を高める
- EU-OSHA 欧州労働安全衛生機関
- EWAS Exposome-wide association studies エキスポソームワイド関連研究
- GIS 地理情報システム

優良試験所規範
全地球測位システム
健康労働者効果
試験と評価に関する統合的アプローチ
国際疾病分類
国際保健規則
フランス国立保健医療研究所
定量化限界
単クローン性ガンマグロブリン血症
標的分子への作用
作用機序
非ホジキンリンパ腫
国立労働安全衛生研究所
ニューカッスルオタワスケール
経済協力開発機構
農薬プログラム局
毒物管理センター
個人用保護具
更新評価報告書
食品と飼料をカバーする迅速警報システム
リサーチトライアングル研究所
構造活性相関
遺伝学的関連研究への STROBE 拡張
疫学における観察研究の報告の強化
不確実性因子
世界保健機関
エビデンスの重み付け

# 付属書 A-EFSA の外部科学報告書でレビューされた農薬疫学研究及びその他のレビュー

EFSA 外部科学報告書(Ntzani ら、2013 年)によって収集された広範なエビデンスは、疫学研究からの農薬ばく 露と健康影響に関するかなりの量の情報が利用可能であることを強調している。それにもかかわらず、このエビデンス の質は通常低く、多くのバイアスが結果に影響を与え、結論を出すことができない可能性が高い。特に、ばく露疫学は 長い間、測定と定義の貧弱さに悩まされてきたが、特に農薬に関しては、これは常に評価と定義が非常に難しいもので あった。

## A.1. EFSA の外部研究報告書

## A.1.1. 方法論的品質評価

外部研究報告書は、2006年1月1日から2012年9月30日までに発表されたすべての疫学研究の包括的なシ ステマティックレビューから構成されており、農薬ばく露ととト健康関連影響の発生との関連性を調査している。

対象となる研究の方法論的評価(各研究に関連するバイアスのリスクを評価する)は、研究デザイン、研究対象集団、 ばく露の定義の詳細度、ばく露の測定方法、測定の特殊性に焦点を当てた。マッチングモデルや多変量モデル、盲検 化されたばく露評価、十分に説明された有効な結果評価を通じた交絡因子の説明などの取り組みが検討された。

方法論的評価の要素は、Research Triangle Institute (RTI; Research Triangle Park, NC, USA)の項目バンク で検討されたもので、観察による研究の偏りのリスクと精度を評価するための実用的で検証済みのツールである。これ らの要素を以下に示す(表 A.1)。

# 表 A.1: Research Triangle Institute (RTI; Research Triangle Park, NC, USA)の疫学研究の方法論的評価のための項目バンクの要素

質問	- 高リスク	低リスク
研究デザイン(有望、回顧的、混合、断面的)	回顧的、混合、該当なし	有望
除外基準が明確に記載されている(はい、部分的に、いいえ)	いいえ	はい
著者は電力計算について言及しています(はい、いいえ)		はい
暴露の記述の詳細レベル(高、中、低)	低	高
暴露のロバストな測定(バイオマーカー(有);小面積生態学的尺度、職	いいえ	はい
種、アンケート(部分的);大面積生態学的尺度(無)に基づいている。)		
曝露の尺度は特定のものだったか? はい;より広範な化学的に関連した	いいえ	はい
グループに基づいて(部分的)、多様な化学的および毒性学的特性の広範		
なグループに基づいて(いいえ)。		
グループ間の配分のバランスを図る(層別化、マッチングなど)。	いいえ	はい
潜在的な交絡因子の調整を行った(はい、いくつか、いいえ)。	いいえ	はい
被ばく状態に盲検化された評価者(コホート研究の場合)	いいえ	はい
有効かつ信頼性の高い尺度を用いて評価された結果は、すべての研究参加	いいえ	はい
者に一貫して実施されているか?		
サンプルサイズ	低	最大
ラフな品質評価	6 以上の回答で高リスク	6以上の回答で低リスク

結果の定量的な統合は、対象となる結果ごとに 5 件以上の適格な研究があり、発表されたエビデンス間に実質的な 異質性がない場合に試みられた。出版バイアスは、10 件以上の研究がメタアナリシスに含まれている場合に、非対称 性を視覚的に確認できる漏斗プロットを用いて評価した。

毒性学的データは、外部科学研究報告書ではレビューまたは議論されなかった。

## A.1.2. 除外基準

レビューの時点で入手可能な疫学的証拠の総合的に評価するために、EU で禁止されているものを含むすべての 種類の農薬を検討した。

除外基準。

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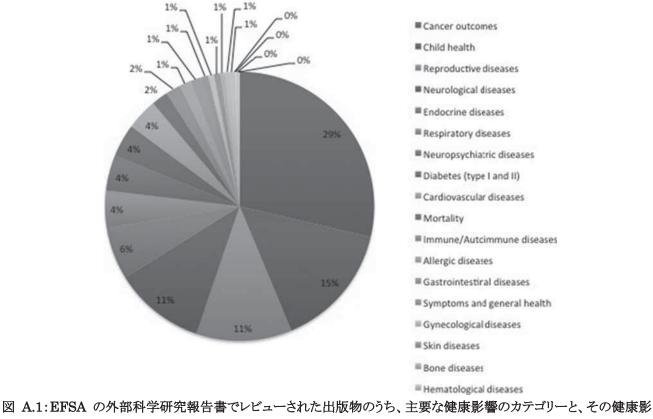
- ・ 対照集団のない研究(症例報告、症例シリーズ)及び生態学的研究
- ・農薬中毒または偶発的な高用量ばく露
- ・影響の推定に関する定量的な情報がない研究
- ・データの重複を避けるために、追跡期間が異なり、同じ健康影響(アウトカム)を調査している研究については、 追跡期間が最も長いもののみを残した。
- ・様々な病状の治療に使用される物質の有害影響について言及した研究(例:ワルファリンをベースとした抗凝固 薬)。
- ・農薬中の溶剤やその他の非有効成分(補助剤など)の研究
- ・ばく露とばく露のバイオマーカーとの関連性を検討した研究は、健康影響を調べないため対象外とされた。
- ・ 農薬へのばく露を調査した研究/解析:ヒ素、ヘキサクロロシクロヘキサン(HCH)aまたはb、鉛、ダイオキシン 類及びポリ塩化ビフェニル(PCB)を含むダイオキシン様化合物は考慮されなかった。
- ・ ナラティブレビューは除外したが、システマティックレビューやメタアナリシスは除外しなかった。

急性中毒または臨床症例のシリーズである出版物、健康影響とは無関係のバイオモニタリング研究、または動物また はヒトの細胞システムで実施された研究は含まれず、ヒトの健康影響を扱った疫学的研究のみが選ばれた。また、関連 性を測定するための定量的データを欠く出版物も除外した。

コホート研究、症例対照研究及び横断研究が含まれた。各研究は、研究デザイン、除外・包含基準の正確な記述、 ばく露の記述の詳細度、ばく露の測定の強固性、潜在的な交絡因子の調整、健康影響の評価方法、サンプルサイズ 等の12の基準を含む方法に基づいて適格性の評価を受けた。これら12の基準のうち、3つの基準はばく露の記述・ 測定の精度に関連しており、多くの疫学研究が選ばれなかった理由を説明することができるかもしれない。

# A.1.3. 結果

全体では、602の個別の論文が科学的レビューに含まれている。これら 602の出版物は、6,479の異なる解析に対応していた。エビデンスの圧倒的多数は後ろ向きまたは横断研究(それぞれ 38%と32%)であり、前向きな研究は 30%のみであった。ばく露評価は研究によって大きく異なり、全体の 46%が農薬ばく露のバイオマーカーを測定し、さらに 46%が農薬ばく露を推定するためにアンケートを使用していた。研究のほぼ半数(49%)がアメリカを拠点としていた。 ほとんどの研究では、農薬への職業性ばく露と健康影響との関連性が調査されていた。農薬に関連する疾患の全領域を対象とした研究はこれまで行われていなかった。報告書では、さまざまなアウトカムを調査している(図 A.1)。最も多いのは、がんのアウトカム(N=164)と子どもの健康に関するアウトカム(N=84)である。



# 図 A.1: EFSA の外部科学研究報告書でレビューされた出版物のうち、主要な健康影響のカテゴリーと、その健康影響を調査した研究の割合(Ntzaniら、2013 年)

利用可能な大量のデータと大量の分析(>6,000 件)にもかかわらず、研究されたアウトカムの大部分については確 固たる結論が出ていない。これは、収集したデータのいくつかの限界と、レビュー自体の固有の限界によるものである。 上述したように、レビューでは約5年間に農薬に関連して検討されたアウトカムの全範囲が調査された。したがって、最 近のエビデンスのみがレビューされ、実施されたメタアナリシスの結果は、利用可能なすべてのエビデンスを含んでい るわけではないため、慎重に解釈されるべきである。したがって、文献全体(5年以上)をみて、選択したエビデンスの 信頼性を評価することに焦点を当てることで、農薬に関連してさらに詳細な解析を行う価値のある結果を浮き彫りにす ることができる。研究自体の限界は、環境疫学の他の分野と一致しており、ばく露評価、研究デザイン、統計解析、報 告に焦点を当てている。特に以下の点が挙げられる:

a) ばく露評価: ばく露の評価は、おそらく ESR でレビューされた研究の中で最も重要な方法論的限界である。研究では、ばく露の評価と割り付けにさまざまな方法が用いられている。ほとんどの研究では、「これまでに使用したことがあるかないか」または「定期的に使用したことがあるかないか」という自己申告による農薬へのばく露に基づいていた。このような方法では、高い誤分類率に悩まされ、用量反応分析を行うことができない。これは特に後ろ向き研究の場合で、病気のある参加者で報告されるより高いばく露量の差が誤分類を生じる(想起バイアス)(Raphael、1987年)。アンケートは非常に高いばく露レベルと非常に低いばく露レベルの被験者を区別することができるかもしれないが、ばく露濃度による有効な分類を行うことはできず、その結果、用量反応関係の研究を行うことができない。また、ばく露評価のためのアンケートは、疫学研究で使用するために検証される必要がある。それにもかかわらず、多くの研究では検証されていないアンケートを使用しているが、これには内容(アンケートが対象とする有害なばく露源をすべて網羅していない)や基準妥当性(例えば、不正確な想起や質問の誤解)に問題があるかもしれない(Coggon、1995年)。

調査対象とした農薬のカテゴリーの範囲は広いが、研究では多くの場合、広く定義された農薬のカテゴリーに集中しているため、対象集団がどのような種類の農薬にばく露されているのかを知ることは困難である。

農薬へのばく露は、研究参加者による農薬の使用報告または政府の登録データとして定義された。これらのデータ

は、自己記入式アンケート、質問者が管理するアンケート、職業性ばく露マトリックス(JEM)、居住状況(農薬ばく露に 近接しているかどうか)、農薬ばく露に関連するバイオマーカーの検出、または各研究によって定められたその他の方 法から得られたものである。

- 研究では、欧米の集団や EU ですでに禁止されている農薬を調査することが多い。ばく露評価の手段としてバイオ マーカーを使用することはまれであるが、ほぼ半数の研究ではまだ利用可能である。
  - b)研究デザイン:上述したように、エビデンスの大部分は症例対照研究と横断研究から得られている。横断研究や一部の症例対照研究では、時間的関係を完全に評価することができないため、関連性の因果関係に関する裏付け を提供することができない。
  - c) 調査された健康影響:臨床健康影響の定義は、適格な疫学研究において大きなばらつきを示しており、これが結果のばらつきの原因となっている。おそらくこのような状況において最も重要なのは、調査された多数の代替健康 影響の使用である。代替健康影響とは、特定の臨床転帰の代替または予測因子として一般的に受け入れられているバイオマーカーまたは身体測定値のことである。しかし、多くの場合、これらの代替健康影響は検証されておらず、代替健康影響の厳密な定義を満たしていない。このような健康影響は、臨床健康影響の予測因子の可能性があるとされているが、代替健康影響の基準を満たしていない。これらの健康影響の暗黙の仮定を考慮に入れることにより、検証されていない代替健康影響に関するエビデンスを評価することが不可欠である。

広範囲の病態生理をカバーすると評価されたアウトカムは非常に多様だった。データベースに含まれる多くの代替 健康影響と同様に「ハード」な臨床健康影響は、評価された臨床研究の課題にアプローチするために支持された異な る方法論を反映している。さまざまな健康影響は 23 の主要な疾患カテゴリーに分類されており、がんと小児の健康影響を扱った研究が最も多くを占めている。

評価された健康への悪影響には以下のものが含まれる。

- a) がん、呼吸器(アレルギー)、生殖(受精率低下、先天性疾患)、神経変性(パーキンソン病)などの主要な臨床転 帰;
- b)神経発達障害(神経認知スケールで評価)などの臨床代替健康影響;
- c)検査での代替健康影響(例:肝酵素の変化)。

農薬ばく露に起因する多くの有害な健康影響については、矛盾した、あるいは曖昧な研究が存在する。この結果が 一貫性の欠如からくるものなのか、真の不均一性からくるものなのかは、さらなる解明が必要である。

d)統計的解析。

異なる供給源からの複数の物質(重金属、溶剤、浮遊粒子状物質など)への同時ばく露は一般的である。それらの すべてが有害な健康影響をもたらす可能性があるため、結果にさらなるバイアスがかかる可能性がある。したがって、 真の関連性を明らかにするためには、複数の物質へのばく露による交絡を考慮することが不可欠であるが、EFSAの 外部科学研究報告書で評価された圧倒的多数のエビデンスでは、これは不可能であった。

さらに、EFSA の外部科学研究報告書(Ntzani ら、2013 年)で収集・評価されたエビデンスは、都合の良い報告と 複数の試験に悩まされる可能性が高い。研究は非常に広範な解析を報告しており、602 の出版物で 6,000 の解析が 行われた。多重仮説検定の量は膨大である。これらの解析は、複数の仮説検定のために調整されていなければならず、 そうでなければ結果は高い偽陽性率に悩まされる。研究が 1 つの解析しか行われていない場合でも、他の疫学的研究 でも示されているように、都合の良い報告の可能性が常にある。さらに、結果を解釈する際には、特に特定の健康影響 (がんなど)については、エビデンスの大部分が単一の研究集団そして特に農業健康調査から得られていることも考慮 に入れるべきである。

#### A.1.4. EFSA 外部科学研究報告書の結論

上記で強調された限界にもかかわらず、外部科学研究報告書(Ntzani ら、2013 年)では農薬ばく露とパーキンソン病及び小児の白血病との関連、これらは先行するメタアナリシスでも裏付けられている、について首尾一貫したエビデンスを示した。さらに、肝臓がん、乳がん、II 型糖尿病など、これまであまり研究されてこなかった多様な健康影響につ

いてもリスクの増加が認められた。内分泌疾患、喘息、アレルギー、糖尿病、肥満などの他の健康影響への影響は、リ スクの増加を示しており、今後さらに調査が必要である。

小児白血病とパーキンソン病は、2006 年以降のメタアナリシスで一貫して農薬ばく露に関連したリスクの増加を示した2 つの健康影響である。それにもかかわらず、特定の農薬クラスや個々の農薬の影響を解きほどくためには、ばく露をよりよく研究する必要がある。他の健康影響についても、有意な要約推定値が報告されている(表 A.2 に要約)。しかし、これらは2006 年以降の研究であるため、結果は関連性を示唆するものとみなすべきであり、特にばく露の不均一性に関する限界は常に考慮されるべきである。存在しうるバイアスの量を定量化し、バイアスの推定値を考慮に入れても農薬との関連性が十分に支持される結果を分離するために、2006 年以前の出版物を含むように結果を更新した後、特定の健康影響に関連してこれらのデータにデータ統合と統計ツールを適用すべきである。同様に、結論を出すためにさらなるエビデンスが必要な健康影響は、強調される必要がある。

健康面での成果	N	メタアナリシスの	<sup>2</sup>
	studies	結果	
白血病	6	1.26 (0.93; 1.71)	59.40%
ホジキンリンパ腫	7	1.29 (0.81-2.06)	81.60%
小児白血病(妊娠中の農薬ばく露)	6	1.67 (1.25-2.23)	81.20%
小児白血病(妊娠中の殺虫剤ばく露)	5	1.55 (1.14–2.11)	65%
小児白血病(妊娠中に殺虫剤にばく露された場合-ターナー,2010 年の更新)	9	1.69 (1.35–2.11)	49.80%
小児白血病(妊娠中に不特定多数の農薬にばく露された場合	5	2.00 (1.73-2.30)	39.60%
小児白血病 (妊娠中の特定されていない農薬へのばく露 - ターナー、2010 年の更新)	11	1.30 (1.06–1.26)	26.50%
小児白血病(小児期の農薬ばく露)	7	1.27 (0.96-1.69)	61.10%
小児白血病(小児期の殺虫剤ばく露-小児期の殺虫剤ばく露,更新 ターナー, 2010年	8	1.51 (1.28–1.78)	0%
の更新)			
小児白血病 (小児期に特定されていない農薬へのばく露 - ターナー、2010 年の更新)	11	1.36 (1.19–1.55)	0%
乳がん(DDE ばく露)	5	1.13 (0.81–1.57)	0%
乳がん	11	1.24 (1.08–1.43)	0%
精巣がん(DDE ばく露)	5	1.40 (0.82-2.39)	59.50%
胃がん	6	1.79 (1.30-2.47)	0%
肝臓がん	5	2.50 (1.57-3.98)	25.40%
停留精巣	8	1.19 (0.96–1.49)	23.90%
停留精巣(DDT ばく露)	4	1.47 (0.98-2.20)	51%
尿道下裂(一般的な農薬ばく露)	6	1.01 (0.74-1.39)	71.50%
尿道下裂(特定の農薬へのばく露)	9	1.00 (0.84–1.18)	65.90%
流産	6	1.52 (1.09–2.13)	63.10%
パーキンソン病	26	1.49 (1.28–1.73)	54.60%
「 パーキンソン病(DDT ばく露)	5	1.01 (0.78-1.30)	0%
パーキンソン病(パラコートばく露)	9	1.32 (1.09–1.60)	34.10%
筋萎縮性側索硬化症	6	1.58 (1.31-1.90)	10%
喘息(DDT ばく露)	5	1.29 (1.14–1.45)	0%
喘息(パラコートばく露)	6	1.40 (0.95-2.06)	53.30%
喘息(クロルピリホスばく露)	5	1.03 (0.82–1.28)	0%
1 型糖尿病(DDE ばく露)	8	1.89 (1.25–2.86)	49%
1 型糖尿病(DDT ばく露)	6	1.76 (1.20-2.59)	76.30%
2 型糖尿病(DDE ばく露)	4	1.29 (1.13–1.48)	0%

#### 表 A.2:報告書で実施されたメタアナリシスの概要

N=メタアナリシスのために検討された研究の数;メタアナリシス結果の列では、数字は効果の大きさ(オッズ比(OR)または相対リ スク(RR))の統計的推定値を、対応する 95%信頼区間(Cl)とともに表している。

l<sup>2</sup>は、研究間の総変動のうち、不均一性に起因する割合を示す。

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#### A.2. INSERIM レポート

2013 年 9 月、フランス国立保健医療研究所(INSERM)は、農薬へのばく露によるとト健康影響について専門家グ ループと共に実施した文献レビューを発表した<sup>22</sup>。2012 年 6 月までの科学文献に発表された疫学的または実験的デ ータが解析された。報告書には、文献的解析を概説し、主要な結論と政策及び勧告を強調した要約が添付されている。

INSERM の報告書は4つの部分から構成されている。(1) ばく露評価、疫学研究におけるばく露を評価するための 直接的及び間接的な方法の詳細な説明、(2) 疫学、2012 年までの文献で利用可能な疫学研究のインベントリと解析 及び推定される関連性の強さを評価するためのスコアリングシステム、(3) 毒性学、いくつかの物質の毒性学的データ (代謝、作用機序、分子経路)のレビューと生物学的妥当性の評価、(4) 推奨事項。

健康影響の発生と推定される中等度または強い関連性を持つと INSERM の報告書で特定された物質の大部分は、 現在禁止されている化学物質である。これは主に、調査された疾患の大部分が高齢者の疾患であるという事実によっ て推進されている。したがって、これまでに実施された研究は、研究の時点で高齢であり、何年も前にばく露された人に 基づいている。結論から言うと、最近の製品の多くの潜在的な長期的影響を調査することはまだ可能ではない。

これらの物質は、DDT やトキサフェンのような有機塩素系殺虫剤や、テルブフォスやプロポキサーのようなコリンエス テラーゼ阻害作用を持つ殺虫剤のグループに属している。

INSERM の専門家評価報告書で確認された 7 つの承認された有効成分(除草剤 2,4-D、MCPA、メコプロップ、グ リホサート、殺虫剤クロルピリホス、葉の殺菌剤マンコゼブとマネブ)のうち、すべてが造血器がんとの中等度または弱い 関連性があると推定されていた。そのうち 2 つ(葉状殺菌剤マンコゼブとマネブ)はパーキンソン病との関連性が弱いと 推定され、2 つ(クロルピリホスとグリホサート)は専門家の評価で弱い、または中等度とされた発達障害との関連性があ ると推定された。

#### A.2.1. 疫学研究におけるばく露評価方法の説明

ばく露を評価するために、生物学的または環境モニタリングデータ、その場しのぎのアンケート、職業別または作物 別のばく露マトリクス、専門家のカレンダーの分析、販売データ、土地利用データなど、さまざまな方法(直接的及び間 接的)が開発されてきた。著者らによると、これらの様々なツールは互いに組み合わせることができるが、現在までのとこ ろ、職業性農薬ばく露評価の背景でばく露を推定するための基準となる方法として有効性が確認されていない。

#### A.2.2. 疫学

INSERMの専門家グループは、文献で入手可能な疫学研究の目録作成と解析を行い、農薬ばく露と健康影響との 関連性の可能性を検討した。8 つのがん部位(非ホジキンリンパ腫、白血病、リンパ腫、多発性骨髄腫、前立腺、精巣、 脳、メラノーマ)、3 つの神経変性疾患(パーキンソン病、アルツハイマー病、筋萎縮性側索硬化症)、認知・抑うつ障害、 生殖能への影響(受胎性、胎児と出生児の発生)、小児がんである。これらは、以前の研究で農薬ばく露に関連する可 能性があるとして同定されている健康影響である。

主に農家、農薬散布者、農薬製造業の労働者を対象とした疫学的研究と、関連性がある場合には一般集団を対象 とした研究が選ばれた。

INSERM の専門家グループは、研究の関連性における階層を設定し、メタアナリシスを最上位に置き、次にシステ マティックレビュー、コホート研究、そして最終的には症例対照研究とした。この階層に基づいて、研究結果の解析から、 ばく露と健康影響の発生との間の関連性の推定の強さを評価するためにスコアリングシステムが定義された;調査され た各疾患や病態について、このスコアは、例えば、利用可能な研究の質、種類、数によって異なる。

(++):強い推定:メタアナリシスの結果に基づく、または複数のコホート研究、または少なくとも1 つのコホート研究と2 つの症例対照研究、または2 つ以上の症例対照研究。

(+):中程度の推定:コホート研究または入れ子になった症例対照研究または2つの症例対照研究の結果に基づ

<sup>&</sup>lt;sup>22</sup> INSERM. 農薬。 sante への影響。 Collection expertise collective, Inserm, Paris, 2013.

く。

(±):弱い推定:1件の症例対照研究の結果に基づく。この統合により、この作業は単純なマッピング作業の状態を 超えたものとなった。

## A.2.3. 毒性学的データ

文献レビューで検討した毒性データは、主に代謝、作用機序、分子経路に関するものであった。製品を上市するための手続きの一部として提供された研究は、公表された文献で発表された場合を除き、考慮されなかった。

疫学研究で物質が明らかに同定された場合には、研究結果から生物学的に妥当であるかどうかを評価するためのス コアリングシステムを設定した:病態生理学的データとの整合性と健康影響の発生。

(++):3つの毒性メカニズムで支持された仮説。

(+):少なくとも1つの毒性メカニズムによって支持された仮説。

## A.2.4. 所見

INSERM 報告書の主な結果は、表 A.3-A.6 にまとめられている。

健康面での成果	リスク過剰が顕著な集団のタイプ	推定の強さ (a)
エヌエイチエル	農家、農薬散布者、製造工場関係者	++
前立腺がん	農家、農薬散布者、製造工場関係者	++
多発性骨髄腫	農家、農薬散布者	++
パーキンソン病	職業上ばく露と非職業上ばく露	++
白血病	農家、農薬散布者、製造工場関係者	+
アルツハイマー病	農家	+
認知障害 <sup>(b)</sup>	農家	+
妊孕性・胎動性障害	職業上ばく露	+
ホジキンリンパ腫	農業従事者	±
精巣がん	農業従事者	±
脳腫瘍(神経膠腫、髄膜腫	農業従事者	±
メラノーマ	農業従事者	±
筋萎縮性側索硬化症	農家	±
不安、うつ病 (b)	農家、急性中毒の既往歴のある農家、農薬散布者	±

表 A.3: 農薬への職業上ばく露と成人の健康影響との間の統計的に有意な関連(レビューで解析された健康影響)

(a):スコアリングシステム:強い推定(++)、中程度の推定(+)、弱い推定(±)。

(b):ほとんどの農薬が有機リン酸塩であった。

# 表 A.4:職業または家庭生活での農薬ばく露と小児のがんまたは発達障害(レビューで解析された健康影響)との間の 関連(統計的に有意な関連のみを示す)

健康面での成果	<b>戈果 リスク超過が顕著なばく露の種類と集団</b>		
白血病	妊娠中の職業上ばく露、出生前ばく露(住居)	++	
脳腫瘍	妊娠中の職業上ばく露	++	
先天性奇形	妊娠中の職業上ばく露。	++	
	妊娠中の住居ばく露(農地、家庭生活での使用)	+	
胎児の死	妊娠中の職業上ばく露	+	
神経発達	妊娠中の住居ばく露(農地、家庭生活、食品) <sup>(b)</sup> 。	++	
	妊娠中の職業上ばく露	±	

(a):スコアリングシステム:強い推定(++)、中程度の推定(+)、弱い推定(±)。

(b):有機リン酸系。

#### 表 A.5:承認された有効成分に関する知見:疫学的評価と生物学的妥当性

有効成分	分類	推定の強さ <sup>(a)</sup>	生物学的妥当性(b)
有機リン酸系殺虫剤	1		
クロルピリホス	急性毒性 CAT3	白血病(+)	Yes (++)
		神経発達(+)	Yes (++)
		NHL (±)	Yes (++)
ジチオカルバメート	系殺菌剤		
マンコゼブ/マネブ	生殖毒性 CAT2	白血病(+)	?
		メラノーマ (+)	?
		パーキンソン病	Yes (+)
		(パラコートと併用して)(±)	
フェノキシ系除草剤	1		
2,4-D	急性毒性 CAT4	NHL (+)	?
MCPA	急性毒性 CAT4	NHL (±)	?
メコプロップ	急性毒性 CAT4	NHL (±)	?
アミノホスホン酸ク	リシン除草剤		
グリホサート		NHL (+)	?
		胎児死亡 (±)	?

(a):スコアリングシステム:強い推定(++)、中程度の推定(+)、弱い推定(±)。

(b):スコアリングシステム。(++):毒性の3つの異なる既知のメカニズムによって支持された仮説、(+):毒性の少なくとも1つのメカニズムによって支持された仮説。

有効成分	Ban in the EU	IARC classfication	推定の強さ <sup>(a)</sup>	生物学的妥当性 <sup>(b)</sup>
ディルドリン	1978	3or2 (US-	NHL (c)	Yes (+)
		EPA)	前立腺がん (±)	Yes (+)
			パーキンソン病 (±)	?
DDT/DDE	1978	2B	NHL (++)	Yes (+)
			精巣がん(+)	?
			子供の成長(++)	?
			神経発達(±)	?
			精子パラメータ異常(+)	?
クロルデン	1978	2B	NHL	Yes (+)
			白血病(+)	Yes (+)
			前立腺がん(±)	Yes (+)
			精巣がん(+)	?
リンデン(c-HCH	2002/2004/2006/2007	2B(d)	NHL (++)	Yes (++)
			白血病(+)	Yes (++)
b-HCH	2002/2004/2006/2007	2B(d)	前立腺がん(±)	?
トキサフェン	2004	2B	NHL (c)	Yes (++)
			白血病(+)	Yes (++)
			メラノーマ (+)	Yes (+)
クロルデコン	2004	2B	前立腺がん (++)	Yes (+)
			精子パラメータ異常(+)	?
			神経発達(+)	?
ヘプタクロル	1978	2B	白血病(+)	Yes (+)
エンドスルファン	2005	Not classified	?	Yes (+)
ヘキサクロロベン	1978	2B	子供の成長(+)	?
ゼン (HCB)	1010	20		
テルブフォス	2003/2007		NHL (+)	?
			白血病(+)	?
ダイアジノン	2008		NHL (+)	?
			白血病(+)	?
マラチオン	2008	3	NHL (++)	Yes (+)
			白血病(+)	Yes (+)
			神経発達(+)	?
			精子パラメータ異常(+)	?
フォノフォス	2003		NHL (±)	?
			白血病(+)	?
			前立腺がん(+)	?
パラチオン	2002	3	メラノーマ (+)	?
クマフォス	EU では届出認可されてい	ない	前立腺がん(+)	?
カルバリル	2008	3	NHL (±)	?
			メラノーマ(+)	?
			精子パラメータ異常(+)	?
プロポキサー	2002		神経発達(+)	?
			胎児発育(+)	?
カルボフラン	2008		NHL (±)	?
			前立腺がん(+)	?
ブチル酸塩	2003		NHL (+)	?
			前立腺がん(+)	?
EPTC	2003		白血病(+)	?
アトラジン	2005	3	NHL	Yes (+)
			胎児発育(+)	?
シアニジン	2002/2007		NHL (c)	?
ペルメトリン	2002	3	前立腺がん(+)	Yes (+)

表 A.6:非承認有効成分に関する知見:疫学的評価と生物学的妥当性

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フェンバレレート	1998	Not classified	精子パラメータ異常(+)	?
臭化メチル	2010	3	精巣がん(+)	?
ジブロモエタン	Banned	2A	精子パラメータ異常(+)	?
ジブロモクロロプ ロパン(DBCP)	Banned	2B	精子パラメータ異常/不妊 (++++)(因果関係)	Yes (+++) (作用機 序解明)
パライバット	2007		パーキンソン病(+)	Yes (++)
ロテノン	2011		パーキンソン病(+)	Yes (++)
アラクロール	2008		白血病(+)	Yes (++)

(a):スコアリングシステム:強い推定 (++)、中程度の推定 (+)、弱い推定 (±)。

(b):スコアリングシステム。(++):3つの毒性メカニズムに支持された仮説、(+):少なくとも1つの毒性メカニズムに支持され た仮説。

(c):t(14,18) 転座を有する母集団のみ。(d):技術的混合物(α-、β-及び Y-HCH)。

## A.2.5. 推奨事項

いくつかの有効成分に関する利用可能な疫学的・機序学的データを解析した結果、さらなる研究開発のためのいく つかの推奨事項が示唆された。

- a) 農薬への集団ばく露に関する知識は改善されるべきである。
  - 1) 農家の有効成分使用に関する情報収集
  - 2) 実際のばく露レベルを測定するための農地での研究の実施
  - 3) 生涯労働期間のばく露を監視すること
  - 4)空気(屋外・屋内)、水、食品、土壌中のばく露レベルの測定
  - 5) 急性中毒に関する情報収集
  - 6) バイオモニタリングや外部ばく露量測定のための解析方法の改善
  - 7)研究者が広範な製剤データ(溶剤、共配合製剤など)にアクセスできるようにする。
- b) ばく露と健康影響との間の潜在的な関連性を研究する。
  - 1)健康影響をもたらす物質または物質群の特性を把握する
  - 2)影響を受けやすい個人または集団に焦点を当てる(酵素の遺伝子多型など)
  - 3) ばく露枠と感受性(妊娠期間、発育期間)に焦点を当てた研究
  - 4) 疫学と毒物学のギャップを埋める(作用機序)
  - 5) 混合物の毒性に関する知識の向上

6)研究の新しいアプローチ(in vitroや in silicoモデル、オミクスなど)を育成する。

#### A.3. EFSA 外部科学研究報告書と INSERM 報告書の類似点と相違点

ここで議論されている2つの報告書は、異なる方法論を使用している。しかし、多くの場合、それらの結果と結論は一致している。INSERMの報告書は、事前に調査された結果に限定されており、毒物学的データもレビューすることで疫学研究の生物学的妥当性を調査しようとしているのに対し、EFSAの報告書は、約5年の期間に発表されたすべての利用可能な疫学研究の包括的なシステマティックレビューである。

両報告書の違いは表 A.7 に示されており、検索期間(すなわち、両報告書は同じ出版データを評価していない)、研究の適格性の基準の違い、健康影響全体と健康影響内のエビデンスを要約するアプローチの違いに関連している。

全体的に、INSERM の報告書は EFSA の報告書よりも多くの健康被害との関連を特定している。しかし、同じ健康 影響(小児白血病、パーキンソン病)については、両方の報告書で農薬ばく露との関連性が十分に証明されていると主 張されている。

表 A.7: EFSA 外部科学報告書と INSERM 報告書で使用された方法の比較	
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	EFSA External report	INSERM report
レビューされた論文	602/43,000	NR
言語	Yes	NR
検索戦略(キーワード、MeSH)	Yes	NR
検索データベース	Yes (4)	NR
出版年	2006-2012 (Sep)	? to 2012 (Jun)
評価された疫学研究の種類	Cross-sectional	Cross-sectional
	Case-control	Case-control
A / ++>//	Cohort	Cohort
含有基準	Yes	NR
除外基準	Yes	NR
方法論的品質評価	Yes (12 criteria)	NR
ばく露グループ(a)	Yes	Yes
ばく露評価	Yes	Yes
定量的統合(メタアナリシス)	Yes	No
質的統合(c)	Yes	Yes
毒物学的データのサポート	NI	Yes
個々の農薬との関連性	Yes	Yes
健康影響研究		
血管がん	Yes	Yes
充実性腫瘍	Yes	Yes
小児がん	Yes	Yes
神経変性疾患	Yes	Yes
神経発達影響	Yes	Yes
精神障害(b)	No	Yes
生殖と発生	Yes	Yes
内分泌	Yes	NI
代謝	Yes	Yes
免疫学的	Yes	NI
呼吸器	Yes	NI

NR:報告されていない、NI:調査されていない。

(a): ばく露の種類(環境、職業など)及び期間(一般集団、児童など)。

(b):例:うつ病性障害。

(c):説明を追加する。

# A.4. The Ontario College of Family Physicians の文献レビュー(OCFPLR)

2004 年、カナダの The Ontario College of Family Physicians は、1992 年から 2003 年の間に発表された、農 薬ばく露に関連した主要な健康影響に関する文献をレビューした。著者らは、表 A.8 に示すように充実性腫瘍と農薬 ばく露との間には正の関連が存在すると結論づけた。著者らは、よく計画された大規模コホート研究では、これらの関 連性は一貫して統計的に有意であり、その関係は高ばく露レベルで最も一貫していたと指摘している。また、用量反応 関係がしばしば観察され、研究の質は概ね良好であるとした。

#### 表 A.8:オンタリオ州家庭医大学のレビューで考慮された健康影響、2004年

エンドポイント	オンタリオ大学で同定された関連性、農薬(差異化されている場合)、研究の 種類、(研究数/総研究数)			
A) がん				
1. 肺	-ve cohort (1/1)			
	+ve case-control (1/1)			
	+ve carbamate, phenoxy acid, case-control (1/1)			
2. 乳房	+ve case-control (2/4)			
	+ve ecological (1/1)			
	+ve triazine, ecological (1/1)			
	-ve atrazine, ecological (1/1)			
3. 大腸直腸				
4. 膵臓	+ve cohort $(1/1)$			
	+ve case-control (2/2)			
5. 非ホジキンリンパ腫	+ve cohort (9/11)			
	+ve case-control (12/14)			
	+ve ecological (2/2)			
6. 白血病	+ve cohort $(5/6)$			
	+ve case-control (8/8)			
	-ve ecological $(1/1)$			
7 01/2	+ve lab study $(1/1)$			
7. 脳	+ve cohort (5), similar case-control (5)			
8. 前立腺	+ve cohort (5/5) case-control (2/2) ecological (1/1)			
9. 胃				
10. 卵巣				
11. 腎臓	+ve pentachlorophenol cohort (1/1)			
	+ve cohort $(1/1)$			
10 // / // / / / / / / / / / / / / / / /	+ve case-control (4/4)			
12. 精巣 P) まぜく				
B) 非がん 1) 生 時 制 細				
1) <b>生殖影響</b>	+ve glyphosate			
先天性奇形	+ve pyridyl derivatives			
分娩/妊娠までの期間	Suggest impaired			
受胎性				
発育影響	Possible +ve association, but further study required			
胎児死亡	Suggested association			
混合した健康影響				
2) 遺伝毒性・免疫毒性	+ve Synthetic pyrethroids (1)			
染色体異常	+ve organophosphates (1)			
NHL再編成	+ve fumigant and insecticide applicators +ve fumigant and herbicide applicators			
2) 史虞利尚的	+ve runngant and herbicide applicators			
3)皮膚科学的				
4)神経毒性の精神及び感情への影響	+ve			
機能的神経系の影響	+ ve organophosphate/carbamate poisoning			
神経変性の影響(PD)	+ve cohort $(4/4)$			
	+ve case-control (2/2)			
	+ve ecological (1/1)			

+ve: positive; -ve: negative.

報告書は、農薬ばく露と非ホジキンリンパ腫(NHL)の発症との関連性を示す説得力のあるエビデンスがあり、また農 薬ばく露と白血病との間に正の関連性があるという明確なエビデンスがあると結論づけている。著者らはまた、様々な ばく露時間の経過から生じる多くの神経系への影響についても一貫した結果が得られたと主張している。

このような断定された結論は、非政府組織(NGO)の支持を得て、いくつかの規制当局の間で疑問が生じた。当時の

英国政府の独立諮問委員会である農薬諮問委員会(ACP)は、オンタリオ大学レビューの結果の評価を依頼された。 委員会のメンバーには1人の疫学者が含まれており、委員会は、他の政府の委員会に独立した助言を提供することに 関与している他の5人の疫学者に相談した。彼らはすべてのレビューが主要な欠点を持っていたことに同意した(例え ば、正確な検索戦略と特定されていない選択基準、結果の選択的な報告、不適切な理解と関連する毒性学の考慮、 ばく露のルートとレベルへの不十分な注意、正当化された結論、等)。全体的に、オンタリオ大学レビューの結論は、提 示された解析によってサポートされていないと考えられた。2012年には、オンタリオ大学レビューの著者は、彼らの評 価の更新を発表した;彼らの2番目の報告書では、彼らは非常に似たようなアプローチを使用したが、使用される包含 基準に関するより詳細を提供した。この例は、疫学研究の過剰解釈のリスクを思い起こさせるものである。特に、ばく露 と有害な性健康影響の発生との間の因果関係を推論することはよくあるが、これはさらに評価されるべき関連性を示し ている。 Epidemiological studies and pesticides

### 付属書 B-EFSA が委託したヒト・バイオモニタリング・プロジェクト23

2015 年、EFSA は、疫学研究におけるばく露評価のためのツールとして、また、農薬への職業上ばく露による潜在 的な健康リスク評価に貢献するために、労働安全衛生戦略における HBM の役割をさらに調査するためのプロジェクト を外部委託した。実際、ばく露評価はすべての疫学研究の重要な部分であり、ばく露の誤分類や単純な分類法の使用 は、接触と健康被害の結果との間に関連性があるかどうかを判断する研究の能力を弱めてしまうことが知られている。

Risk & Policy Analysts Limited (RPA)、IEH Consulting Limited (IEH)、Health & Safety Laboratory (HSL)からなるコンソーシアムは、1990 年から 2015 年までの期間、系統的な文献レビューを実施した。その目的は、 作業ばく露評価の再開発のためのツールとしての HBM の使用に関する概要を提供し、長所、短所、さらなる開発の 必要性を特定することであった(第一の目的)。検索では、農薬(またはその代謝物)への職業上ばく露を評価するため の HBM の使用に関連する 2096 の文献を特定した。検索の結果(Bevan ら、2017 年)は、過去 10~20 年の間に HBM の使用が拡大してきたこと、特に環境や消費者のばく露分析の分野にまで拡大してきたことを示している。しかし、 農薬ばく露評価のための HBM の使用については、特に、分析品質の向上や標準化のための戦略の開発、代謝物の ための標準物質の利用可能性の向上、数学的モデリングへの HBM データの統合、ばく露の再構築、分析機器の改善、ヒト毒性データの利用可能性の向上など、さらなる改善が必要とされている。

請負業者は、EU/米国の作業環境で実施された利用可能な HBM 研究/サーベイランスプログラムのレビューを 実施し、残留性のあるものと残留性のないものの両方の農薬(または代謝物)を特定した。最も関連性の高い研究を特 定するために、HBM、疫学的、毒性学的側面の品質スコアリングを含む 2 段階のスクリーニングプロセスを利用し、 178 件の研究をクリティカルレビューの対象とした。特定された研究のスクリーニングと並行して、これらの文献からのデ ータを照合するためにマスタースプレッドシートが計画され、その中には、研究タイプ、研究参加者、調査対象の化学 物質、バイオマーカーの品質チェック、分析方法、ばく露評価、健康影響/毒性エンドポイント、追跡期間、結果の説 明、バイアスのリスク、その他のコメントに関する情報が含まれている。

HBM は、様々な農薬への作業者のばく露を監視するために広く使用されている。職業上の農薬の使用に関する疫 学的研究では、不十分なばく露情報や後ろ向きなばく露情報が制限されていることが見受けられる。職業別または作 物別のばく露マトリックスの使用例も報告されている。しかし、実際のばく露データに対するこれらのマトリックス研究の 検証はほとんど行われていない。季節的なばく露と PPE の影響を調査したデータは非常に限られており、多くの研究 では、1 つまたは 2 つの特定化合物のみを評価するために HBM を使用している。現在、健康リスク評価には多種多 様なばく露モデルが採用されており、モデルによって予測されたばく露推定値を評価するためにバイオマーカーもしば しば使用されている。

関連性があると判断された 178 の出版物から、41 の個別研究が除草剤を含み、そのうち 34 の個別除草剤が同定され、そのうちの 15 が現在 EU での使用が承認されている。同様に、殺虫剤を含む 90 件の個別研究のうち、79 件の殺虫剤が同定され、そのうち 18 件は現在 EU での使用が承認されている。20 の個別研究には殺菌剤が含まれており、34 種類の殺菌剤が確認され、そのうち 22 種類が現在 EU での使用が承認されている。最も研究された除草剤は(順に)、2,4-D>アトラジン>メトラクロール=MCPA>アラクロール=グリホサートであることが示されている。同様に、最も研究された殺虫剤(順に)は、クロルピリホス>ペルメトリン>シペルメトリン=デルタメトリン>マラチオンであり、最も研究された殺菌剤は、キャプタン>マンコゼブ>フォルペットであった。

現在の限界は、特にとトを対象とした個々の農薬の ADME に関して、とトからの動特性データの数が限られているこ とに起因しており、これにより、すべてのばく露経路についてより正確な HBM サンプリングが可能になる。このことは、 毒物動態データに依存する農薬のリスク評価のための PBPK モデルの開発や、現在使用されているばく露評価モデ ルのバリデーションにも影響を及ぼす。現在、この分野での HBM の使用に影響を与えているさらなる限界は、現在使 用されている農薬への長期ばく露を評価するための大規模な前向きコホート研究が不足していることである。

特定されたエビデンスは、ヨーロッパにおける農薬の労働衛生サーベイランスの一環としての HBM の実施に関する

<sup>23</sup> Bevanら

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推奨事項を策定するために使用されている。実施を可能にするために克服しなければならないいくつかの重要な問題 が検討された。その中には、新しいスペックや感度の高いバイオマーカーの開発の優先順位の設定、健康に基づいた ガイダンス値の導入と採用、研究間の計測値を検証するための QA スキームの開発、野外作業やアンケートの作成に おける良好な実施、バイオバンキングの使用範囲の拡大、農薬の安全性の承認後のモニタリングにおける HBM の使 用などが含まれている。

# 付属書 C-ハザードの特定のための疫学研究の統合に関する国際規制機関の経験 C.1. WHO-国際がん研究機関(IARC)

国際がん研究機関(IARC)の「ヒトに対する発がん性リスク評価に関する IARC モノグラフ」は、ヒトのがんリスクを増加させる可能性のある環境ばく露を評価するために 40 年前に設立されたプログラムである。これらには、個々の化学物質や化学物質の混合物、職業上ばく露、物理的要因、生物学的要因、生活様式の要因が含まれる。

IARC は、科学者からなる国際的な学際的作業部会を組織し、科学的出版物からのエビデンスの質と強度をレビューして評価し、懸念される物質がヒトに発がんリスクをもたらす可能性を評価するためのハザード評価を実施している。 特に、IARC ワーキンググループのメンバーの役割は、がんに関する疫学的研究やその他の実験的研究の結果の評価、発がんのメカニズムに関するデータの評価、ヒトへのばく露による発がん性の総合的な評価を行うことである。

モノグラフは、世界中の政府、組織、公衆衛生の予防・管理措置を設定するために広く利用され、参照されている。

IARC モノグラフの前文<sup>24</sup>は、プログラムの範囲、モノグラフの開発に使用される科学的原理と手順、考慮されるエビ デンスの種類、評価の指針となる科学的基準を説明している。モノグラフの範囲は、単一の化学物質だけでなく、関連 する化学物質のグループ、複雑な混合物、職業上ばく露、物理的・生物学的物質、生活様式の要因を含むように拡大 された。そのため、モノグラフのタイトルは「ヒトに対する発がん性リスクの評価」となっている。

関連する疫学研究、実験動物を用いた発がんバイオアッセイ、メカニズムデータ、ばく露データなどが批判的にレビューされている。公表されている科学文献に掲載された、または掲載が認められた報告書のみが含まれる。しかし、研究を含めることは、研究デザインの妥当性や結果の分析と解釈を受け入れることを意味するものではない。利用可能な研究の質的側面は慎重に精査されている。

モノグラフではハザードの特定を強調しているが、がんのハザードを評価するために用いられた疫学研究や実験研 究と同じものを、用量反応関係を推定するためにも用いることができる。モノグラフは、利用可能な疫学データの範囲内 で用量反応関係を推定することもあれば、実験研究と疫学研究の用量反応情報を比較することもある。

モノグラフの構成は以下のようになっている。

1)ばく露データ

2) ヒトにおけるがんの研究

3)実験動物を用いたがんの研究

4)メカニズム等の関連データ

6)評価と根拠

ヒトの疫学的データは、関連するすべての疫学的研究が評価されている 2)に記載されている。バイオマーカーの研究は、ヒトに対する発がん性の評価に関連する場合に含まれる。

疫学研究の IARC 評価には、以下の基準の評価が含まれる:検討された研究の種類(例:コホート研究、症例対照 研究、相関(または生態学的)研究及び介入研究、症例報告)、研究の質(例:バイアス、交絡、生物学的変動及び影 響の推定精度に対するサンプルサイズの影響)、メタアナリシス及びプール分析、時間的影響。例えば、最初のばく露 時の年齢、最初のばく露からの時間、ばく露の期間、累積ばく露、ピークばく露などの時間的変数)、疫学研究におけ るバイオマーカーの使用(例えば、ばく露のエビデンス、初期効果のエビデンス、細胞、組織または生物の反応のエビ デンス)、因果関係の基準。

因果関係に関する特定の基準では、問題の薬剤がとトに対して発がん性があるというエビデンスの強さに関して判断 がなされる。

判断を下す際に、作業部会は因果関係についていくつかの基準を考慮している(Hill、1965 年)。強い関連性(例 えば、大きな相対リスク)は因果関係を示す可能性が高い。しかし、疾患やばく露が一般的な場合には、弱い関連性が 重要であることが認識されている。異なるばく露条件で計画の異なる複数の研究で再現された関連性は、単一の研究

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<sup>5)</sup>まとめ

<sup>&</sup>lt;sup>24</sup> http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf

からの孤立した観察よりも因果関係を示す可能性が高い。異なる研究間で一貫性のない結果が得られた場合には、考 えられる理由(例えば、ばく露の違い)を探り、方法論的に健全でない研究よりも質の高い研究の方が重要視される。明 確な用量反応効果がないからといって必ずしも因果関係を否定するエビデンスにはならないが、ばく露に伴ってリスク が増加することは因果関係を示す強いエビデンスと考えられている。ばく露の停止または減少後にリスクが低下してい ることが示された場合も、結果の因果関係の解釈を支持するものである。時間性、影響の推定精度、生物学的妥当性、 全体的なデータの一貫性が考慮される。バイオマーカー情報は、疫学的観察の生物学的妥当性の評価に使用される ことがある。被験者と非被験者でがんの発生率が異なることを示している無作為試験は、特に因果関係を示す強力な エビデンスとなる。

疫学的研究でばく露とがんとの間の関連性がほとんど、または全く示されない場合には、発がん性がないと判断する ことができる。このような場合、研究は、バイアスの可能性、交絡の可能性、またはばく露の誤分類を含めて、上述の計 画と解析の基準を評価するために精査される。さらに、方法論的に健全な研究は、観察されたばく露レベルのどのよう なばく露についても、影響の推定値が一致していること、相対リスクのプール推定値がほぼ一致すること、そして狭い信 頼性間隔を持つことに一貫性があるべきである。さらに、個々の研究も、すべての研究のプール結果も、ばく露レベル の増加に伴うリスクの増加を示すべきではない。発がん性がないというエビデンスは、研究されたがんの種類、報告され たばく露量及びこれらの研究で観察された最初のばく露と疾患発症の間の期間にのみ適用できる。ヒトのがんの経験 から、最初のばく露から臨床症状のがんの発生までの期間が20年よりも長いことがあり、30年よりも実質的に短い潜伏 期間は、発がん性の欠如のエビデンスを提供できないことが示されている。

最後に、疫学研究の結果、標的臓器または組織、用量反応関連、ヒト及び動物のデータのエビデンスの強固さの評価、メカニズムのエビデンスの強固さをまとめた総合評価に到達するために、エビデンスの全体像を検討する。

総合評価の最後に、以下のいずれかのグループに分類される。グループ 1、その薬剤はヒトに対して発がん性がある;グループ 2A、その薬剤はおそらくヒトに対して発がん性がある;グループ 2B、その薬剤はヒトに対する発がん性に関して分類できない;グループ 4、その薬剤はおそらくヒトに対して発がん性がない。

薬剤の分類は、とト及び実験動物での研究、ならびにメカニズム及びその他の関連データから得られたエビデンスの 強固さを反映する科学的判断の問題である。これらの分類は、ばく露が発がん性であるというエビデンスの強固さにの み言及しており、発がん性(可能性)の程度には言及していない。

例えば、グループ 1:その薬剤はヒトに対して発がん性がある。このカテゴリーは、ヒトにおける発がん性の十分なエビ デンスがある場合に使用される。例外的に、ヒトにおける発がん性のエビデンスが十分ではないが、実験動物における 発がん性の十分なエビデンスがあり、ばく露されたヒトにおいて、その薬剤が発がん性の関連メカニズムを介して作用 するという強いエビデンスがある場合には、薬剤はこのカテゴリーに分類される。国際的に広く受け入れられているが、 過去には特定の薬剤の分類に対する批判があり、より最近の批判は、そのような評価のために IARC が採用した一般 的なアプローチに向けられており、反論の発表を動機づける可能性がある(Pearce ら、2015 年)。

#### C.2. リスク評価における疫学的研究の統合に関する US-EPA の経験

米国環境保護庁の農薬プログラム(OPP)は、農薬製品の登録と規制を担当する米国の政府機関である<sup>25</sup>。この活動の一環として、また農薬の使用が許可される前に、OPPは農薬がビト健康と環境に及ぼす影響を評価している。

EPAは、連邦殺虫剤・殺菌剤・殺鼠剤法(FIFRA)及び連邦食品・医薬品・化粧品法(FFDCA)を通じて、農薬製品のリスクを特性評価するための広範なハザード及びばく露情報を入手している。農薬の毒性影響に関する情報は、一般的に、農薬登録者が実施し、EPAに提出する実験動物を用いた研究から得られている。

これまでは、農薬へのばく露に関連する可能性のある潜在的なリスクを EPA が評価するための情報として、農薬に 関する十分に計画された疫学研究から得られた情報は一般的には得られていなかった。農薬へのばく露と健康影響と

www.efsa.europa.eu/efsajourna

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<sup>&</sup>lt;sup>25</sup> 農薬科学及び農薬リスクの評価に関する一般的な情報については、https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks を参照のこと。

の間に考えられる関連性を調査する疫学研究が文献に掲載されることが増えてきたため、EPA はこの情報源をさらに 重視している。これは、20 年以上にわたって 9 万人近くの個人を追跡した大規模でよく実施された前向きなコホート研 究である農業健康調査(Agricultural Health Study<sup>26</sup>(AHS)や、小児環境保健・疾病予防研究センターから得られ た豊富な研究に特に当てはまる。EPA<sup>27</sup>は、このような疫学的情報を最も科学的に強固で明白性の高い方法で利用す ることを目標に、ヒトの健康リスク評価においてこれらの疫学的研究をより多く利用することを意図している。

# C.2.1. OPP 疫学的フレームワーク文書

このプロセスの初期段階として、EPA-OPPは、2010年に「健康リスク評価にヒトの疫学的データ及びインシデントデ ータを組み込むためのフレームワーク(Framework for incorporated human Epidemiologic and Incident Data in Health Risk Assessment)」(US-EPA、2010 年 a)という疫学的枠組み文書案を作成した。2010 年のフレームワ ーク草案は、2010年2月にFIFRA科学諮問委員会(SAP)によって好意的にレビューされた(US-EPA、2010年b)。 この文書は最近、2016 年に「Office of Pesticide Programs' Framework Document for Incorporating Human Epidemiology and Incident Data in Risk Assessments for Pesticides」(US-EPA、2016年)に更新された。改訂 及び更新された2016年のフレームワーク文書は、疫学研究(ヒト事例データベース、バイオモニタリング研究に加えて) でみられるようなヒトの情報が、実験的な毒性学的情報とともに、実際の化学物質ばく露によって引き起こされる影響に ついての予測を提供することで、この新しいアプローチにおいて重要な役割を果たすことが提案されている。さらに、疫 学的/分子疫学的データは、追加解析の指針となり、潜在的に影響を受けやすい集団や新たな健康影響を特定し、 既存の毒物学的観測を補完する可能性がある。2016 年版フレームワークの概念は、専門家の査読を経た強固な原則 とツールに基づいており、疫学データのレビューと評価のための多くの既存のガイダンス文書とフレームワーク(表 C.1) に依存している。また、問題設定の重要性と生物学的組織の異なるレベルでの情報統合の必要性を強調した世界保 健機関/国際化学安全計画(MOA)/ヒト関連性フレームワークの更新とも一致している(Meek ら、2014 年)。さらに、 このフレームワークは、2009年の報告書「Science and Decisions (NRC、2009年)」の中で、複雑な科学的分析の最 初に問題の定式化を使用することの重要性を説明しているという点で、全米科学アカデミーの全米研究評議会(NAS /NRC)の勧告と一致している。問題の定式化の段階は、解析の目標と可能なリスク管理戦略を特定するためのリスク 管理者との計画的な対話から始まると想定されている。この最初の対話は、科学的解析のための規制の背景を提供し、 そのような解析の範囲を明確にするのに役立つ。問題設定の段階では、農薬の使用/使用、懸念される毒性学的影 響、ばく露経路、持続時間、データや科学的情報の主要なギャップに関する利用可能な情報を考慮することも含まれ ている。

<sup>26</sup> https://aghealth.nih.gov/を参照

<sup>27</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers を参照し てください。

	1983	連邦政府におけるリスクアセスメントプロセスの管理			
NAS	1994	科学と判断			
	2007	21 世紀の毒性試験			
	2009	科学と政策決定。リスク評価の推進			
WHO/IPCS	2001–2007	行動様式/ヒトとの関連性のフレームワーク			
	2005	化学物質調整係数(CSAF)			
	2014	行動様式/種のコンコーダンス解析に関する WHO/IPCS フレームワークの 進化と応用における新展開			
EPA 1991–20	1991–2005	リスクアセスメントフォーラムリスクアセスメントのためのガイダンス (例:発がん性、生殖、発生、神経毒性、生態学的、ばく露評価のためのガ イドライン、ベンチマーク用量モデリングのためのガイダンス、参照用量と 参照濃度プロセスのレビュー			
		http://www.epa.gov/risk_assessment/guidance.htm			
	2000	リスク特性評価に関する科学政策ハンドブック			
		http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=40000006.txt			
	2006	生理的薬物動態(PBPK)モデルのリスク評価への応用のためのアプローチ とその裏付けとなるデータ			
	2014	政策決定に役立つヒトの健康リスク評価の枠組み			
2014	2014	種間・種族内の外挿のためのデータ由来の外挿係数を開発するための定量デ ータ適用の手引き			
	2001	総合的なリスク評価			
		https://www.epa.gov/sites/production/files/2015-			
		07/documents/aggregate.pdf			
OPP	2001 and 2002	累積リスク評価 http://www.epa.gov/ncer/cra/			
OECD	2013	経済協力開発機構 (Organisation for Economic Co-operation and Development) 有害性転帰経路の開発と評価に関するガイダンス文書			

表 C.1: OPP が使用している主なガイダンス文書とフレームワーク(US-EPA、2016 年より)

EPA フレームワーク文書は、このような疫学的研究及び科学的情報を農薬化学物質のリスク評価にどのように組み 込むことができるかを評価する際に、また、有害性転帰経路(または MOA)の理解との関連で複数の科学的エビデン スを評価するための基盤を提供する際に、EPA が考慮する科学的考察を記述している。このフレームワークは、疫学、 毒性学、リスク評価の標準的な手法に依存し、それを支持しているが、新しい情報源や追加の情報源からの情報を取り 入れることも可能である。この機関のフレームワークの重要な構成要素の一つは、実験研究と観察研究の両方で観察 された因果関係の特性を知るために、異なる情報源からの情報を整理して統合するためのツールとして、MOA フレー ムワーク/有害性転帰経路の概念を使用することである。MOA(Boobis ら、2008 年; Simon ら、2014 年; Meek ら、 2014 年)と有害性転帰経路の概念を使用することである。MOA(Boobis ら、2008 年; Simon ら、2014 年; Meek ら、 2014 年)と有害性転帰経路(Ankley ら、2010 年)は、フレームワーク文書で議論されている統合解析において重要な 概念を提供する。MOA と有害性転帰経路の両方とも、化合物へのばく露によって引き起こされる毒性影響は、ヒトの毒 性影響をもたらす一連の因果関係のある生物学的重要事象によって記述できるという前提に基づいており、その目的 は、環境物質へのばく露がこれらのパスウェイをどのようにかく乱させ、それによって毒性影響につながる後続の重要事 象のカスケードを引き起こすかを決定することである。

フレームワークの多くの概念は、全米科学アカデミー(National Academies, Science and Decisions)の2つの報告書:「リスクアセスメントの進展」(Advancing Risk Assessment: NAS 2009 年)と「21世紀の毒性試験」(Toxicity Testing on the 21st Century: NAS 2007 年)から引用されている。これら2つのNRC報告書は、毒性試験の実施方法、データの解釈方法、そして最終的に規制上の政策決定の方法を大幅に変更することを提唱している。特に、21世紀の毒性試験に関する2007年の報告書では、毒性試験、リスク評価、政策決定をより良く伝えるために、現在の先毒性エンドポイントの頂点の使用に焦点を当てたものから、毒性経路を使用することへの決定的な変化を提唱している。

MOA のフレームワークは、原因経路に沿って、そして用量反応、時間的一致、生物学的妥当性、一貫性、一貫性などの要素を考慮に入れ、Bradford Hill によって記述されたものに基づいた基準を使用して、エビデンスの重み付け

に基づいて確立された一連の重要事象を特定することから始まる。特に、修正された Bradford Hill 基準(Hill、1965年)は、MOA または有害な影響の現経路内の重要事象を立証する実験的支持を評価するために使用され、エビデンスの重み付け分析において、強度、一貫性、用量反応、時間的一致、生物学的妥当性などの概念を明示的に考慮している。この分析的アプローチを用いることで、疫学的結果は、報告された結果の一貫性、再現性、生物学的妥当性を評価し、不確実性の領域と将来の研究を特定するために、他のヒト情報との関連で評価することができる。以下の図C.1(NRC、2007年より引用)は、異なるタイプの情報が生物学的組織の複数のレベル(分子レベルから集団ベースのサーベイランスに至るまで)でどのように相互に関連しているかを示唆しており、遺伝子、タンパク質、低分子がヒトの細胞機能を維持する分子経路を形成するためにどのように相互作用するかという急速に発展している科学的理解に基づいている。

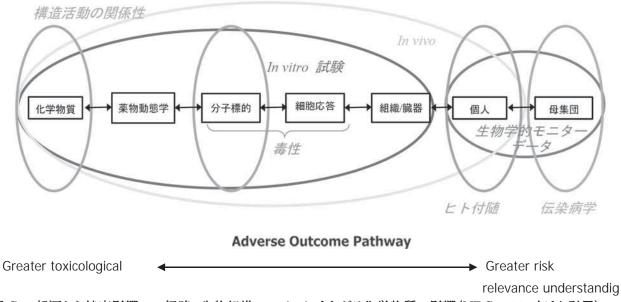


図 C.1:起源から健康影響への経路。生物組織のレベルにまたがる化学物質の影響(NRC、2007年より引用)

# C.2.2. システマティックレビュー:目的にかなった

全米アカデミーの全米研究評議会(NRC)は、EPA の IRIS プログラムのレビューにおいて、システマティックレビュ ーを「特定の問題に焦点を当て、明確かつ事前に特定された科学的方法を用いて、類似しているが別個の研究の結 果を特定、選択、評価、要約する科学的調査」と定義している<sup>28</sup>。近年、NRC は、規制上の政策決定に情報を提供す るために化学的特異性リスク評価をサポートする科学的な文献レビューの明白性を高めるシステマティックレビュープロ セスに移行することを EPA に勧めている<sup>6</sup>。

NRC の勧告に沿って、EPA-OPP は、政策決定を支える科学的データの収集、評価、統合のための明白性の高い 方法に依拠した、目的にかなったシステマティックレビューを採用している。そのため、各システマティックレビューの複 雑さと範囲はリスク評価ごとに異なる。EPA-OPP は、対象範囲/問題策定から始まり、データ収集、データ評価、デー タ統合及び重要なデータギャップが特定された結果の要約が行われる。

システマティックレビューでは、対象となる研究の結果をまとめるために統計的手法(メタアナリシスなど)やその他の 定量的手法を使用することが多く、利用可能なエビデンスのレベルや存在する可能性のあるバイアスの程度を評価す るために半定量的な採点システムを使用することができる。EPAの農薬プログラム管理局の場合、規制審査プロセスの 一環として実施されるこのような Tier III(システマティックレビュー)評価には、審査中の農薬化学物質と、(最初の Tier II 評価で示唆されたように)特定の関連する健康影響が疑われる農薬化学物質の審査が含まれることになる。

米国の多くの連邦政府やその他の組織が、このようなシステマティックレビューの実施方法を評価したり、ガイダンス

<sup>&</sup>lt;sup>28</sup> http://dels.nas.edu/Report/Review-Integrated-Risk/18764 www.efsa.europa.eu/efsajourna

文書を発行したりしており、多くのフレームワークが開発されている。これらには、EPA IRIS プログラムのアプローチ<sup>29</sup>、 National Toxicology Programs'Office of Health Assessment and Translation (NTP/OHAT) アプローチ<sup>30</sup>、 Cochran Collaboration のアプローチ<sup>31</sup>、Campbell Collaboration 及び Navigation Guide<sup>32</sup>が含まれ、後者については Environmental Health Perspectives 誌の一連の記事で説明されている。それぞれのアプローチは、データ収集、データ評価、データ統合、要約/更新という 4 つのステップを大まかに共有している。例えば、The Cochrane Collaboration は、The Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine の中で、システマティックレビューの重要な主要特性の多くをリストアップしている(US-EPA、2016 年)。

- ・ 目的が明確に示されており、研究の適格性基準があらかじめ定義されていること
- ・明示的で再現性のある方法論
- ・ 適格性基準を満たすすべての研究を特定するための系統的な検索
- ・ 特定された研究から得られた知見の妥当性の評価
- ・収録された研究の特性と知見を体系的に提示し、総合的にまとめたもの。

この付属書の以下のセクションで説明・詳述されているように、農薬リスク評価への疫学的データのレビューと統合に 対する OPP のアプローチは、「作成された評価が必要な政策決定を報告するのに適しており有用であることを確認し (US-EPA、2012 年)、必要な供給源が、より詳細な研究から得られる予測または予測される情報と一致しているか、バ ランスが取れていることを確認する」という意味で、各段階が目的に応じて適切に実施される段階的なアプローチを採 用している。Tier I 評価は、調査及び評価が AHS に由来する研究に限定されている場合の、調査実施または調査実 施の更新のいずれかである。Tier II 評価では、疫学的文献の広範な検索、包括的なデータ収集、より深く、より関与し たデータ評価が行われ、より広範であるが、一般的には範囲が疫学に限定されており、疫学、ヒト中毒事例、動物毒物 学、有害な影響経路を横断した学際的な統合には至らない。Tier III 評価は、データ統合とより広範なデータ評価と抽 出を伴う完全なシステマティックレビューであり、メタアナリシスやメタ回帰、因果関係推論/因果関係図、定量的バイア ス解析や感度分析などのより高度な疫学的手法を含むことがある。

# C.2.3. 現在及び将来予想される EPA 疫学レビューの実施

## C.2.3.1. Tier I (scoping と問題の定式化)とTier II (より広範な文献検索)

現在 EPA では、農薬の疫学的レビューは、上述の通り、リスク評価の進展に応じて段階的なプロセスで実施されて いる。この初期段階の Tier I/scoping 疫学報告書の目的は、プロセスの問題の定式化/scoping 段階で関連性の高い 疫学研究が検討され、適切な場合には、プロセスの(後期の)リスク評価段階で十分に検討されるようにすることである。 Tier I 段階では、EPA-OPP は、農薬問題に焦点を当てた質の高い有名なコホート研究、特に農業健康調査 (Agricultural Health Study:AHS)に焦点を当てている。AHS は、農薬ばく露とがん及びその他の健康影響との関 連を評価する連邦政府出資の研究であり、米国国立がん研究所(NCI)、国立環境衛生科学研究所(NIEHS)、CDC の国立労働安全衛生研究所(NIOSH)及び米国環境保護庁との共同研究を代表するものである。AHS 参加者コホー トには、アイオワ州とノースカロライナ州の 89,000 人以上の免許を持つ商業及び民間の農薬散布者とその配偶者が含 まれている。登録は 1993 年から 1997 年まで行われ、データ収集は現在も継続中である。AHS は、AHS コホートに 関連した、またそれを利用した出版物のリストをウェブサイトに掲載している (https://aghealth.nih.gov/news/publications.htmlを参照)。

対象となる農薬がAHS(www.aghealth.org)の一部として調査されている場合、EPAの「scoping」解析の一環として内容摘要(または「調査資料」)が公表されるため、評価の早い段階でこれらの研究の予備的な(Tier I/scoping)レビ

<sup>&</sup>lt;sup>29</sup> <u>https://www.epa.gov/iris/advancing-systematic-review-workshop-December-2015</u>年を参照。

<sup>&</sup>lt;sup>30</sup> http://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html 及び NTP の「系統的レビューとエビデンス統合のための OHAT アプローチを 用いた文献ベースのアセスメントを実施するためのハンドブック」https://ntp.niehs.nih.gov/ntp/ohat/pub s/handbookjan2015\_508.pdf を参 照。

<sup>&</sup>lt;sup>31</sup> http://handbook.cochrane.org/を参照。

<sup>&</sup>lt;sup>32</sup> http://ehp.niehs.nih.gov/1307175/を参照

www.efsa.europa.eu/efsajourna

ューが実施される。この初期の段階では、AHS研究の基本的な疫学的結果と結論は、審査中の農薬に関連するAHS 結果がある場合には、様々な AHS 研究の著者による適切な結論を簡潔に要約することを目的とした Tier I/scoping 文書に記載されている;この Tier I scoping レビューは詳細な内容、批判的な評価またはエビデンスの統合を提供す るようになっていなくて AHS に関連する学術論文の要約されたハイライトに触れる可能性があるだけである。子ども環 境保健・疾病予防研究センター(Children's Environmental Health and Disease Prevention Research Centres) の研究のように、AHS 以外の高品質な研究がある場合は、これらの研究も同様にこの Tier I/scoping 疫学レビューに 要約されているかもしれない。繰り返しになるが、文献の批評や統合は行われていない。場合によっては、Tier I/scoping レビューでは、利用可能なエビデンスの追加的な疫学的レビューはこれ以上必要ないと結論付けられること がある。あるいは、より詳細な Tier I/update または Tier II 評価の一部として、更なるレビューが必要であると勧告する 場合もある。

Tier I/update 評価は通常、Tier I/ scoping 評価の完了から1年から3年後に完了し、後述するTier II と同様に、 ヒト健康リスクアセスメント案(Draft Human Health Risk Assessment)と一緒に、またその一部として発行される。 Tier I/update アセスメントでは、AHS で利用可能な文献の徹底的なレビューが行われる。Tier I/update 評価では、 AHS のウェブサイトに掲載されている該当する研究を質的、叙述的な要約(報告された関連性の尺度を含む)でレビュ ーし、要約し、評価する<sup>33</sup>。レビューは一般的に叙述の形式で行われ、研究の主要な側面とその結論に焦点を当て、必 要に応じて EPA OPP の結論の要約及び更なる研究のための勧告に加えて EPA OPP の解説を含む。

# C.2.3.2. Tier II(より広範な文献検索)

Tier II 評価は、利用可能な疫学的証拠のより完全なレビューであり、一般的には、初期の Tier I/scoping 文書が特 定の懸念の可能性を示唆している場合にのみ実施される(例えば、特定で信頼できるばく露・疾病仮説が進められてお り、より詳細な評価の一部としてさらに評価する必要がある)。Tier II 疫学評価は、Tier I/update と同様に、一般的に Tier I 評価の完了から 1 年から 3 年後に完了し、OPP のヒトの健康リスク評価案 (Draft Human Health Risk Assessment)と一緒に、またその一部として発行される; Tier II 評価はシステマティックレビューの特定の要素を組み 込む定性的で叙述的なレビューだと考えられる。例えば、Tier II 評価では、AHS データベースだけでなく、PubMed、 Web of Science、Google Scholar、Science Direct などのデータベースや、場合によっては標準化された明白で再現 性のある照会言語を使用して、専門の図書館や情報科学の支援を得て、Tier I 評価よりも広範囲に及ぶ徹底した完全 な文献検索が含まれている<sup>34</sup>。EPA によるエビデンス統合 (一般的には定性的かつ叙述的な形式で行われるが)も Tier II 評価では行われ、疫学的文献に関する全体的な結論が出されている。さらに、Tier II 評価は、特別な仮説とし てばく露・健康影響に関する更なる疫学的データや研究が将来の研究のための興味深い分野を示す可能性がある。 Tier II 評価文書は一般的に、疫学的知見を、動物毒性試験や、リスク評価の一部としてある程度(別個にに)行われる MOAs/AOPs からの情報などの他のエビデンスと統合しようとはしない。特定の農薬に関連すると考えられる特定の健 康影響が特定される範囲までの Tier II 評価に対して、その後のより包括的な Tier III 評価では、分野を超えた更な る調査及び統合を行うことができる(下記参照)。

# C.2.3.3. Tier 3(データ統合を伴う完全なシステマティックレビュー)

Tier II 評価では、ある農薬化学物質との関連性があると仮説が立てられている疫学的文献に現れる広範な健康影響を検討するが、Tier III 評価では、より広範な(学際的な)疫学的根拠に基づく、時にはより定量的/統計学的な評

<sup>34</sup> 疫学とバイオモニタリング/ばく露の項目の下で行われた追加検索は、NHANES Exposure Reports (http://www.cdc.gov/exposurereport/); TOXNET (http://toxnet.nlm.nih.gov/); CDC NBP Biomonitoring Summaries

(http://www.cdc.gov/biomonitoring/biomonitoring\_summaries.html); ICICADS (http://www.inchem.org/pages/cicad s.html); ATSDR Toxicological Profiles (http://www. atsdr.cdc.gov/toxprofiles/index.asp), IARC モノグラフ (http://monographs. iarc.fr/ENG/Monographs/PDFs/; EFSA's Draft Assessment Report Database (http://dar.efsa.europa.eu/dar-web/provision); and

arc.tr/ENG/Monographs/PDFs/; EFSA's Draft Assessment Report Database (http://dar.etsa.europa.eu/dar-web/provision); and Biomonitoring Equivalents (https://blog.americanchemistry.com/2014/07/biomonitoring-equivalents-a-valuable-scientific-tool- formaking-better-chemical-safety-decisions/)

<sup>&</sup>lt;sup>33</sup> https://aghealth.nih.gov/news/publications.html

価を行い、これを動物毒性学や MOA/AOP 情報とより正式に統合しようとするものである。このような Tier III 評価は、 疫学的文献のシステマティックレビューの形をとり、毒性及び有害な転帰の経路の評価と併せて実施される。

AHS 由来の農薬化学物質については、Tier III 解析では、他の質の高い疫学調査の評価結果を取り入れ、より多 様な情報源を再調査するために「エビデンスの重み付け」をより多く取り入れることが理想的である。これらの調査の結 果は、AHS の結果との再現性と一貫性を評価するために使用される。多くのケースにおける初期の AHS の結果は、 特定の健康影響を示した少数の参加者、または参加者を追跡した年数が比較的少ない参加者に基づいている。AHS コホートの高齢化に伴い、AHS からのいくつかの化学物質の 2 回目の評価の発表は、さらなる追跡調査の年数と、ば く露と健康影響の間の正負の関連を解釈するためのより強固な根拠となると期待されるより多くの症例数に基づいて行 われることになるであろう。さらに、AHS では、疫学研究の結果の解釈を助けるために、かなりの量の生化学的、遺伝 的マーカー及び分子データの生成が増加している。このような結果は、AHS の結果をさらに明確にしたり、ばく露と健 康影響を結びつける生物学的基盤のエビデンスを提供したり、あるいは因果関係の経路の基礎となるメカニズムのエビ デンスを強化する可能性のある追加の実験研究や観察研究を示唆したりする可能性がある。さらに Tier III 解析では、 AHS がメンバーである国際農業コホートコンソーシアム(AgriCOH)の国際的なコホート研究からの情報と結果をまと める努力を利用することもできる。AgriCOH は、研究間のデータを蓄積するための機会と方法を特定するために積極 的に取り組んでおり、これらの他のコホートデータの利用可能性は、EPA が疫学的データを検討、評価、重み付けする 際に、ばく露ー健康影響の関係の再現性と反復を評価するのに役立つはずである。

## C.2.4. OPP の公表文献検索戦略及び研究の質の評価

システマティックレビューアプローチの重要な側面は、確立された適格基準を満たす多くの文献をみつけることがで きるように、公表されている疫学的文献を徹底的に、体系的に、再現可能に検索することである<sup>35</sup>。OPP は文献検索の 一部として特定のデータベースを使用しており、その実施に関する特定のガイダンスがある(例えば、ヒト健康リスク評価 に関する OPP の公表文献検索ガイダンス<sup>36</sup>)。すべての関連文献の評価、エビデンスの強固さを評価するための標準 化されたアプローチの適用、明確で一貫性のある総括的表現は、一般的に重要な要素となる(NRC、2011年)。さらに、 質の高いばく露評価は、環境疫学研究や職域疫学研究において特に重要である。

上記のシステマティックレビューのアプローチの第二の重要な要素は、同定された研究から得られた結果の妥当性 の評価である。一般的に言えば、疫学研究の質、研究の文書化の妥当性(研究の計画と結果)、リスク評価との関連性 は、政府機関のリスク評価に使用するために公表されている文献から疫学研究を評価する際に考慮される。個々の研 究の質を検討する際には、疫学研究の計画、実施、解析、解釈の様々な側面が重要である。これらには以下が含まれ る(US-EPA、2016年より)。

- 1) 仮説を明確に明示することで、たとえその研究が本質的に仮説生成的なものであったとしても、その仮説を明確に示されていること。
- 2)健康影響の関連する臨界期、リスク評価対象集団の関係あるばく露範囲、試験から得られる用量/ばく露反応の 傾向の入手可能性など、ばく露評価の資質の中で、適切なばく露評価が十分であること。
- 3) 合理的に有効で信頼性の高い結果の確認(研究集団における健康影響の有無を正しく識別されていること)。
- 4)対象集団を代表するサンプル集団となり、系統的な偏りがない適切な組み入れ基準と除外基準。
- 5)観察されたリスク推定値における複数の農薬ばく露、または混合物ばく露の役割の評価または考察を含む、潜在 的な交絡変数の適切な評価及び解析。

<sup>35</sup> 出版バイアスに関連する潜在的な問題を軽減するために、はっきりしない文献や未発表の文献を見ることを提唱する者もいる。

<sup>36</sup> 疫学的データに関する特別な注意事項については、

<sup>&</sup>lt;u>https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open</u>及び2012年 8月28日付けの文書「Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment」の10ページを参照のこと。

- 6)参加者の選択や情報収集における誤りを含む、研究における潜在的な系統的な偏りの全体的な特性。これには、 提示されたリスク推定値に対する系統的誤差の潜在的な影響を調査するための感度分析の実施を含む。
- 7) ばく露・健康影響評価のための適切な統計的検出力、または観察された影響に対して検出力が不足している場合 の研究の統計的検出力の影響の評価及び検出力推定値の適切な考察及び/または提示。
- 8)研究デザインと対象となる結果の性質を考慮した適切な統計的モデル化技術の使用。

### 参考文献

- Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730-741.
- Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36, 781-792.
- Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C, 2008. IPCS framework for analyzing the relevance of a noncancer mode f action for humans. Critical Reviews in Toxicology, 38, 87–96.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295-300.
- Meek, ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J and Vickers C, 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. Journal of Applied Toxicology, 34, 595–606. Meek, ME, Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance
- (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of Applied Toxicology, 34, 1–18. NAS (National Academy of Sciences), 2007. Toxicity Testing on the 21st Century: A Vision and a Strategy.
- Board on Environmental Studies and Toxicology, Available online: https://www.nap.edu/catalog/11970/
- toxicity-testing- in-the-21st-century-a-vision-and-a NAS (National Academy of Sciences), 2009. Science and decisions: advancing Risk Assessment. Board on Environmental Studies and Toxicology. Available online: http://dels.nas.edu/Report/Science-Decisions-Advanc ing-Risk-Assessment/12209
- NAS (National Academy of Sciences), 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Board on Environmental Studies and Toxicology. Available online: https:// www.nap.edu/download/13142
- Simon TW, Simons SS, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Crisp PF, McMullin TS, McQueen CA and Rowlands JC, 2014. The use of mode of action information in risk assessment: Quantitative key events/dose response framework for modelling the dose response for key events. Critical Reviews in Toxicology, 44 (Suppl 3), 17–43. US-EPA (Environmental Protection Agency), 2010a. Draft Framework for Incorporating Human
- Epidemiologic and Incident Data in Health Risk Assessment. Presented to FIFRA Scientific Advisory Panel on February 2-4 2010a. January 7. Available online: https://www.regulations.gov/document?D= EPA-HQ-OPP-2009-0851-0004
- US-EPA (Environmental Protection Agency), 2010b. Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. MEMORANDUM dated 22 April, 2010b. SAP Minutes No. 2010-03. Available online: https://www.re gulations.gov/document?D=EPA-HQ-OPP-2009-0851-0059 US-EPA (Environmental Protection Agency), 2012. Office of the Science Advisor. Risk Assessment Forum. Draft Framework for Human Health Risk Assessment to Inform Decision Making. July 12, 2012.
- US-EPA Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic and Incident ata in Risk Assessments for Pesticides. December 28, 2016. Available online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

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#### 付属書 D-影響量の拡大/膨張

この文書の本文で説明されているように、研究の検出力が低い場合には、バイアスの潜在的な原因が生じる可能性 がある。このあまり知られていないタイプのバイアスは、「影響量の規模」として知られています。一般的に、小規模で検 出力の低い研究では、研究の検出力が意味のある影響量を確実に検出するには不十分であるため、偽陰性が生じる 可能性があることは広く知られているが、推定された影響が重要、関連性がある、または「発見された」と判断されるため に、統計的閾値(例えば、統計的有意性に使用される一般的な p<0.05 の閾値)を通過する必要がある場合、これらの 研究が影響量の膨張をもたらす可能性があることはあまり知られていない。この影響は、影響量の拡大、「勝者の呪い」、 真実の膨張、または影響量の膨張として様々に知られているが、これは、「発見された」関連性(すなわち、意味がある と判断されるために統計的有意性の所定の閾値を通過した関連性)が、その発見を行うために最適ではない検出力を 持つ研究から得られる現象であり、その結果、意図的かつ体系的に膨張した影響量が生じることになる。

このような真偽の不明確化は、検出力の低い研究で統計的有意差を達成する研究では、帰無値から離れる(系統的 な)バイアスとして現れる(Reinhart、2015年)。これは、検出力の低い(したがって一般的には小さい)研究は、結果が 大きく変動する可能性が高く、大規模な研究よりも個人の間のランダムな変動の影響を受けやすいからである。より具 体的には、どのような研究でも観察されるかもしれない影響量の拡大の変化の程度は、研究の結果がどの程度広く変 化すると予想されるかに部分的に依存しており、これは研究の検出力に依存する;検出力の低い研究は影響量の拡大 の程度を大きくする傾向があり、その結果検出力の高い研究よりも統計的有意性(または他の閾値基準をパスする)を 見出す。

この「影響量の拡大」の概念とその理由の例として、可変のサンプルサイズで何千回も試験を実施した場合を想像す ると便利である。この場合、観察された影響量の広い分布がある。これらの推定影響量の観察された中央値は、真の影 響量に近いと予想されるが、小規模な試験では、大規模な試験に比べて、観察された影響量のばらつきが必然的に大 きくなる。しかし、低検出力の研究では、観察された影響のうち、統計的に有意な(高い) 閾値を通過するのはごく一部 であり、これらの影響は最大の影響量を持つものだけである。このように、一般的に小規模で、ランダム変動が大きい低 検出力の研究では、与えられた統計的閾値を通過した結果、実際に有意性起因の関連を発見した場合、その影響の 大きさを過大評価する可能性が高くなる。これが意味するのは、低検出力で統計的に有意な研究の結果は、膨張効果 となるように偏っているということである。Gelman 及び Carlin(2014 年)が要約しているように、「研究者が小さな影響 を研究するために小さな[検出力不足]<sup>37</sup>サンプルとノイズの多い測定を使用した場合、有意な結果はしばしば驚くほど 間違った方向に行き、影響を大幅に過大評価する可能性が高い」のである。一般的に、バックグラウンド(または対照ま たは無処置)率が低い、対象となる影響量が小さい、研究中のサンプルサイズが小さいと、研究の検出力が低下し、そ の結果、(あらゆる)膨張した影響量の傾向と規模が大きくなることが示されている。

影響量の膨張現象は、発見科学全般に適用される一般的な原則であり、疫学の特殊な現象や弊害ではないことに 注意することが重要である(Ioannidis、2005年;Lehrer、2010年;Button、2013年;Buttonら、2013年;Gelman 及び Carlin、2014年;Reinhart、2015年)。これは、薬理学の研究、遺伝子研究、心理学の研究、そして最もよく引 用される医学文献の多くでよくみられる。ほとんどの疫学研究のように、研究者がサンプルサイズを増加させる能力が限 られている場合、影響効果量の拡大は、研究や研究デザインの機能や欠陥ではなく、むしろ、その研究の結果がユー ザーコミュニティによってどのように解釈されるかという機能である。したがって、疫学研究における選択や情報バイアス のような他のバイアスの可能性とは異なり、バイアスは研究やその計画に内在するものではなく、むしろその研究がどの ように解釈されるかに特徴がある。

統計的に有意な結果をもたらす研究について、影響量の規模の潜在的な程度を決定(定量化)するために、査読者 は様々な検出力の計算を実行しなければならない。より具体的には、化学物質ばく露と疾患との間の関連が統計的に 有意であることが判明した場合、検出力解析は、統計的に有意な影響量の推定値(例えば、オッズ比、相対リスクまた は率比)がどの程度人工的に膨張しているかを決定するために行われる。

<sup>37 [</sup>斜体を付けた]

必要な検出力計算を行うために、査読者は以下の4つの値を知っているか、または得なければならない。

1) 非ばく露群の被験者数。

2) ばく露群の被験者数。

3) 非ばく露群の対象疾患を持つ個人の数(または症例数) 及び

4)2 つのグループ(例えば、ばく露群 vs.非ばく露群)の比較において、所定の(事前決定の)量の差を検出するための対象となる目標値。

最初の3 つの値は文献に記載されているか、文献から入手しなければならないが、対象となる目標値(一般的に疫 学研究ではORまたはRR)はリスク管理者によって選択される(最終的には政策決定である)38。本付属書では、シミュ レーションを用いて、この影響量の膨張現象を定量的に検討する。本付属書では、2 つの公表された研究例と数百件 の試験のシミュレーションを用いて、検出力が低いために偏った影響量(オッズ比、率比、相対リスクなど)を生み出す のに影響量の拡大がどの程度の役割を果たしているかを評価している。

最初の例では、ダイアジノンばく露と肺がんを調査した Agricultural Health Study の前向きコホート出版物からの データを使用し、計算された RR の影響量の偏りの問題を説明している。2番目の例は、マラチオンばく露とNHLを研 究した症例対照研究からの ever-never データを使用して、推定 OR の観点から影響量の拡大の概念を説明する。

#### 影響量の拡大の説明と相対リスクを説明する例(Jones ら、2015 年)

ダイアジノンにばく露されていないものと、最も高い三分位(T)でばく露されているものとの間の比較に関連した検出 力は、肺がんに対する Jones ら(2015 年)の AHS 研究発表「Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study - an updated analysis」で提供された情報から計算することができる。各ばく露量での被験者数は文献中に記載されてい た(非ばく露群。N=17710及びT(ertile)1、T2及びT3は、ばく露分布に基づいて分類された;具体的には、各三分 位の N=(2,350 + 2,770)/3=1,710文献の表1から。(a)2,350の値は最も低いばく露量を表し、(b)2,770の値は、 ばく露された被験者を二分法で分類したときの、2つの最も高いばく露量を表している。(i)参照非ばく露群の被験者数 =17,710; (ii) ばく露群(三分位)のそれぞれの被験者数=1710; (iii)参照非ばく露群の罹患者数(肺がん)=199人 (引用文献の表3より)とすると、真の率比=1.2、1.5、または2.0と仮定すると、文献に示されたT1対非ばく露群、T2 対非ばく露群、T3対非ばく露群の比較の検出力を計算することができる。

ここで、我々は、199/17710(=0.011237)の推定バックグラウンド率及び感度分析の形態として、このバックグラウン ド率の1/2(または0.005617)及びダイアジノンにばく露された個人の各三分位の被験者の間で1.2、1.5、2.0及び3.0 の(可能な規制関係の)相対率を検出するためのこの率の2倍(0.022473)に関連する検出力を評価することに注目し ている。この解析はStata統計ソフトウェアを使用して行われ、1.2、1.5、2.0及び3.0の真の率比について、199人の 罹患者/17,710人のバックグラウンド率に対して1/2x・、1x・(太字/網掛けで以下に示す)及び

<sup>&</sup>lt;sup>38</sup>この目標値は対象となる効果量であり、相対リスク(コホート研究の場合)またはオッズ率(症例対照研究の場合)のいずれかで表されることが多い。 すなわち、目標値は一般的に、リスク管理者が一定の確信度で検出したいと考えている一定の大きさの OR または RR である。OR または RR が 高ければ高いほど、ばく露と健康影響との間の推定関連性の規模が大きくなる。何が「弱い」関連性と「強い」関連性を構成するかについての厳密 なガイドラインはないが、約1以下(時には1.2以下)の値は「帰無」または「本質的に帰無」と考えられる(これは、いくつかの背景(例えば、ワクチ ン接種の有効性など)では考慮することが適切であるかもしれない保護効果の可能性を無視している)。2または3未満の値は、しばしば「弱い」と 考えられている。2(または3)より大きく約5までの値は「中等度」と考えられ、5より大きい値は「大」であると考えられている。Monson(1990)は、 関連性の強さの目安として、1.0~1.2を「なし」、1.2~1.5を「弱」、1.5~3.0を「中程度」、3.0~10.0を「強」としている。他の著者は疫学において は Cohenの基準を用いて、1.5を「小」、5を「大」、3.5を「中」と表現している(Cohen and Chen、2010)。また、1.5を「小」、2.5を「中」または「中 程度」、4を「大」または「強」、10を「非常に大きい」または「非常に強い」と表現する人もいる(Rosenthal、1996年)。Taube(1995年)は、弱い関 連性を検出する上での環境疫学の限界について議論している(Wynder(1997年)の反論を示す招待解説も参照のこと)。これらの境界線はどれ も「難しい」ものではなく、どこに線が引かれ、どのように考えられ、どのように解釈されるかについては、正当な意見の相違があり得ることを認識す べきである。それにもかかわらず、これらの境界線は背景に大きく依存するものであり、上記のような境界線は、いかなる意味でも、公式なものでも、 決定的なものでもあると考えるべきではない。

N <sub>control</sub>	$N_{exposed}$	Proportion control <sup>(b)</sup>	Proportion exposed	Relative risk	Power	
17,710	1,710	0.00562	0.00674	1.2	0.1634	
17,710	1,710	0.00562	0.00843	1.5	0.4353	
17,710	1,710	0.00562	0.01124	2.0	0.8182	
17,710	1,710	0.00562	0.01685	3.0	0.9935	
17,710	1,710	0.01124	0.01348	1.2	0.2259	
17,710	1,710	0.01124	0.01685	1.5	0.6379	
17,710	1,710	0.01124	0.02247	2.0	0.9652	
17,710	1,710	0.01124	0.03371	3.0	1	
17,710	1,710	0.02247	0.02697	1.2	0.3353	
17,710	1,710	0.02247	0.03371	1.5	0.8632	
17,710	1,710	0.02247	0.04495	2.0	0.9991	
17,710	1,710	0.02247	0.06742	3.0	1	

2x-(観察された)を表形式及びグラフ形式の両方で以下に示す39。

上記の検出力計算結果を生成するために使用される Stata コード:検出力2比例('=0.5\*199/17710'=199/17710'=2\*199/17710), test(chi2) RR (1.2 1.5 2.0 3.0) n1(17710) n2

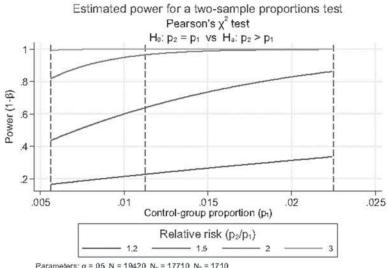
(1710)片側表(N1:"N コントロール" N2:"N エクスポージャー" p1:"割合コントロール" p2:"割合エクスポージャー" RR:"相対リスク" power:"検出力')。

(a):片側検定 a=0.05 Ho:p2=p1 vs Ha:p2>p1; Ncontrols=17,710、Nexposed=1,710;反復回数=1,000回(データセッ ト)。

(b): Jones ら(2015)の199/17710の肺がんの観察されたバックグラウンド率を1/2倍-、1倍-、2倍-を表す。

上表の強調表示/太字の領域は、引用研究における肺がんのこの 1x 観測されたバックグラウンド率に関連した検出力を表してい る。

これらの値は以下のようにグラフ化することができる40。



Parameters: a = .05, N = 19420, N<sub>1</sub> = 17710, N<sub>2</sub> = 1710

<sup>&</sup>lt;sup>39</sup> 1.2、1.5、2.0、3.0のRRは、リスク管理者や意思決定者が関心を持ちそうな一連の相対リスクに関連する検出力検出力を示すために、やや恣意 的に選択されたものである。RR または OR=2.0 と 3.0 の値は、弱い影響量の大きさと強い影響量の間の境界線であると考えられている。RR 値 1.2は、一部の人が「帰無に近い、または本質的に帰無」と考えるものであり、RR値1.5はこれらの間の中間値である。疫学的証拠がばく露と健康 影響との間の関係を示唆しているかどうかを判断する際に、リスク管理者は、許容可能な統計力(一般的には 80-90%と考えられている)を持つ強 固な研究から得られた「本質的には帰無」RR1.2 を、関連性を見いだせなかったことを示す十分なエビデンスであり、事実上、ばく露と健康影響と の間に観察可能な関連性がないという結論を支持するエビデンスを提供していると判断されることがあるかもしれない。

<sup>40</sup> 上のグラフを生成するための Stata コード:乗 2 比例(' = 0.5 \* 199/17710'(0.0001) '= 2 \* 199/17710'), test(chi2) rrisk(1.2 1.5 2.0 3.0) n1(17710) 0) n1(17710) n2(1710)グラフ(recast(line) xline(= 0.5 \* 199/17710" '= 199/17710" '= 2 \* 199/17710', lpattern(dash)) legend(rows(1)size(small)) ylabel(0.2(0.2)1.0))片側グラフである。

1.2-、1.5-、2.0-及び 3.0 の真の RR における制御群比率の関数としての検出力を評価する(片側)2 標本比率検定 の推定検出力を示すグラフ。検定では、1.2-1.5-2.0、2.0-3.0 の真の RR でのコントロールグループ比率の関数として の検出力を評価している。

上の表とグラフからわかるように、本研究では、バックグラウンド率(対照比率、199人の罹患者/17,710人=0.011237) の1倍で約23%の検出力でRR1.2を検出することができた。約64%の検出力が1.5のRRを検出する。真のバック グラウンド率が実際には観測されたバックグラウンド率の2倍(290.011237=0.022473)であれば、RR1.5を検出でき る検出力は約86%、RR2.0を検出できる検出力は実質的に100%となる<sup>41</sup>。

上記を考えると、真の相対リスクを1.2、1.5、2.0、3.0とした場合に、どの程度の影響量の拡大(別名、影響量の膨張) があるかをシミュレーションするために SAS が使用された。下の表は、ダイアジノンと肺がんの検出力解析を示しており、 シミュレーション結果から影響量の拡大の程度を示している。下の表に示された解析は、Ioannidis(2008 年)によって 行われたものと類似していて、彼の表2に示された、影響量の拡大の概念を説明するための正式な統計的有意性の閾 値を通過した仮説的な結果のセットである。


真値		解析した								
対照における罹患者 の割合	RR	データセ ット数 (N)	検出力 <sup>(b)</sup>	観測された有意な RR の分布						
				N	10 <sup>th</sup> percentile	Median (% inflation)	90 <sup>th</sup> percentile			
0.005617	1.2	1,000	0.16	157	1.6	1.7 (42)	2.0			
(1/29background)	1.5	1,000	0.4	401	1.6	1.8 (20)	2.3			
	2	1,000	0.82	823	1.7	2.1 (5)	2.8			
	3	1,000	1	997	2.3	3.0 (0)	3.9			
0.011237	1.2	1,000	0.22	224	1.4	1.6 (33)	1.8			
(19 background)	1.5	1,000	0.63	627	1.4	1.6 (7)	2.0			
	2	1,000	0.98	977	1.6	2.0 (0)	2.5			
	3	1,000	1	1,000	2.5	3.0 (0)	3.6			
0.022473 (2 9 background)	1.2	1,000	0.33	331	1.3	1.4 (17)	1.6			
	1.5	1,000	0.87	871	1.3	1.5 (0)	1.8			
	2	1,000	1	1,000	1.7	2.0 (0)	2.3			
	3	1,000	1	1,000	2.6	3.0 (0)	3.4			

ポアソン回帰モデルが、グループ間の(相対リスク)率を比較するために使用された. EXACT 検定は、一般化ヘシアン行列が正のデフィニートでない場合(グループの1つでゼロのケースがあるため)、いくつかのデータセットの解析に使用された。

(a):片側検定、a=0.05、N コントロール=17,710、N ダイアジノンばく露者=1,710、反復回数=1,000回(データセット)。

(b):このシミュレーションから得られた検出力は、SAS (PROC POWER)や Stata (power two-proportion)のような統計ソフトの組み 込まれた手順から計算された検出力に近いかもしれないが、正確には一致しないかもしれない。これは、シミュレーションされたデ ータセットの数が十分でないためかもしれない。しかし、1,000回の反復は、検出力を適切に推定し、統計的に有意な結果(ここで は、≤0.05)を与えられた影響量の拡大の程度を説明するのに十分である。

p<0.05 で統計的に有意な結果が得られた場合、統計的に有意な結果の中央値での影響量の拡大の変化率は、ダイアジノンにばく露されていない個人の肺がんの割合(すなわち、非ばく露グループの罹患者の割合)と真の相対リスク(1.2 から 3.0 までの範囲)の両方に応じて 0%から 42%まで変化することに注意する。例えば、ばく露対非ばく露の三分位の真の RR が 1.2 であった場合、非ばく露グループの肺がんの割合は 0.011237(上記の表の太字の行)で、観察された統計的に有意な RR の半分は 1.6 の中央値を超えており、半分は 1.6 以下になるだろう;これは、シミュレーションで使用される 1.2 の真の RR の上に 33%の膨張があることを表している。

Jones ら(2015 年)の研究(0.011237)でみつかったバックグラウンド率については、統計的に有意であることが判明

<sup>&</sup>lt;sup>41</sup> 別の言い方をすると、真の(しかし未知の)バックグラウンド率が実際に観測されたバックグラウンド率の2倍であった場合、統計的に有意な関係 がみつからなかった場合、我々は合理的に(86%の信憑性で)真のOR が1.5を超えていないと結論付けることができる。

した真の RR の 1.2 は、前述の中央値 1.6 の代わりに 1.4(10%のパーセンタイルで)から 1.8(90%のパーセンタイル で)に変化することが観察される研究が繰り返されるだろう。 真の RR が 2 または 3 のときには、検出力は 80%以上(上 記の表に見られるように)であり、観測された RR の中央値は真の RR に近く、観測された RR の範囲は狭い。 真の RR が 3 になると、影響量の膨張がなくなり、シミュレーションの中央値が真の RR に回帰するように検出力が増加する。

エバー/ネバー解析における影響量の拡大とオッズ比を示す例(Waddellら、2001 年)

ばく露群と非ばく露群の比較は、三分位や四分位などの他の分類やグループ分けに基づいた比較とは対照的に、 「これまでありと、決してない」の比較で示されることがある。このようなばく露カテゴリーベースの解析は、ばく露カテゴリ ーを小さな(より均質な)ばく露分類やグループに分けるには十分な数の症例数がないため、あるいはばく露の測定値 が利用できないか、あるいは信頼性が低いため(症例対照研究のような)に行われるかもしれない。これらの状況では、 (i)非ばく露群の被験者の総数、(ii)ばく露群の被験者の数、(iii)ばく露群と非ばく露群の間の比較の検出力を計算 するために、非ばく露群の罹患者の数、(iv)与えられた、または事前に選択されたオッズ比が同様に必要となる。

エバー/ネバー分類を用いた症例対照研究において、検出力と影響量の拡大の解析がどのように行われるかを説明するために、マラチオンとNHLの関連性を調査した研究(Waddellら、2001)を選択した。ここでは、(i)基準非ばく 露群の被験者数=1,018人(表1より:非農家=243人の罹患者+775人の非罹患者)、(ii)ばく露群の被験者数=238 人(表4より:マラチオンばく露者=91人の非ばく露者+147人の非ばく露コントロール)、(iii)参照非ばく露群の罹患 者数=243人(表1より:非農家または非ばく露群の243人の罹患者)とすると、真の率比=1.2、1.5、または2.0と仮定 すると、「ばく露ありと、ばく露なし」の比較の検出力が計算できた。

肺がんとダイアジノンについて上述したように、非農家(非ばく露者)では、以下の表に示すように、研究で推定され	/
た NHL の割合が 0.2387 で 1.2 の OR を検出する検出力を 30.5%と推定した。	

片側 2 標本の比率検定の検出力解析の結果 $(a=0.05)^{(a)}$								
N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Odds Ratio	Power			
1,018	238	0.1194	0.1399	1.2	0.2279			
1,018	238	0.1194	0.1689	1.5	0.647			
1,018	238	0.1194	0.2133	2.0	0.9693			
1,018	238	0.1194	0.2891	3.0	1			
1,018	238	0.2387	0.2734	1.2	0.3047			
1,018	238	0.2387	0.3199	1.5	0.8149			
1,018	238	0.2387	0.3854	2.0	0.9971			
1,018	238	0.2387	0.4847	3.0	1			
1,018	238	0.4774	0.523	1.2	0.3522			
1,018	238	0.4774	0.5781	1.5	0.8779			
1,018	238	0.4774	0.6463	2.0	0.9992			
1,018	238	0.4774	0.7327	3.0	1			

上記の結果を生成するために使用される Stata コード:2乗比例('=0.5\*243/1018' '=2\*243/1018' ), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1,018) n2(238) 片側

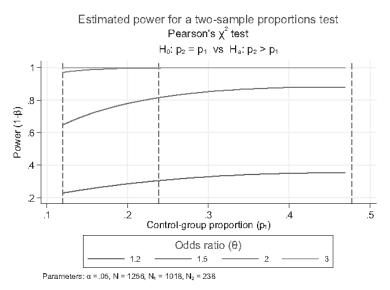
table(N1:"N コントロール" N2:"N ばく露" p1:"割合コントロール" p2"割合ばく露" OR:"オッズ比" power:"検出力')

 (a):片側検定 a = 0.05 Ho: p2 = p1 vs Ha: p2 > p1; Ncontrols = 1,018、Nexposed = 238、反復回数 = 1,000 回 (データセット)。
 (b):Waddell ら (2001) の 243/1018 の肺がんの観察されたバックグラウンド率を 1/2 倍、1 倍、2 倍を表す。上表のハイライトされ 太字の領域は、引用された研究におけるこの1 倍の NHL の観測されたバックグラウンド率に関連した検出力を表している。

このようなマラチオンと NHL の検出力関係は、上記の AHS 前向きコホート研究(ダイアジノンと肺がん)と同様に以下のようにグラフ化されている42。グラフの中央縦点線は非農家/非ばく露群の NHL 比が 0.2387 での検出力を示し、

<sup>&</sup>lt;sup>42</sup> グラフを生成するための Stata コード:2 乗比例 ('= 0.5 \* 243/1018'(0.01) '= 2 \* 243/1018'), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1018) 0) n1(1018) n2(238)graph(再キャスト(線) x-line('= 0.5 \* 243/1018'' '= 243/1018'' '= 2 \* 243/1018', lpattern(dash)) legend(rows(1)size(small)) y-label(0.2(0.2)1.0))片側。

左端と右端の縦点線は非農家/非ばく露群のNHL比が1/2と2倍での感度分析の枠を示している。



1.2-、1.5-、2.0-及び 3.0 の真の RR におけるコントロール群比率の関数としての検出力を評価する(片側)2 標本比率検定の推定検出力を示すグラフ。赤い破線の縦線は、観測された比率の 1/2 倍、観測された比率の 1 倍及び観測された比率の 2 倍におけるコントロールグループの比率を表し、これらのバックグラウンドレートの仮定に対する検出力の感度を示している。

非農業者/非ばく露者の NHL 割合を 0.2387 と推定した場合、OR 1.2、1.5、2.0、3.0 を検出する検出力(片側) は、それぞれ 30.5%、81.5%、99.7%、99.9%以上となる。なお、Waddellら(2001 年)は、マラチオンを使用した NHL 症例 91 例と使用しなかった非農家 243 例を対象に、OR は 1.6、95% CI は 1.2・2.2 と報告している。

以上のことから、真のオッズ比が 1.2、1.5、2.0、3.0 の場合に、影響量の拡大の差がどの程度存在するかをシミュレーションするために SAS が使用された。以下は、マラチオンと NHL の検出力解析のために SAS で作成された表で、 SAS ベースのシミュレーション結果から、影響の拡大のマグニフィケーションの大きさを示している。

1.2、1.5、2.0 及び 3.0 の真のオッズ比を与えられた影響量の拡大を示す SAS シミュレーション結果 <sup>(a)</sup>								
真値				観察された有意な OR の分布				
非ばく露群における罹	OR	N 個の解析 データセット	Power <sup>(b)</sup>	Ν	10%台	中央値	000/4	
患者の割合						(%inflation)	90%台	
0.1194	1.2	1,000	0.22	220	1.4	1.5 (25)	1.8	
(1/2 background)	1.5	1,000	0.66	661	1.5	1.7 (13)	2.0	
	2	1,000	0.97	972	1.6	2.0 (0)	2.5	
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7	
0.2387	1.2	1,000	0.32	323	1.3	1.4 (17)	1.6	
(19 background)	1.5	1,000	0.81	812	1.4	1.6 (7)	1.8	
	2	1,000	1.0	997	1.6	2.0 (0)	2.4	
	3	1,000	1.0	1,000	2.5	3.0 (0)	3.6	
0.4774	1.2	1,000	0.34	337	1.3	1.4 (17)	1.6	
(29 background)	1.5	1,000	0.87	872	1.3	1.5 (0)	1.8	
	2	1,000	1.0	1,000	1.6	2.0 (0)	2.5	
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7	

ロジスティック回帰モデルが、2 つのグループのオッズ比を計算するために使用された。最尤推定値が存在しない場合(おそらくいずれ かのグループの症例がゼロであったため)、EXACT 検定をいくつかのデータセットの解析に使用した。

(a):片側検定、a = 0.05、非ばく露群=1,018、マラチオンばく露群=238、反復 N=1,000 (データセット)。(b):このシミュレーション から得られた検出力は近いかもしれないが、組み込みの SAS (PROC POWER)や Stata (power 2-proportion)のような統計ソフトウェア のプロシージャを使用している。これは、シミュレーションされたデータセットの数が十分でないためかもしれない。しかし、1,000 回 の反復は、検出力を十分に推定し、統計的に有意な結果(ここでは、≤0.05)が得られた場合の影響量の拡大を説明するのに十分である

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p<0.05 で統計的に有意な結果が得られた場合、影響量の中央値は、非ばく露群の NHL の割合と真のオッズ比 (1.2 から 3.0 まで)によって、1.4 から 3 まで変化することに注意する。例えば、非農家の NHL の割合が 0.2387 の場 合、真の OR が 1.2(表中の太字の行)とすると、統計的に有意な OR の半分は中央値 1.4 を超え、半分は下回ってい ることになる。さらに、統計的に有意な OR の大部分(90%)は 1.3 以上であることが観察され、少数(10%)は 1.6 以上 であることさえ観察された。

まとめると、疫学研究の検出力は、規制当局や他の人がそのような研究を評価する際に考慮すべき重要な要素であ る。十分な検出力を持つ研究は、与えられた規模の真の効果が実際に存在する場合に検出する可能性が高くなるだ けでなく(検出力の古典的な定義は、II型エラーや偽陰性の問題に関連している)、影響が存在しないが(偶然にも) 事前に選択された閾値(統計的有意性の0.05レベルなど)を超えた場合に、影響を拡大したり誇張したりする可能性 が低くなるだろう。研究が適切な検出力を持っている場合(例えば、80%以上)、観察された影響量は真の影響量を再 現する可能性が高く、この影響量の観察された偶然の変動は、未知の真の値を中心に対称的に分布を再現する。これ らのシミュレーションと Ioannidis によるオリジナルの研究及び Gelman と Carlin(2014年)による拡張研究から得ら れるメッセージは、研究は偽陰性(タイプ II エラー)を回避するために適切な検出力が必要であるだけでなく、統計的 に有意な(または他の閾値を通過した)影響量のために影響量の拡大を回避するために適切な検出力が必要であると いうことである。Gelman と Carlin(2014年)はさらに進んで、そのような「後ろ向き計画の計算は、統計的に有意でな い影響量よりも統計的に有意な影響量に関連しているかもしれない。統計的に有意な結果の解釈は、基礎となる影響 のもっともらしい規模によって大きく変わる可能性がある」と述べている。研究が適切な検出力を持っていれば体系的な リスクの膨張は皆無であるが、統計的に有意な効果をもたらす検出力不足の研究の影響推定値は、実質的なリスク膨 張の可能性があり、その解釈は真の(基礎となる)影響の現実的な推定値に依存することに注意することである。

理想的には、公表されている文献研究は、検出力解析を実施し、文書化するべきである。それ以外にも、公表されて いる文献は、読者がこのような検出力計算(あるいは Gelman と Carlin(2014 年)が言うところの(後ろ向き)デザイン 計算)を行うのに十分な情報を提供すべきである。上記の2つの例では、著者は読者に検出力と影響量の拡大を計算 することができる十分な情報を提供していた。これは常にそうとは限らない。検出力の計算に使用された情報が文献に 部分的にしか提供されていなかったり、提供された情報がそのような計算ができない方法で構成されていたりすること がある<sup>43,44</sup>。例えば、著者が三分位または四分位を決定するためにばく露量の代わりに症例数を使用している場合(こ れはグループ間での症例数が一定であることから証明される)、または著者が複数のがん症例をまとめてグループ化し、 その数を使用して三分位を決定している場合、必要な入力が得られないため、ここに示されている検出力(またはデザ イン)の計算は不可能である。集積及び報告される数値及びデータは、著者及び文献の間で必ずしも標準化されてい るわけではないので、一つの強い推奨事項は、文献が(補足的またはオンラインデータであっても)検出力を推定する ために必要な情報を報告することを義務付けることであり、このような評価を査読者及び関心のある読者の両方ができ るようにすることであろう。

以上の解析から、影響量の膨張現象の潜在的な意味合いは、疫学研究を評価する上で重要な考慮事項であること が示唆されたが、この現象に関するいくつかの注意点を覚えておくことが重要であり、疫学研究の解釈にどのように考

<sup>&</sup>lt;sup>43</sup> 例えば、Stella Koutros ら(2012年)の出版物「Risk of Total and Aggressive Prostate Cancer and Pesticide Use in the Agricultural Health Study」で発表されたマラチオンばく露と侵攻性前立腺がんの関連性のレビューでは、発表された論文に重要な情報が提供されていなか ったため、パネルはマラチオンばく露群と非ばく露群の比較の力を算出できなかった。文献及び文献の補足文書から、非ばく露群の症例数を容 易に把握することができたが(本文中の表 2)、非ばく露群または各ばく露量(四分位)の被験者数は入手できなかったようである。我々は、文献の 補足文書の表 1 の情報から非ばく露者群の被験者数及び各四分位の被験者数を導入しようとしたが、表 1 の情報は、ばく露者を症例数の四分 位に基づいてグループに分類するという他の多くの AHS の出版物と一致しない方法で示されていたため、それを行うことができなかった。

<sup>&</sup>lt;sup>44</sup> 検出力の計算に使用された情報は、文献では部分的にしか提供されていないことがある。例えば、我々は Laura Beane-Freeman ら(2011)に よる AHS 研究文献「Atrazine and Cancer Incidence Among Pesticide Applicators in the Agricultural Health Study(1994-2007)」に記 載されている情報から、様々な甲状腺がんの比較に関連した検出力を計算した。この文献では、著者らは被験者をばく露に基づいて四分位に分 類するのではなく、その代わりに、すべてのがん症例を合わせた総症例数に基づいて被験者を分類またはグループ化した。このようにして、すべ てのタイプのがんの症例数は、分類されたグループ間で同じであり、したがって、対象となる任意の特定のがん(例えば、甲状腺、ここでは)の症例 数は、グループ間で同じではなく、被験者の数は、グループ間で同じではなかった。この例では、文献は、(i)参照 Q1:N=9,523、(ii)Q2、Q3 及 び Q4 の総被験者数を提供した。N=26,834 人(表 1)及び(iii)参考 Q1 の甲状腺がん症例数=3 人(表 2)を提供した。しかし、比較群(Q2、Q3 または Q4)のそれぞれの正確な被験者数は得られなかった。

慮すべきであるかについても留意すべきである。

- -第一に、この現象は、対象となる影響が統計的(またはその他の)閾値を通過するような検出力不足の研究では影響量が膨張する傾向があるが、他にも推定値を帰無に向かって逆方向に偏らせるバイアスが存在する可能性がある。このバイアスは、影響量抑制と呼ばれることがある。おそらく、これらのバイアスの中で最もよく知られているのは、本文で議論されている非差異的誤分類バイアスである。これは、一般的に(常にではないが)帰無値に向かって予測可能なバイアスを生じさせ、それによって影響量を体系的に過小予測する。これが常に正しいとは限らず、(少なくとも小規模の低い検出力の研究については)影響量の拡大のような対抗要因や相殺要因が存在する可能性があることを認識することは、重要な前進である。特に、検出力不足の研究では、例えば、非差異的誤分類バイアスから生じるかもしれない帰無値へのバイアスを潜在的に相殺する(そして、多分相殺以上に)ことができる程度に、帰無値から離れた方向に偏った推定値をもたらすことがある。いずれにしても、重要なことは、統計的に有意な結果を得るための影響量の推定値に少なくともいくつかの最低限の一致度を持たせることができるようにするためには、十分な検出力のある研究が必要であるということを認識することである。
- -第二に、そして本文で述べられているように、影響量の拡大は、研究者(またはそのような研究を解釈する規制者)の側で、その研究が検出力不足の場合に、与えられた有意性の閾値(例:p<0.05)を通過するか、または一定の大きさ(例:OR>3)を達成する影響を特定することに焦点を当てた努力に関連している。この現象は、統計的有意性(または影響量)の「事前スクリーニング」を行う際に最も懸念される現象である。規制者や政策決定者などが、事前に決められた統計的閾値を「通過」した関連性のみに焦点を当てて行動することを避け、研究が検出力不足の場合に効果量の拡大を評価して判断するためにその影響量を使用する場合、この現象はあまり懸念されない。影響量の拡大の決定は、研究や研究デザインの機能や欠陥ではなく、むしろその研究がユーザーコミュニティによってどのように解釈されるかの機能であることに注意する。

残念なことに、与えられた量よりも大きい、またはある統計的閾値を通過して「発見された」影響量に注目が集まる 傾向が時々ある。これらの「発見」がどのように考慮されるべきかに関しては Ioannidis によって推奨されている (Ioannidis、2008年)。

「最初に仮定された発見の時点では、影響量を判断することはおろか、関連性が全く存在するかどうかもわからないのが普通である。最初の原則として、影響量については慎重にならなければならない。不確実性は、単に CI (95%、99%、99.9%であるかどうかは関係ありません)だけでは伝わらない。

新たに提案された関連性については、提案された影響の信頼性と正確性はケースによって異なる。とトは次のよう な質問をするだろう:この分野の研究コミュニティは、研究成果を主張するために、広く統計的な有意性や同様の選 択の閾値を採用しているのか?発見は小規模な研究から生まれたのか?分析に大きな変動の余地があるか?選択 的な報告から保護されていないか(例:プロトコールが前もって完全に利用可能ではなかったか)?特定の「ポジティ ブな」結果を発見し、促進することに興味を持っている人や組織はあるか?最後に、影響を相殺する力は最小限に 抑えられているか?

- 第三に、影響量の膨張現象は、発見科学全般に適用可能な一般的な原則であり、疫学の特異的悩みや弊害で はないことを覚えておくべきである(Ioannidis、2005年;Lehrer、2010年;Button、2013年;Buttonら、2013 年;Reinhart、2015年)。先に示したように、これは薬理学、遺伝子研究、心理学研究、そして最も頻繁に引用さ れる医学文献の多くでしばしば見られる。このような真実性の膨張は、研究の規模が小さく、検出力が不足してい る場合に起こり、そのような研究では結果に大きなばらつきがあるからである。これは、多くの研究者が同様の研究 を行っており、「新しい」または「刺激的な」結果を発表するために競争している場合には特に問題となる (Reinhart、2015年)。

# まとめと結論

影響量の拡大または「真実の膨張」とは、影響を「発見」するために統計的またはその他の閾値を満たす必要がある

検出力不足の研究から得られた効果測定値の場合に、オッズ比、相対リスク、または率比の推定値が誇張されることが ある現象である。この現象は、疫学や疫学研究に特有のものではなく、むしろ、影響が存在するかどうかを判断するた めに、影響量や統計的有意性に関連するような、研究の規模が小さく、事前に設定された閾値が使用される傾向にあ るあらゆる科学に見られる。このように、疫学研究の利用者がこの問題とその潜在的な解釈の結果を認識することが重 要である。特に、検出力不足の研究から発見された関連性は、統計的または他の同様の閾値を通過したことに基づい て強調されたり、注目されたりするが、それは帰無値から系統的にバイアスがかかっている。統計的閾値を通過した「発 見された」」関連性の結果として、特定の研究で観測された影響量が帰無値から遠ざかるかどうかはわからないが(非差 異的な誤分類を示す特定の研究が必ずしも帰無値に向かって偏るとは言えないのと同じように)、ここで提示された説 明と実行されたシミュレーションによって説明されているように、偶然がそのような偏りをある程度有利にすることはわか っている。別の言い方をすると、統計的またはその他の閾値を通過した影響量に基づいて影響量に注目したり、報告し たり、行動したりすることを選択することで、バイアスが導入される(Yarkoni、2009 年)。繰り返しになるが、これは研究 がユーザーによってどのように解釈されるかに関連する問題であり、研究デザインに内在するものでもなければ、優れ た科学技術の原則や実践に関連するものでもない。

上記の問題に対する(部分的な)解決策の 1 つは、観察された影響量が帰無値から系統的に偏ってしまう可能性が あることを認識した上で、事前に定められた閾値を通過した疫学研究の影響量または検出力不足の研究から生じた場 合の統計的に有意な影響量を慎重に解釈することである。このようなアプローチでは、著者が研究の検出力を報告す るか、著者が読者に十分な情報を提供する必要がある。検出力が実質的に 80%未満の研究から得られた影響量は、 おそらく実質的に(特に検出力が 50%未満の場合)誇張する可能性があることを認識した上で、適切な程度の疑いを 持って解釈する必要がある。この誇張の潜在的な程度は、対象となる健康影響のバックグラウンド率、研究のサンプル サイズ、対象となる影響量など、多くの問題に依存する。より具体的には、(a)対象となる健康影響のバックグラウンド率 が低い場合、(b)研究のサンプルサイズが小さい場合、(c)対象となる影響量が弱い場合、研究の検出力(その影響量 を検出するための)は低く、統計的に有意な結果において影響量が誇張される傾向が高くなる。対象とする健康影響 のバックグラウンド率が低い集団で、小さな、または弱い影響を調査する低検出力研究では、影響量の誇張が最も大き くなる傾向がある。その結果、PPRパネルは、疫学的文献にこのような計算を組み込むか、または読者が計算を実行で きるような重要な情報を含めることを推奨している。具体的には以下の通り。

特定の農薬ばく露と疾病との間の関連が統計的に有意であることが判明した場合、特に(推定される)検出力の低い 研究では、データ利用者は、統計的に有意な影響量推定値(OR または RR)がどの程度人工的に拡大、または誇張さ れているかを判断するために、様々な検出力計算(または検出力解析)を実行すべきである。これは、疫学研究で明確 に報告される3つの値を必要とする。(i)非ばく露群の被験者数(罹患者と非罹患者を含む)、(ii)ばく露群の被験者数 (罹患者と非罹患者を含む)、(iii)非ばく露群の罹患者数である。リスク管理者は、次に、ばく露群と非ばく露群の間の 所定の(予め決められた)影響量の差を検出するために、対象となる目標値(典型的には、OR または RR)を選択でき、 影響量の規模が、対象となる研究で推定された影響量をどの程度説明できるかを評価することができる。

(i)多くの疫学研究はしばしば検出力不足であり、(ii)著者が検出力の計算や(場合によっては)計算に必要な情報 を文献の中で提供することは一般的ではなく、(iii)影響量の拡大の現象は一般的に疫学分野ではほとんど認識され ていないようであるため、上記の PPR パネルの勧告を実施するためには、研究者/助成機関、出版社、研究資金提 供者の側での努力が必要である。上記のように、この分野での実践の現状は悲観的になるかもしれないことを示唆して いるが、研究者である Kate Button (Button、2013 年)が Nature Reviews Neuroscience 誌に掲載したこのトピック に関するオピニオン・ピース(Button ら、2013 年)で、楽観について慎重な理由を提供した。

これらの問題に対する認識は高まっており、問題を認識することは、現在の実践を改善し、解決策を特定するための 第一歩である。出版バイアスの問題を一朝一夕に解決するのは難しいが、研究者は確立された(しかし、しばしば無視 される)科学技術の原則を採用することで、研究の信頼性を向上させることができる。また、研究者は、確立された(しか し無視されることが多い)科学技術の原則を採用することで、研究の有用性/信頼性を向上させることができる。

1)研究の計画や結果の解釈において、統計的な検出力を考慮する。

2)研究方法と結果の開示には誠実さを高める。

3)研究計画書、解析計画、さらにはデータまでもが公表されるようにする。

4)供給源を共有し、サンプルサイズと再現力を向上させるために協力して作業する

上記の一連の推奨事項と考えは、サンプルサイズと神経毒性学の背景で設定されているが、疫学を含むあらゆる発 見科学に広く適用可能である。まとめると、疫学研究の実施と報告が公衆衛生に基づいた選択をする際に規制機関に とって有用なものとなるためには、改善の余地が大いにあるが、問題点はより明確にされ、認識され始めており、今後も 楽観的に考えられる理由がある。

# 参考文献

- Beane Freeman, LE, Rusiecki, JA, Hoppin, JA, Lubin, JH, Koutros, S, Andreotti, G, Hoar Zahm, S, Hines, CJ, Coble, JB, Barone Adesi, F, Sloan, J. Sandler, DP, Blair, A, and Alavanja, MCR. Atrazine and cancer incidence among pesticide applicators int eh agricultural health study (1994–2007). Environ Health Perspect, 119, 1253–1259.
- Button K, 2013. Unreliable neuroscience? Why power matters. The Guardian newspaper (UK). 10 April 2013 Available online: https://www.theguardian.com/science/sifting-the-evidence/2013/apr/10/unreliable -neuroscie nce-power-matters [Accessed 6 September 2017]
- Button K, Ioannidis JPA, Mokrysz C, Nosek BA, Flink J, Robinson ESJ and Munafo MR, 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nature Reviews Neuroscience, 14, 365-376.
- Cohen P and Chen S, 2010. How big is a big odds ratio: interpreting the magnitudes of odds ratios in epidemiological studies. Communications in Statistics: Simulation and Computation, 39, 860–864.
- Gelman A and Carlin J, 2014. Beyond power calculations: assessing type S (sign) and type M (magnitude) errors. Perspectives on Psychological Science, 9, 641-651.

Ioannidis JP, 2005. Why most published research findings are false. PLoS Med, 2, e124. Ioannidis JP, 2008. Why most discovered true associations are inflated. Epidemiology, 19, 640–648.

- Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC and Beane Freeman LE. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis.
- Occupational and Environmental Medicine, 72, 496–503. Koutros, S, Beane Freeman, LE, Lubin, JH, Heltshe, SL, Andreotti, G, Hughes-Barry, K, DelllaValle, CT, Hoppin, JA, Sandler, DP, Lynch, CF, Blair, A and Alavanja, MCR, 2013. Risk of total and aggressive prostate cancer and pesticide use in the agricultural health study. American Journal of Epidemiology, 177, 59–74.
- Lehrer J. 2010. The truth wears off is there something wrong with the scientific method. New Yorker, 13 December, 2010. Available online: http://www.newyorker.com/magazine/2010/12/13/the-truth-wears-off [Accessed September 2017]
- Reinhart A, 2015. Statistics Done Wrong: the WOEfully complete guide. No Starch Press (San Francisco, CA).

Rosenthal JA, 1996. Qualitative descriptors of strength of association and effect size. Journal of Social Service Research, 21, 37-59.

Taubes G, 1995. Epidemiology faces its limits. Science, 269, 164–169.

- Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP and Blair A, 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male
- farmers (United States). Cancer Causes Control, 12, 509–517. Wynder EL, 1997. Epidemiology Faces its Limits Reply. Invited Commentary: Response to Science Article, "Epidemiology Faces Its Limits". American Journal of Epidemiology, 143, 747–749.
- Yarkoni T, 2009. Ioannidis on effect size inflation, with guest appearance by Bozo the Clown. 21 November 2009. Available online: http://www.talyarkoni.org/blog/2009/11/21/ioannidis-on-effect-size-inflationwith-guest-appea rance-by-bozo-the-clown/ [Accessed on 6 September 2017]

# **SCIENTIFIC OPINION**



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# Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects'

EFSA Panel on Plant Protection Products and their Residues (PPR), Colin Ockleford, Paulien Adriaanse, Philippe Berny, Theodorus Brock, Sabine Duquesne, Sandro Grilli, Susanne Hougaard, Michael Klein, Thomas Kuhl, Ryszard Laskowski, Kyriaki Machera, Olavi Pelkonen, Silvia Pieper, Rob Smith, Michael Stemmer, Ingvar Sundh, Ivana Teodorovic, Aaldrik Tiktak, Chris J. Topping, Gerrit Wolterink, Matteo Bottai, Thorhallur Halldorsson, Paul Hamey, Marie-Odile Rambourg, Ioanna Tzoulaki, Daniele Court Marques, Federica Crivellente, Hubert Deluyker and Antonio F. Hernandez-Jerez

# Abstract

In 2013, EFSA published a comprehensive systematic review of epidemiological studies published from 2006 to 2012 investigating the association between pesticide exposure and many health outcomes. Despite the considerable amount of epidemiological information available, the quality of much of this evidence was rather low and many limitations likely affect the results so firm conclusions cannot be drawn. Studies that do not meet the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009 are thus not suited for risk assessment. In this Scientific Opinion, the EFSA Panel on Plant Protection Products and their residues (PPR Panel) was requested to assess the methodological limitations of pesticide epidemiology studies and found that poor exposure characterisation primarily defined the major limitation. Frequent use of case-control studies as opposed to prospective studies was considered another limitation. Inadequate definition or deficiencies in health outcomes need to be avoided and reporting of findings could be improved in some cases. The PPR Panel proposed recommendations on how to improve the quality and reliability of pesticide epidemiology studies to overcome these limitations and to facilitate an appropriate use for risk assessment. The Panel recommended the conduct of systematic reviews and meta-analysis, where appropriate, of pesticide observational studies as useful methodology to understand the potential hazards of pesticides, exposure scenarios and methods for assessing exposure, exposure-response characterisation and risk characterisation. Finally, the PPR Panel proposed a methodological approach to integrate and weight multiple lines of evidence, including epidemiological data, for pesticide risk assessment. Biological plausibility can contribute to establishing causation.

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**Keywords:** epidemiology, pesticides, risk assessment, quality assessment, evidence synthesis, lines of evidence, weight-of-evidence

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# 「農薬へのばく露と健康影響に関連する疫学研究の文献レビュー」の結果のフォローアップ(追跡調査)に関する PPR パネルの意見書

植物保護製剤(農薬)とその残留物に関する EFSA パネル(PPR)

Colin Ockleford, Paulien Adriaanse, Philippe Berny, Theodorus Brock, Sabine Duquesne, Sandro Grilli, Susanne Hougaard, Michael Klein, Thomas Kuhl, Ryszard Laskowski,

Kyriaki Machera, Olavi Pelkonen, Silvia Pieper, Rob Smith, Michael Stemmer, Ingvar Sundh, Ivana Teodorovic, Aaldrik Tiktak, Chris J. Topping, Gerrit Wolterink, Matteo Bottai, Thorhallur Halldorsson, Paul Hamey, Marie Odile Rambourg, Ioanna Tzoulaki,

Daniele Court Marques, Federica Crivellente, Hubert Deluyker and Antonio F. Hernandez-Jerez

# 抄録

2013年に EFSA は、2006年から 2012年までに発表された疫学研究の包括的な システマティックレビューを発表 し、農薬ばく露と多くの健康影響との関連性を調査した。かなりの量の疫学的情報が得られたにもかかわらず、これらの エビデンスの多くはかなり質が低く、多くの制限が結果に影響している可能性が高いため、確固たる結論を出すことは できなかった。このように、規則(EU)No 1107/2009に記載されている「認可基準」を満たしていない研究は、リスク評 価には適していない。この科学的意見書では、植物保護製剤(農薬)とその残留物に関する EFSA パネル(PPR パネ ル)は、農薬疫学研究の方法論的限界を評価するよう求められており、その主な限界はばく露の特徴付けが不十分で あることが原因であることが判明した。また、前向き研究ではなく症例対照研究を頻繁に使用していることも限界と考え られた。健康影響の不適切な定義や不正確さは避ける必要があり、結果の報告はいくつかのケースで改善される可能 性がある。PPR パネルは、これらの限界を克服し、リスク評価への適切な利用を促進するために、農薬疫学研究の質と 信頼性を向上させる方法についての勧告を提案した。パネルは、農薬の潜在的な有害性、ばく露シナリオ、ばく露評 価の方法、ばく露ー反応特性、リスク特性を理解するための有用な方法として、農薬観察研究のシステマティックレビュ ーとメタアナリシスの実施(必要に応じて)を推奨した。最後に、PPR パネルは、農薬のリスク評価のために疫学的デー タを含む複数のエビデンスを統合し、重み付けする方法論的アプローチを提案した。生物学的妥当性は因果関係の 立証に寄与することができる。

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キーワード:疫学、農薬、リスク評価、品質評価、エビデンスの統合、複数のエビデンス、エビデンスの重み付け

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#### Epidemiological studies and pesticides

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Terron、Andrea Altieri、Arianna Chiusolo。パネルと EFSA は、以下のヒアリング専門家の意見に謝意を表する。(1)
David Miller (US-EPA)は US-EPA の経験を共有し、効果量の算出を行った。(2) 農業健康調査のための Kent
Thomas (US-EPA)、(3) the INSERM Report のための Marie Christine Lecomte (INSERM), Sylvaine Cordier
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(Risk & Policy Analysts Ltd), Ruth Bevan (IEH Consulting Ltd), Kate Jones (UK Health & Safety Laboratory)。
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#### Summary

The European Food Safety Authority (EFSA) asked the Panel on Plant Protection Products and their Residues (PPR Panel) to develop a Scientific Opinion on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (Ntzani et al., 2013). This report was based on a systematic review and meta-analysis of epidemiological studies published between 2006 and 2012 and summarised the associations found between pesticide exposure and 23 major categories of human health outcomes. Most relevant significant associations were found for liver cancer, breast cancer, stomach cancer, amyotrophic lateral sclerosis, asthma, type II diabetes, childhood leukaemia and Parkinson's disease. While the inherent weaknesses of the epidemiological studies assessed do not allow firm conclusions to be drawn on causal relationships, the systematic review raised a concern about the suitability of regulatory studies to inform on specific and complex human health outcomes.

The PPR Panel developed a Scientific Opinion to address the methodological limitations affecting the quality of epidemiological studies on pesticides. This Scientific Opinion is intended only to assist the peer review process during the renewal of pesticides under Regulation (EC) 1107/2009 where the evaluation of epidemiological studies, along with clinical cases and poisoning incidents following any kind of human exposure, if available, is a data requirement. Epidemiological data concerning exposures to pesticides in Europe will not be available before first approval of an active substance and so will not be expected to contribute to a draft assessment report (DAR). However, there is the possibility that earlier prior approval has been granted for use of an active substance in another jurisdiction and epidemiological data from that area may be considered relevant. Regulation (EC) No 1107/2009 requires a search of the scientific peer-reviewed open literature, which includes existing epidemiological studies. This type of data is more suited for the renewal process of active substances, also in compliance with Regulation (EC) 1141/2010 which indicates that 'The dossiers submitted for renewal should include new data relevant to the active substance and new risk assessments'.

In this Opinion, the PPR Panel proposed a methodological approach specific for pesticide active substances to make appropriate use of epidemiological data for risk assessment purposes, and proposed recommendations on how to improve the quality and reliability of epidemiological studies on pesticides. In addition, the PPR Panel discussed and proposed a methodology for the integration of epidemiological evidence with data from experimental toxicology as both lines of evidence can complement each other for an improved pesticide risk assessment process.

First, the opinion introduces the basic elements of observational epidemiological studies<sup>1</sup> and contrasts them with interventional studies which are considered to provide the most reliable evidence in epidemiological research as the conditions for causal inference are usually met. The major observational study designs are described together with the importance of a detailed description of pesticide exposure, the use of validated health outcomes and appropriate statistical analysis to model exposure-health relationships. The external and internal study validity is also addressed to account for the role of chance in the results and to ascertain whether factors other than exposure can distort the associations found. Several types of human data can contribute to the risk assessment process of pesticides, particularly to support hazard identification. Besides formal epidemiological studies, other sources of human data such as case series, disease registries, poison control centre information, occupational health surveillance data and post-marketing surveillance programmes, can provide useful information for hazard identification, particularly in the context of acute, specific health effects.

However, many of the existing epidemiological studies on pesticides exposure and health effects suffer from a range of methodological limitations or deficiencies (Terms of Reference (ToR) 1). The Panel notes that the complexity of studying associations between exposure to pesticides and health outcomes in observational settings among humans is more challenging than in many other disciplines of epidemiology. This complexity lies in some specific characteristics in the field of pesticide epidemiology such as the large number of active substances in the market (around 480 approved for use in the European Union (EU)), the difficulties to measure exposure, and the frequent lack of quantitative (and qualitative) data on exposure to individual pesticides. The systematic appraisal of epidemiological evidence carried out in an EFSA external scientific report (Ntzani et al., 2013) identified a number of methodological limitations. Poor exposure characterisation primarily defines the major limitation of most existing studies because of the lack of direct and detailed exposure assessment to specific pesticides (e.g. use of generic pesticide definitions). Frequent use of case-control studies as

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Epidemiological studies and pesticides

#### 概要

欧州食品安全機関(EFSA)は、植物保護製剤(農薬)とその残留物に関するパネル(PPR,パネル)に、外部科学報 告書「農薬へのばく露と健康影響を関連付ける疫学研究の文献レビュー」(Ntzani ら、2013 年)の結果のフォローアッ プ(追跡調査)に関する科学的意見書の作成を依頼した。この報告書は、2006年から2012年の間に発表された疫学 研究のシステマティックレビューとメタアナリシスに基づいており、農薬ばく露と 23 の主要なカテゴリーのヒト健康影響と の間に見出された関連性をまとめたものである。最も関連性が高いのは、肝臓がん、乳がん、胃がん、筋萎縮性側索硬 化症、喘息、II型糖尿病、小児白血病、パーキンソン病であった。評価された疫学研究に内在する弱点があるため、因 果関係についての結論を導き出すことはできないが、システマティックレビューでは、特定の複雑なとトの健康に関する 影響について情報を提供するための規制研究の適合性についての懸念が提起された。

PPR パネルは、農薬に関する疫学研究の質に影響を与える方法論的限界に対処するために、科学的意見書を作 成した。この意見書は、規制(EC)1107/2009の下での農薬の更新時のピアレビュープロセスを支援することのみを目 的としており、あらゆる種類のヒトばく露による臨床例や中毒事故(入手可能であれば)を加えた疫学的研究の評価デ ータが必要である。欧州における農薬へのばく露に関する疫学的データは、有効成分の最初の認可前には入手でき ないため、評価報告書草案(DAR)に提供することは期待できない。しかし、他の管轄区域では有効成分の使用につ いて先行して認可されている可能性があり、その地域の疫学的データが適切だと考えられる。規則(EC)No 1107/2009 では、既存の疫学研究を含む学術的に査読された公表文献を検索することを要求している。このタイプの データは、「更新のために提出された書類には、有効成分に関連する新しいデータと新しいリスク評価を含めるべきで ある」という規則(EC)1141/2010にも準拠しており、有効成分の更新プロセスに適している。

本意見書では、疫学データをリスク評価に適切に活用するための農薬有効成分に特化した方法論的アプローチを 提案し、農薬の疫学研究の質と信頼性を向上させるための提言を行った。さらに、PPR パネルは、農薬のリスク評価プ ロセスを改善するために、疫学的と実験毒性学のデータを統合するための方法論を議論し、提案した。

まず、本意見書では、観察による疫学研究1の基本的な要素を紹介し、因果関係を推論するための条件が通常満た されていることから、疫学研究において最も信頼性の高いエビデンスを提供すると考えられている介入研究との対比を 行っている。主な観察による研究の計画については、農薬ばく露の詳細な記述の重要性、有効な健康影響の使用及 びばく露と健康影響の関係をモデル化するための適切な統計解析の重要性が説明されている。また、外部及び内部 研究の妥当性については、結果における偶然の役割を説明し、ばく露以外の要因が発見された関連性を歪めないか どうかを確認するために取り上げられてもいる。いくつかの種類のヒトのデータは、農薬のリスク評価プロセス、特にハザ ードの特定をサポートするのに貢献することができる。正式な疫学研究以外にも、症例集積、疾病登録、毒物管理セン ター情報、労働衛生監視データ、市販後の監視プログラムなどのヒトのデータの他の情報源は、特に急性の特定の健 康影響の場合には、ハザードの特定に有用な情報を提供することができる。

しかし、農薬ばく露と健康影響に関する既存の疫学研究の多くは、さまざまな方法論の限界や不完全性に悩まされ ている(Terms of Reference(ToR)1)。パネルは、ヒトの観察環境における農薬ばく露と健康影響との関連を研究する ことは複雑で、疫学の他の多くの分野よりも困難であると指摘している。この複雑さは、市場に出回っている有効成分の 数の多さ(欧州連合 EU で使用が認可されているものは約 480 種類)、ばく露の測定の難しさ、個々の農薬へのばく露 に関する定量的及び定性的データが頻繁に欠如していることなど、農薬疫学の分野におけるいくつかの特殊な特徴に 起因する。EFSA の外部科学報告書(Ntzani ら、2013 年)で実施された疫学的証拠の系統的評価では、多くの方法 論的限界が指摘されている。特定の農薬に対する直接かつ詳細なばく露評価が行われていない(例えば、ジェネリック 農薬の使用に対して情報不足)ため、主にばく露の特徴付けが不十分であることが、ほとんどの既存の研究の主な限 界となっている。前向き研究ではなくて症例対照研究を頻繁に使用していることも限界となっている。健康影響の不十 分な定義、統計解析がないこと、研究結果の質の低い報告が、いくつかの農薬疫学研究の他の限界として確認されて いる。これらの限界は、因果関係に関する強固な結論を導き出すことを困難にするデータの不均一性や矛盾の原因と

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<sup>&</sup>lt;sup>1</sup> This Opinion deals only with observational studies (also called epidemiological studies) and vigilance data. In contrast, interventional studies (also called experimental studies, such as randomised clinical trials) are outside the scope of this Opinion. 3

<sup>1</sup> 本意見書は、観察研究(疫学研究ともいう)と警戒データのみを扱う。これに対し、介入研究(無作為化臨床試験などの実験研究とも呼ばれる)は 本意見書の対象外である。

opposed to prospective studies is also a limitation. Inadequate definition or deficiencies in health outcomes, deficiencies in statistical analysis and poor quality reporting of research findings were identified as other limitations of some pesticide epidemiological studies. These limitations are to some extent responsible for heterogeneity or inconsistency of data that challenge drawing robust conclusions on causality. Given the small effect sizes for most of the outcomes addressed by Ntzani et al. (2013), the contribution of bias in the study design can play a role.

The PPR Panel also provides a number of refinements (ToR 2) and recommendations (ToR 3) to improve future pesticide epidemiological studies that will benefit the risk assessment. The quality and relevance of epidemiological research can be enhanced by (a) an adequate assessment of exposure, preferentially by using personal exposure monitoring or biomarker concentrations of specific pesticides (or combination of pesticides) at an individual level, reported in a way that minimises misclassification of exposure and allows for dose-response assessment; (b) a sufficiently valid and reliable outcome assessment (well defined clinical entities or validated surrogates); (c) adequately accounting for potentially confounding variables (including other known exposures affecting the outcomes); (d) conducting and reporting subgroup analysis (e.g. stratification by gender, age, etc.). A number of reporting guidelines and checklists developed specifically for studies on environmental epidemiology are of interest for epidemiological studies assessing pesticide exposures. This is the case for extensions of the modified STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) criteria, among others, which includes recommendations on what should be included in an accurate and complete report of an observational study.

Exposure assessment can be improved at the individual level (direct and detailed exposure assessment to specific pesticides in order to provide a reliable dosimeter for the pesticide of concern that can be supplemented with other direct measures such as biomonitoring). Besides, exposure can be assessed at population level by using registered data that can then be linked to electronic health records. This will provide studies with unprecedented sample size and information on exposure and subsequent disease. Geographical information systems (GIS) and small area studies might also serve as an additional way to provide estimates of residential exposures. These more generic exposure assessments have the potential to identify general risk factors and may be important both informing overall regulatory policies, and for identification of matters for further epidemiological research. The development of -omic technologies also presents intriguing possibilities for improving exposure assessment through measurement of a wide range of molecules, from xenobiotics and metabolites in biological matrices (metabolomics) to complexes with DNA and proteins (adductomics). Omics have the potential to measure profiles or signatures of the biological response to the cumulative exposure to complex chemical mixtures and allows a better understanding of biological pathways. Health outcomes can be refined by using validated biomarkers of effect, that is, a guantifiable biochemical, physiological or any other change that, is related to level of exposure, is associated with a health impairment and also helps to understand a mechanistic pathway of the development of a disease.

The incorporation of epidemiological studies into regulatory risk assessment (ToR 4) represents a major challenge for scientists, risk assessors and risk managers. The findings of the different epidemiological studies can be used to assess associations between potential health hazards and adverse health effects, thus contributing to the risk assessment process. Nevertheless, and despite the large amount of available data on associations between pesticide exposure and human health outcomes, the impact of such studies in regulatory risk assessment is still limited. Human data can be used for many stages of risk assessment; however, a single (not replicated) epidemiological study, in the absence of other studies on the same pesticide active substance, should not be used for hazard characterisation unless it is of high quality and meets the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009. As these 'recognised standards' are not detailed in the Regulation, a number of recommendations should be considered for optimal design and reporting of epidemiological studies to support regulatory assessment of pesticides. Although further specific guidance will be helpful, this is beyond the ToR of this Opinion. Evidence synthesis techniques, such as systematic reviews and meta-analysis (where appropriate) offer a useful approach. While these tools allow generation of summary data, increased statistical power and precision of risk estimates by combining the results of all individual studies meeting the selection criteria, they cannot overcome methodological flaws or bias of individual studies. Systematic reviews and meta-analysis of observational studies have the capacity of large impact on risk assessment as these tools provide information that strengthens the understanding of the potential hazards of pesticides, exposure scenarios and methods for assessing exposure, exposure–response characterisation and risk characterisation. Although systematic reviews

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なっている。Ntzaniら、(2013年)が取り上げたほとんどの健康影響の効果量が小さいことを考えると、研究デザインに おけるバイアスの寄与が一役買っている可能性がある。

PPR パネルはまた、リスク評価に有益な将来の農薬疫学研究を改善するための多くの再修正(ToR 2)と勧告(ToR 3)も提供している。疫学研究の質と妥当性は、以下によって高められる。(a) ばく露の適切な評価、好ましくは個人のば く露モニタリングや特定の農薬(または農薬の組み合わせ)のバイオマーカー濃度を個人レベルで使用し、ばく露の誤 分類を最小化し、用量反応評価を可能にする方法で報告すること、(b) 十分に有効で信頼性の高い健康影響(アウトカ ム)の評価(十分に定義された臨床データまたは有効な代替物)、(c) 交絡変数(健康影響(アウトカム)に影響を与える 他の既知のばく露を含む)を適切に考慮すること、(d) サブグループ解析(例:性別、年齢などによる層別解析)を実施 し、報告すること、環境疫学の研究のために特別に開発された多くの報告ガイドラインとチェックリストは、農薬ばく露を 評価する疫学研究にとっても有用なものである。これは、修正された STROBE(STrengthening the Reporting of OBservational studies in Epidemiology)基準の拡張版が特に該当し、観察による研究の正確で完全な報告書に 何を含めるべきかについての推奨事項が含まれている。

ばく露評価は、個人レベル(バイオモニタリングのような他の直接的な手段で補足することができる信頼性の高い線 量計を目的とする農薬に使用することにより、特定の農薬に対する直接かつ詳細なばく露評価を行う)で改善すること ができる。さらに、登録されたデータを電子カルテにリンクさせることにより集団レベルでのばく露を評価することができ る。これにより、これまでにないサンプルサイズの研究が可能となり、ばく露とその後の疾患に関する情報を得ることがで きるようになる。地理情報システム(GIS)や小規模地域調査も、住居ばく露の推定値を提供するための追加的な方法と して役立つかもしれない。これらのより一般的なばく露評価は、一般的なリスク因子を特定する可能性があり、規制政策 全体への情報提供と、さらなる疫学研究の対象を特定することの両方で重要になる可能性がある。オミクス技術の開発 はまた、生物学的マトリックス中の外来物質や代謝物(メタボロミクス)から DNA やタンパク質との複合体(アダクトミクス) まで、幅広い分子の測定を通じてばく露評価を改善するための興味深い可能性を提示している。オミクスは、複雑な化 学物質の混合物への累積ばく露に対する生物学的反応の特性やシグネチャーを測定する可能性があり、生物学的経 路の理解を深めることができる。つまり、ばく露レベルに関連して、健康障害に関連する生化学的、生理学的、またはそ の他の変化を定量化できるバイオマーカーを使用することで、健康影響を再発見することができ、また、病気の発生の メカニズムを理解するのに役立つ。

規制リスク評価(ToR 4)に疫学的研究を組み込むことは、科学者、リスク評価者、リスク管理者にとって大きな課題で ある。様々な疫学研究の知見は、潜在的な健康被害と有害な健康影響との関連性を評価するために使用することがで き、その結果、リスク評価のプロセスに貢献することができる。農薬ばく露ととト健康影響との関連性に関する利用可能 なデータが大量にあるが、それにもかかわらず、規制上のリスク評価へのこのような研究の影響はまだ限られている。ヒ トのデータはリスク評価の多くの段階で利用できるが、同じ農薬有効成分に関する他の研究がない場合には、単一の (反複されていない)疫学研究は、質が高く、規則(EU)No 1107/2009 に記載されている「認可基準」を満たしていな い限り、ハザードの特性評価に利用すべきではない。これらの「認可基準」は同規則には詳述されていないため、農薬 の規制評価を支援するための疫学的研究の最適な計画と報告のために、多くの勧告が考慮されるべきである。さらなる 特定のガイダンスが有用であるが、これは本意見書の ToR の範囲を超えている。システマティックレビューやメタアナリ シス(必要に応じて)などのエビデンス統合技術が有用なアプローチを提供する。これらのツールは、選択基準を満た すすべての個々の研究の結果を組み合わせることで、要約データを生成し、統計検出力を高め、リスク推定の精度を 向上させることができるが、個々の研究の方法論的な欠陥やバイアスを克服することはできない。観察による研究のシ ステマティックレビューやメタアナリシスは、これらのツールが農薬の潜在的なハザード、ばく露シナリオ、ばく露評価の 方法、ばく露一反応特性、リスク特性に関する理解を強化する情報を提供するため、リスク評価に大きな影響を与える 能力を持っている。システマティックレビューもまた、毒性学的な課題に答えるための潜在的なツールと考えられている が、その方法論は、異なるエビデンスの系統に合わせて対応させる必要がある。

研究の評価はベストエビデンス統合の枠組みの中で行われるべきであり、それによって各特定の研究が持つ可能性のあるバイアスの特性と疫学的データベースの全体的な整合性の評価が示される。本意見書は、単一の疫学研究で www.efsa.europa.eu/efsajourna EFSA Journal 2017:15(10):5007

are also considered a potential tool for answering toxicological questions, their methodology would need to be adapted to the different lines of evidence.

Study evaluation should be performed within a best evidence synthesis framework as it provides an indication on the nature of the potential biases each specific study may have and an assessment of overall confidence in the epidemiological database. This Opinion reports the study quality parameters to be evaluated in single epidemiological studies and the associated weight (low, medium and high) for each parameter. Three basic categories are proposed as a first tier to organise human data with respect to risk of bias and quality; (a) low risk of bias and high/medium reliability; (b) medium risk of bias and medium reliability; (c) high risk of bias and low reliability because of serious methodological limitations or flaws that reduce the validity of results or make them largely uninterpretable for a potential causal association. These categories are intended to parallel the reliability and relevance rating of each stream of evidence according to the EFSA peer review of active substances: acceptable, supplementary and unacceptable. Risk assessment should not be based on results of epidemiological studies that do not meet well-defined data quality standards in order to meet the 'recognised standards' mentioned in the Regulation (EU) No 1107/2009.

Epidemiological studies provide complementary data that can be integrated together with data from *in vivo* laboratory animal studies, mechanistic *in vitro* models and ultimately *in silico* technology for pesticide risk assessment (ToR 4). The combination of all these lines of evidence can contribute to a Weight-of-Evidence (WoE) analysis in the characterisation of human health risks with the aim of improving decision-making. Although the different sets of data can be complementary and confirmatory, and thus serve to strengthen the confidence of one line of evidence on another, they may individually be insufficient and pose challenges for characterising properly human health risks. Hence, all four lines of evidence (epidemiology, animal, *in vitro*, *in silico*) make a powerful combination, particularly for chronic health effects of pesticides, which may take decades to be clinically manifested in an exposed human population.

The first consideration is how well the health outcome under consideration is covered by existing toxicological and epidemiological studies on pesticides. When both types of studies are available for a given outcome/endpoint, both should be assessed for strengths and weaknesses before being used for risk assessment. Once the reliability of available human evidence (observational epidemiology and vigilance data), experimental evidence (animal and *in vitro* data) and non-testing data (*in silico* studies) has been evaluated, the next step involves weighting these sources of data. This opinion proposed an integrated approach where all lines of evidence are considered in an overall WoE framework to better support the risk assessment. This framework relies on a number of principles highlighting when one line should take precedence over another. The concordance or discordance between human and experimental data should be assessed in order to determine which data set should be given precedence. Although the totality of evidence should be assessed, the more reliable data should be given more weight, regardless of whether the data comes from human or experimental studies. The more challenging situation is when study results are not concordant. In such cases, the reasons for the difference should be considered and efforts should be made to develop a better understanding of the biological basis for the contradiction.

Human data on pesticides can help verify the validity of estimations made based on extrapolation from the full toxicological database regarding target organs, dose–response relationships and the reversibility of toxic effects, and to provide reassurance on the extrapolation process without direct effects on the definition of reference values. Thus, pesticide epidemiological data can form part of the overall WoE of available data using modified Bradford Hill criteria as an organisational tool to increase the likelihood of an underlying causal relationship.

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#### Epidemiological studies and pesticides

評価すべき研究の質のパラメータと、各パラメータの関連する程度(低、中、高)を報告している。ヒトのデータをパイア スのリスクと品質に関して整理するための第一段階として、3 つの基本的なカテゴリーが提案されている。(a)パイアスの リスクが低く、信頼性が高い/中程度、(b)パイアスのリスクが中程度で、信頼性が中程度、(c)パイアスのリスクが高く、 信頼性が低いのは、結果の妥当性を低下させたり、潜在的な因果関係をほとんど解釈できないような重大な方法論的 限界や欠陥があるためである。これらのカテゴリーは、EFSAの有効成分のビアレビューに基づく各エビデンスの信頼 性と妥当性の評価(受容可能、補足的、許容できない)と並行して行うことを意図している。規則(EU)No 1107/2009 ヒ トの健康リスクを適切に記載されている「認可基準」を満たすために、明確なデータ品質基準を満たさない疫学研究の 結果に基づいてリスク評価を行うべきではない。

疫学研究は補完的なデータを提供するものであり、農薬リスク評価のために in vivo の実験動物試験、in vitro のメ カニズムモデル及び in silico 技術から得られるデータと統合することができる(ToR 4)。これらすべてのエビデンスを 組み合わせることで、判断の改善を目的としたヒトの健康リスクの特性評価におけるエビデンスの重み付け(WOE, Weight-of-Evidence)解析に貢献することができる。異なるデータセットは補完的であり、結論を出すことができ、その 結果、1 つのエビデンス系統の別のエビデンス系統との整合性を強化するのに役立つが、それらは個別には不十分で あり、ヒトの健康リスクを適切に特性評価するにあたっての課題となる可能性がある。したがって特にばく露されたヒト集 団で臨床的に発現するまでに数十年かかる可能性がある農薬の慢性的な健康影響については、4 つのエビデンス(疫 学、動物実験、in vitro、in silico)は強力な組み合わせとなる。

最初に検討すべき事項は、対象となる健康影響が、農薬に関する既存の毒性学的・疫学的研究でどれだけカバー されているかということである。既知の健康影響/エンドポイントについて両方のタイプの研究が利用可能な場合、リス ク評価に使用する前に、両方の研究の長所と短所を評価すべきである。利用可能な比のエビデンス(観察疫学及び監 視データ)、実験的エビデンス(動物及び in vitro のデータ)、非試験データ(in silico 研究)の信頼性が評価されたら、 次のステップでは、これらのデータソースに重み付けを行う必要がある。この意見書では、リスク評価をより適切にサポ ートするために、すべてのエビデンスを全体的な WOE フレームワークの中で考慮する統合的なアプローチを提案して いる。このフレームワークは、ある系統が他の系統よりも優先されるべき時を強調するいくつかの原則に基づいている。 どのデータセットを優先すべきかを決定するために、ヒトのデータと実験データの一致や不一致を評価すべきである。 エビデンスの全体性を評価すべきであるが、データがヒトと実験のどちらから来たものであるかに関わらず、より信頼性 の高いデータがより重要視されるべきである。より困難な状況は、研究結果が一致しない場合である。このような場合に は、相違の理由を検討し、矛盾の生物学的根拠の理解をより深く理解する努力をすべきである。

農薬に関するとトのデータは、標的臓器、用量反応関係、毒性影響の可逆性に関する完全な毒性学的データベー スからの外挿に基づいて行われた推定値の妥当性を検証するのに役立ち、基準値の定義に直接的な影響を与えるこ となく、外挿の過程を再確認するのに役立つ。このように、農薬疫学的データは、根本的な因果関係の可能性を高める ための組織的なツールとして、改訂 Bradford Hill 基準を使用して、利用可能なデータの全体である WOE の一部を 形成することができる。

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# 1. Introduction

# 1.1. Regulatory data requirements regarding human health in pesticide risk assessment

Regulatory authorities in developed countries conduct a formal human risk assessment for each registered pesticide based on mandated toxicological studies, done according to specific study protocols, and estimates of likely human exposure.

In the European Union (EU), the procedure for the placing of plant protection products (PPP) on the market is laid down by Commission Regulation No 1107/2009<sup>2</sup>. Commission Regulations No 283/2013<sup>3</sup> and 284/2013<sup>4</sup> set the data requirements for the evaluation and re-evaluation of active substances and their formulations.

The data requirements regarding mammalian toxicity of the active substance are described in part A of Commission Regulation (EU) No 283/2013 for chemical active substances and in part B for microorganisms including viruses. With regard to the requirements for pesticide active substances, reference to the use of human data may be found in different chapters of Section 5 related to different end-points. For instance, data on toxicokinetics and metabolism that include *in vitro* metabolism studies on human material (microsomes or intact cell systems) belong to Chapter 5.1 that deals with studies of absorption, distribution, metabolism and excretion in mammals; *in vitro* genotoxicity studies performed on human material are described in Chapter 5.4 on genotoxicity testing and specific studies such as acetylcholinesterase inhibition in human volunteers are found in Chapter 5.7 on neurotoxicity studies. Chapter 5.8 refers to supplementary studies on the active substance, and some specific studies, such as pharmacological or immunological investigations.

Although the process of pesticide evaluation is mainly based on experimental studies, human data could add relevant information to that process. The requirements relating to human data are mainly found in Chapter 5.9 'Medical data' of Regulation (EU) No 283/2013. It includes medical reports following accidental, occupational exposure or incidents of intentional self-poisoning as well as monitoring studies such as on surveillance of manufacturing plant personnel and others. The information may be generated and reported through official reports from national poison control centres as well as epidemiological studies published in the open literature. The Regulation requires that 'relevant' information on the effects of human exposure, where available, shall be used to confirm the validity of extrapolations regarding exposure and conclusions with respect to target organs, dose-response relationships, and the reversibility of adverse effects.

Regulation (EU) No 1107/2009 equally states that, 'where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards, epidemiological studies are of particular value and must be submitted'. However, it is clear that there is no obligation for the petitioners to conduct epidemiological studies specific for the active substance undergoing the approval or renewal process. Rather, according to Regulation (EC) No 1107/2009, applicants submitting dossiers for approval of active substances shall provide 'scientific peer-reviewed public available literature [...]. This should be on the active substance and its relevant metabolites dealing with side-effects on health [...] and published within the last ten years before the date of submission of the dossier'.

In particular, epidemiological studies on pesticides should be retrieved from the literature according to the EFSA Guidance entitled 'Submission of scientific-peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009' (EFSA, 2011a), which follows the principles of the Guidance 'Application of systematic review methodology to food and feed safety assessments to support decision-making' (EFSA, 2010a). As indicated in the EFSA Guidance, 'the process of identifying and selecting scientific peer-reviewed open literature for active substances, their metabolites, or plant protection products' is based on a literature review which is systematic in the approach.

The submission of epidemiological studies and more generally of human data by the applicants in Europe has especially previously sometimes been incomplete and/or has not been performed in

<sup>3</sup> Commission Regulation (EU) No 283/2013, of 1 March 2013, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

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# 1. 序章

# 1.1. 農薬リスク評価におけるとトの健康に関する規制データ要求

先進国の規制当局は、指定された試験プロトコールに基づいて実施される義務づけられた毒性学的研究と、ヒトへ のばく露の可能性の推定値に基づいて、登録された各農薬について正式なヒトのリスク評価を行っている。

欧州連合(EU)では、植物保護剤(農薬)(PPP)の上市手続きは、欧州委員会規則第 1107/2009 号<sup>2</sup>で規定されて いる。欧州委員会規則第 283/2013 号<sup>3</sup>及び第 284/2013 号<sup>4</sup>では、有効成分及びその製剤の評価及び再評価のため のデータ要求が定められている。

有効成分の哺乳類毒性に関するデータ要求は、化学有効成分については欧州委員会規則(EU)No 283/2013 の Part A に、ウイルスを含む微生物については Part B に記載されている。農薬有効成分の要求事項に関しては、ヒトの データ使用に関する言及は、異なるエンドボイントに関連する第5章の異なる章で見られる。例えば、ヒト由来物質(シク ロソームまたは無処置の細胞システム)を対象とした in vitro 代謝試験を含む毒物動態及び代謝に関するデータは、 哺乳類における吸収、分布、代謝及び排泄に関する研究を扱う第5.1章に属し、ヒト由来物質を対象とした in vitro 遺 伝毒性試験は、遺伝毒性試験に関する第5.4章に、ヒトのボランティアにおけるアセチルコリンエステラーゼ阻害などの 特殊な研究は、神経毒性試験に関する第5.7章に記載されている。5.8章では、有効成分に関する補足的な試験や、 薬理学的、免疫学的な試験などのいくつかの特殊な試験について言及している。

農薬の評価プロセスは主に実験研究に基づいているが、ヒトのデータはそのプロセスに関連する情報を追加すること ができる。ヒトのデータに関する要求は、主に規則(EU)No 283/2013 の第 5.9 章「医療データ」にある。これには、偶 発的な職業上のばく露や自傷/自殺の後の医学的報告書や、製造工場の従業員の監視などのモニタリング調査が含 まれる。情報は、国の毒物管理センターからの報告書や、公表文献に掲載されている疫学的研究によって生成され、 報告される。同規則は、ヒトへのばく露の影響に関する「意味のある」情報が入手可能な場合には、ばく露に関する外 挿法の妥当性や、標的臓器、用量反応関係、毒性影響の可逆性に関する結論を導き出すために使用することを要求 している。

規則(EU)No 1107/2009 も同様に、「入手可能で、ばく露レベルとばく露期間に関するデータが裏付けされており、 公認の基準に従って実施されている場合、疫学的研究は特に価値があり、提出しなければならない」としている。しかし、 承認または更新プロセス中の有効成分に特化した疫学的研究を実施する義務が申請者にはないことは明らかである。 むしろ、規則(EC)No 1107/2009 によると、有効成分の承認のための書類(ドシエ)を提出する申請者は、「科学的な ピアレビューを受けた公的に利用可能な文献[.....]」を提出しなければならない。これは、健康への副作用を扱った有 効成分及びその関連代謝物に関するものであり、書類(ドシエ)提出日前の過去 10 年以内に発表されたものでなけれ ばならない[.....]。

特に、農薬に関する疫学的研究は、「規則(EC)No 1107/2009 の下での農薬有効成分の承認のための科学的根拠に基づいた公表文献の提出」(EFSA、2011 年 a)と題する EFSA ガイダンス「政策決定を支援するための食品・飼料安全性評価へのシステマティックレビュー方法論の適用」(EFSA、2010 年 a)の原則に沿って、文献から検索する必要がある。EFSA ガイダンスに示されているように、「有効成分、その代謝物、または植物保護製剤(農薬)のための科学的に査読された公表文献を特定し、選択するプロセス」は、アプローチが体系的な文献レビューに基づいている。

ヨーロッパにおける申請者による疫学研究やより一般的なヒトデータの提出は、特にこれまでに、不完全であったり、 現行の EFSA ガイダンス(EFSA、2011 年 a)に準拠していなかったりすることがあった。これは、特定の EFSA ガイダ ンスに従って(疫学的)文献検索を行うことを義務付けることが比較的最近になって導入(例えば AIR・3 物質に対し)さ

<sup>&</sup>lt;sup>2</sup> Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, OJ L 309, 24.11.2009, p. 1–50.

<sup>&</sup>lt;sup>4</sup> Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ 19 3, 3.4.2013, p. 85–152.

<sup>2</sup> 植物保護製剤(農薬)の上市と理事会指令 79/117/EEC 及び 91/414/EEC の廃止に関する 2009 年 10 月 21 日の欧州議会及び理事会の規則(EC)No 1107/2009。OJ L 309, 24.11.2009, p. 1-50.

<sup>3</sup> 活性物質のデータ要求を定めた 2013 年 3 月 1 日の欧州委員会規則(EU) No 283/2013。

植物保護製剤(農業)の上市に関する欧州議会の規則(EC)No 1107/2009 に基づく。OJL 93, 3.4.2013, p. 1-84. 4 植物保護製剤(農業)のデータ要求を定めた 2013 年 3 月 1 日の欧州委員会規則(EU)No 284/2013.

植物保護製剤(農業)の上市に関する欧洲議会及び理事会の規則(EC)No 1107/2009に基づく。OJL 93, 3.4.2013, p. 85-152. www.efsa.europa.eu/efsajourna EFSA Journal 2017;15(10):5007

compliance with current EFSA Guidance (EFSA, 2011a). This is probably owing to the fact that a mandatory requirement to perform an (epidemiological) literature search according to specific EFSA Guidance is relatively recent, e.g. introduced for AIR-3 substances (Regulation AIR-3: Reg. (EU) No 844/2012; Guidance Document SANCO/2012/11251 – rev.4).

The integration of epidemiological data with toxicological findings in the peer review process of pesticides in the EU should be encouraged but is still lacking. A recent and controversial example is the one related to the evaluation of glyphosate in which significant efforts were made to include epidemiological studies in the risk assessment, but the conclusion was that these studies provided very limited evidence of an association between glyphosate and health outcomes.

In the case of the peer review of 2,4-D, most of epidemiological data were not used in the risk assessment because it was critical to know the impurity profile of the active substance and this information was not available in the publications (as happens frequently in epidemiological studies). In conclusion, within the European regulatory system there is no example of a pesticide active substance approval being influenced by epidemiological data.

Now that a literature search including epidemiological studies is mandatory and guidance is in place (EFSA, 2011a), a more consistent approach can facilitate risk assessment. However, no framework has been established on how to assess such epidemiological information in the regulatory process. In particular, none of the classical criteria used for the evaluation of these studies is included in the current regulatory framework (e.g. study design, use of odd ratios and relative risks, potential confounders, multiple comparisons, assessment of causality). It follows that specific criteria or guidance for the appropriate use of epidemiological findings in the process of writing and peer reviewing Draft Assessment Reports (DARs) or Renewal Assessment Reports (RAR) is warranted. The EFSA Stakeholder Workshop (EFSA, 2015a) anticipated that the availability of more robust and methodologically sound studies presenting accurate information on exposure would bolster the regulation of pesticides in the EU.

Another potential challenge is synchronisation between the process of renewal of active substances and the output of epidemiological studies. Indeed, the planning, conduct, and analysis of epidemiological studies often require a substantial amount of time, especially where interpretation of data is complex.

#### **1.2.** Background and Terms of Reference as provided by the requestor

In 2013, the European Food Safety Authority (EFSA) published an External scientific report 'Literature review on epidemiological studies linking exposure to pesticides and health effects' carried out by the University of Ioannia Medical School (Ntzani et al., 2013). The report is based on a systematic review of epidemiological studies published between 2006 and 2012 and summarises the association between pesticide exposure and any health outcome examined (23 major categories of human health outcomes). In particular, a statistically significant association was observed through fixed and random effect meta-analyses between pesticide exposure and the following health outcomes: liver cancer, breast cancer, stomach cancer, amyotrophic lateral sclerosis, asthma, type II diabetes, childhood leukaemia and Parkinson's disease.

Despite the large number of research articles and analyses (> 6,000) available, the authors of the report could not draw any firm conclusions for the majority of the health outcomes. This observation is in line with previous studies assessing the association between the use of pesticides and the occurrence of human health adverse effects which all acknowledge that such epidemiological studies suffer from a number of limitations and large heterogeneity of data. The authors especially noted that broad pesticides definitions in the epidemiological studies limited the value of the results of meta-analyses. Also, the scope of the report did not allow the in-depth associations between pesticide exposure and specific health outcomes. Nonetheless, the report highlights a number of health outcomes where further research is needed to draw firmer conclusions regarding their possible association with pesticide exposures.

Nevertheless, the outcomes of the External scientific report are in line with other similar studies published in Europe,<sup>5,6</sup> and raise a number of questions and concerns, with regard to pesticide exposure and the associations with human health outcomes. Furthermore, the results of the report

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れたことによるものであろう(規則 AIR-3: Reg. (EU) No 844/2012;ガイダンス文書 SANCO/2012/11251-rev.4)。

EU における農薬のピアレビュープロセスにおける疫学的データと毒性学的結果の統合評価は奨励されるべきであ るが、まだ不足している。最近の大きな議論となった例としては、グリホサートの評価に関連したものがあり、リスク評価 に疫学的研究を含めるために多大な努力がなされたが、結論としては、これらの研究はグリホサートと健康影響との間 の関連性を示す非常に限定的なエビデンスを提供したに過ぎず、十分なエビデンスは得られなかった。

2,4-D のピアレビューの場合、疫学的データのほとんどはリスク評価には使用されなかった。結論として、欧州の規制 システムの中では、疫学的データが農薬有効成分の承認に影響を与えた例はない。

疫学研究を含む文献検索が義務化され、ガイダンスが整備された現在(EFSA、2011 年 a)、より一貫したアプロー チにより、リスク評価が容易になると考えられる。しかし、規制プロセスにおいて、このような疫学的情報をどのように評価 するかについての枠組みは確立されていない。特に、これらの研究の評価に用いられる古典的な基準は、現在の規制 の枠組みには含まれていない(例:研究デザイン、オッズ比と相対リスクの使用、潜在的な交絡因子、多重比較、因果 関係の評価)。評価報告書草案(DAR)や更新評価報告書(RAR)の作成とピアレビューの過程で、疫学的知見を適 切に使用するための特定の基準や指針が必要である。EFSA ステークホルダーワークショップ(EFSA、2015 年 a)で は、ばく露に関する正確な情報を提供する上で、より強固で方法論的に健全な研究が利用可能になれば、EU におけ る農薬規制の向上が図れると予想している。

もう一つの潜在的な課題は、有効成分の更新プロセスと疫学研究の成果との同期化である。実際、疫学研究の計画、 実施、解析には多くの場合、特にデータの解釈が複雑な場合には相当な時間を必要とする。

# 1.2. 依頼者から提供された背景と委託条件

2013 年、欧州食品安全機関(EFSA)は、イオアニナ大学医学部が実施した外部科学報告書「農薬へのばく露と健 康影響に関連する疫学的研究に関する文献レビュー」を発表した(Ntzani 6、2013 年)。この報告書は、2006 年から 2012 年の間に発表された疫学研究のシステマティックレビューに基づき、農薬ばく露と調査したあらゆる健康影響(ヒト の健康影響の 23 の主要カテゴリー)との関連性をまとめたものである。特に、農薬ばく露と以下の健康影響(肝臓がん、 乳がん、胃がん、筋萎縮性側索硬化症、喘息、II 型糖尿病、小児白血病、パーキンソン病)との間の統計学的に有意 な関連性が、固定効果及びランダム効果のメタアナリシスによって観察された。

膨大な数の研究論文と解析(6,000 件以上)が利用可能であるにもかかわらず、報告書の著者は、大部分の健康影響については何ら確かな結論を見出すことができなかった。この観察結果は、農薬の使用ととト健康への悪影響の発生との関連性を評価したこれまでの研究と一致しており、そのような疫学研究は多くの限界とデータの大きな不均一性が問題となっていることを認めている。著者らは特に、疫学研究における農薬の広範な定義がメタアナリシスの結果の価値を制限していることを指摘している。また、本報告書の範囲では、農薬ばく露と特定の健康影響との間の詳細な関連付けを行うことができなかった。しかし、報告書では、農薬ばく露との関連性の可能性について、より詳細な結論を出すためにさらなる研究が必要とされる多くの健康影響を強調している。

とはいえ、外部科学報告書の結果は、ヨーロッパで発表された他の同様の研究5・6と一致しており、農薬ばく露ととト 健康影響との関連性について、多くの疑問や懸念を投げかけている。さらに、本報告書の結果は、疫学研究の結果を どのように農薬リスク評価に統合するかについての議論の道を開くものである。このことは、EU 規則 No 283/2013 に 従って疫学的結果を評価する必要がある植物保護製剤(農薬)の承認評価を扱う EFSA のピアレビューチームにとっ て特に重要である。同規則では、申請者は、利用可能な場合には「意味のある」疫学的研究を提出しなければならない とされている。

この科学的意見書では、PPRパネルは、外部科学報告書(Ntzaniら、2013年)で観察された農薬ばく露ととト健康

<sup>&</sup>lt;sup>5</sup> France: INSERM report 2013: Pesticides – effets sur la santé.

<sup>&</sup>lt;sup>6</sup> UK: COT report 2011: Statement on a systematic review of the epidemiological literature on para-occupational exposure to pesticides and health outcomes other than cancer, and COT report 2006: Joint Statement on Royal Commission on Environmental Pollution report on crop spraying and the health of residents and bystanders.

<sup>5</sup> フランス。INSERM レポート 2013。農薬-人に及ぼす影響

<sup>6</sup> 英国。COTレポート2011年。農薬への准職業上ばく露とがん以外の健康影響に関する疫学的文献のシステマティックレビューに関する声明及びCOT報告書2006。農薬散布と住民や居合わせただけの者(bystanders)の健康に関する環境汚染に関する王立委員会報告書に関する共同声明。

open the way for discussion on how to integrate results from epidemiological studies into pesticide risk assessments. This is particularly important for the peer-review team at EFSA dealing with the evaluation of approval of plant protection products for which the peer-review needs to evaluate epidemiological findings according to EU Regulation No 283/2013. The regulation states that applicants must submit 'relevant' epidemiological studies, where available.

For the Scientific Opinion, the PPR Panel will discuss the associations between pesticide exposure and human health effects observed in the External scientific report (Ntzani et al., 2013) and how these findings could be interpreted in a regulatory pesticide risk assessment context. Hence, the PPR Panel will systematically assess the epidemiological studies collected in the report by addressing major data gaps and limitations of the studies and provide related recommendations.

The PPR Panel will specifically:

- collect and review all sources of gaps and limitations, based on (but not necessarily limited to) those identified in the External scientific report in regard to the quality and relevance of the available epidemiological studies.
- 2) based on the gaps and limitations identified in point 1, propose potential refinements for future epidemiological studies to increase the quality, relevance and reliability of the findings and how they may impact pesticide risk assessment. This may include study design, exposure assessment, data quality and access, diagnostic classification of health outcomes, and statistical analysis.
- 3) identify areas in which information and/or criteria are insufficient or lacking and propose recommendations for how to conduct pesticide epidemiological studies in order to improve and optimise the application in risk assessment. These recommendations should include harmonisation of exposure assessment (including use of biomonitoring data), vulnerable population subgroups and/or health outcomes of interest (at biochemical, functional, morphological and clinical level) based on the gaps and limitations identified in point 1.
- 4) discuss how to make appropriate use of epidemiological findings in risk assessment of pesticides during the peer review process of draft assessment reports, e.g. weight-ofevidence (WoE) as well as integrating the epidemiological information with data from experimental toxicology, adverse outcome pathways (AOP), mechanism of actions, etc.

The PRAS Unit will consult the Scientific Committee on the consensual approach to EFSA's overarching scientific areas,<sup>7</sup> including the integration of epidemiological studies in risk assessment.

#### **1.3.** Interpretation of the Terms of Reference

In the Terms of Reference (ToR), EFSA requested the PPR Panel to write a scientific Opinion on the follow up of the results from the External Scientific Report on a systematic review of epidemiological studies published between 2006 and 2012 linking exposure to pesticides and human health effects (Ntzani et al., 2013). According to EU Regulation No 283/2013, the integration of epidemiological data into pesticide risk assessment is important for the peer review process of DAR and RAR of active substances for EU approval and their intended use as plant protection products.

In its interpretation of the terms of reference, the PPR Panel will then develop a Scientific Opinion to address the methodological limitations identified in epidemiological studies on pesticides and to make recommendations to the sponsors of such studies on how to improve them in order to facilitate their use for regulatory pesticide risk assessment, particularly for substances in the post-approval period. The PPR Panel notes that experimental toxicology studies also present limitations related to their methodology and quality of reporting; however, the assessment of these limitations is beyond the ToR of this Opinion.

This Scientific Opinion is intended to assist the peer review process during the renewal of pesticides under Regulation 1107/2009 where the evaluation of epidemiological studies, along with clinical cases and poisoning incidents following any kind of human exposure, if available, represent a data requirement. Epidemiological data concerning exposures to pesticides in Europe will not be available before first approval of an active substance (with the exception of incidents produced during the manufacturing process, which are expected to be very unlikely) and so will not be expected to contribute to a DAR. However, there is the possibility that earlier prior approval has been granted for use of an active substance in another jurisdiction and epidemiological data from that area may be considered relevant. Regulation (EC) No 1107/2009 requires a search of the scientific peer-reviewed

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影響との関連性と、これらの結果が規制上の農薬リスク評価の背景でどのように解釈されるかを議論する。したがって、 PPR パネルは、報告書で収集された疫学研究を体系的に評価し、研究の主要なデータギャップと限界に対処し、関連 する勧告を提言する。

PPR パネルは特に以下を行う。

- 1)利用可能な疫学研究の質と妥当性に関して外部科学報告書で明らかにされたものに基づいて(必ずしもこれに 限定されないが)、ギャップと限界のすべての情報源を収集し、レビューする。
- 2)上記 1)項で特定されたギャップと限界に基づき、調査結果の質、妥当性、信頼性を向上させ、それが農薬リス ク評価にどのように影響を与えるかについて、将来の疫学調査のための潜在的な改善点を提案する。これには、 研究デザイン、ばく露評価、データの質と評価、健康影響の診断分類、統計解析が含まれる。
- 3)情報及び/または基準が不十分または不足している分野を特定し、リスク評価への適用を改善し最適化するために、農薬疫学的研究をどのように実施するかについての提言を行う。これらの推奨事項には、第1)項で明らかになったギャップと限界に基づいて、ばく露評価(バイオモニタリングデータの利用を含む)、脆弱な集団のサブグループ及び/または対象となる健康影響(生化学的、機能的、形態学的、臨床的レベルでの)の調和を含む。
- 4)評価報告書草案のピアレビューの過程で、疫学的知見を実験毒性学、有害転帰経路(AOP)、作用機序などのデータと統合するとともに、WOEなど、農薬のリスク評価に疫学的知見を適切に利用する方法を議論する。

PRAS ユニットは、リスク評価における疫学的研究の統合を含む EFSA の包括的な科学的分野<sup>7</sup>への合意に基づく アプローチについて、科学技術委員会に諮る。

# 1.3. 委託条件の解釈

EFSAは、検討事項(ToR)の中で、2006年から2012年の間に発表された農薬へのばく露ととトの健康影響を関連 付ける疫学的研究のシステマティックレビューの結果のフォローアップについて、PPR パネルに科学的意見書を作成 するよう要請している(Ntzani 6、2013年)。EU 規則 No 283/2013によると、疫学的データを農薬リスク評価に統合 することは、EU 承認のための有効成分の DAR と RAR 及び植物保護製剤(農薬)としての使用を目的とした有効成 分のピアレビュープロセスにとって重要であるとされている。

PPR パネルは、委託条件の解釈において、農薬の疫学的研究で明らかになった方法論的限界に対処し、規制上の 農薬リスク評価、特に承認後の物質のリスク評価への利用を容易にするためにどのように改善するかについて、そのような研究のスポンサーに勧告を行うための科学的意見書を作成することになっている。PPR パネルは、実験的な毒性 試験にもその方法論と報告の質に関連した限界があることに留意しているが、これらの限界の評価は本意見書の ToR の範囲を超えている。

この科学的意見書は、規制 1107/2009 に基づく農薬の更新時のピアレビュープロセスを支援することを目的として おり、疫学的研究の評価に加えて、なんらかのヒトばく露後の臨床症例や中毒事例(入手可能な場合)がデータ要求と なっている。欧州における農薬へのばく露に関する疫学的データは、有効成分の最初の承認前には入手できない(製 造過程で発生した事故を除いて、その可能性は非常に低いと予想される)ため、DAR に貢献することは期待できない だろう。しかし、他の管轄で有効成分の使用について先行承認を受けている可能性があり、その分野の疫学的データ が有用であると考えられる。EC 規則: (EC) No 1107/2009 では、既存の疫学的研究が有効成分の更新プロセスにおい てより適していることが認識されており、「更新のために提出された書類には、有効成分が指令 91/414/EEC の付録 I に最初に含まれた時から、データ要求の変更や科学的・技術的知識の変化を再確認するために、有効成分に関連す る新しいデータと新しいリスク評価を含めるべきである」という EC 規則 1141/2010 の規定にも準拠している。 PPR パネルは具体的に以下のトピックに取り組む。

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<sup>&</sup>lt;sup>7</sup> According to article 28 of Regulation (EC) No 178/2002.

<sup>&</sup>lt;sup>7</sup> 規則(EC)No 178/2002の第28条による。

open literature, where it is expected to retrieve existing epidemiological studies. It is therefore recognised that epidemiological studies are more suitable for the renewal process of active substances, also in compliance with the provision of the EC regulation 1141/2010 indicating that 'The dossiers submitted for renewal should include new data relevant to the active substance and new risk assessments to reflect any changes in data requirements and any changes in scientific or technical knowledge since the active substance was first included in Annex I to Directive 91/414/EEC'.

The PPR Panel will specifically address the following topics:

- Review inherent weaknesses affecting the quality of epidemiological studies (including gaps and limitations of the available pesticide epidemiological studies) and their relevance in the context of regulatory pesticide risk assessment. How can these weaknesses be addressed?
- 2) What are potential contributions of epidemiological studies that complement classical toxicological studies conducted in laboratory animal species in the area of pesticide risk assessment?
- 3) Discuss and propose a methodological approach specific for pesticide active substances on how to make appropriate use of epidemiological studies, focusing on how to improve the gaps and limitations identified.
- 4) Propose refinements to practice and recommendations for better use of the available epidemiological evidence for risk assessment purposes. Discuss and propose a methodology for the integration of epidemiological information with data from experimental toxicology.

This Scientific Opinion, particularly Section 2–4, is not intended to address the bases of epidemiology as a science. Those readers willing to deepen into specific aspects of this science are encouraged to read general textbook of epidemiology (e.g. Rothman et al., 2008).

It should be taken into account that this Opinion is focussed only on pesticide epidemiology studies in the EU regulatory context and not from a general scientific perspective. Therefore, the actual limitations and weaknesses of experimental toxicology studies will not be addressed herein.

#### **1.4.** Additional information

In order to fully address topics 1–4 above (Section 1.3), attention has been paid to a number of relevant reviews of epidemiological studies and the experience of other National and International bodies with knowledge of epidemiology in general and in applying epidemiology to pesticide risk assessment specifically. Detailed attention has been given to these studies in Annex A and drawn from the experience of the authors that have constructively to understanding in this area. Also Annex A records published information that has been criticised for its lack of rigour showing how unhelpful some published studies may be. The lessons learned from such good (and less-good) practice have been incorporated into the main text by cross-referring to Annex A. In this way, this Scientific Opinion has the aim of clearly distilling and effectively communicating the arguments in the main text without overwhelming the reader with all the supporting data which is nevertheless accessible.

In addition, Annex B contains a summary of the main findings of a project that EFSA outsourced in 2015 to further investigate the role of human biological monitoring (HBM) in occupational health and safety strategies as a tool for refined exposure assessment in epidemiological studies and to contribute to the evaluation of potential health risks from occupational exposure to pesticides (Bevan et al., 2017).

#### 2. General framework of epidemiological studies on pesticides

This section introduces the basic elements of epidemiological studies on pesticides and contrasts them with other types of studies. For more details general textbook on epidemiology are recommended (Rothman et al., 2008; Thomas, 2009).

#### 2.1. Study design

Epidemiology studies the distribution and determinants of health outcomes in human or other target species populations, to ascertain how, when and where diseases occur. This can be done through observational studies and intervention studies (i.e. clinical trials),<sup>8</sup> which compare study

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- 1)疫学研究の質に影響を与える固有の弱点(利用可能な農薬疫学研究のギャップと限界を含む)と、規制上の農薬リスク評価との関連性を検討する。これらの弱点にはどのように対処できるか?
- 2)実験動物を用いた古典的な毒性学的研究を補完する疫学的研究は、農薬リスク評価の分野でどのような貢献 が期待できるか?
- 3) 農薬有効成分に特化した方法論的アプローチとして、疫学的研究をどのように適切に活用するかについて、指摘されたギャップや限界をどのように改善するかを中心に議論し、提案する。
- 4)リスク評価の目的で利用可能な疫学的証拠をより良く利用するための実践への再提案と推奨を提案する。疫学 的情報と実験毒性学のデータを統合するための方法論を議論し、提案する。

本意見書、特にセクション 2・4 は、科学としての疫学の基礎を論じることを意図したものではない。疫学の科学的側面を深めたいと考えている読者には、疫学の一般的な教科書(例:Rothman ら、2008 年)を読むことを勧める。

本意見書は、EU 規制の背景における農薬疫学研究にのみ焦点を当てており、一般的な科学的観点からではない ことを考慮に入れるべきである。したがって、実験的な毒性試験の実際の限界と弱点については、ここでは触れていない。

#### 1.4. 追加情報

上記のトピック 1・4(第 1.3 節)に完全に対応するために、疫学的研究の多くの関連するレビュー及び疫学の知識を 持つ他の国内外の機関の経験に注意を払い、疫学を農薬のリスク評価に特に適用した。付録 A ではこれらの研究に 詳細な注意を払い、この分野の理解に建設的に貢献してきた著者の経験に基づいている。また、付録 A では、いくつ かの公表された研究がどれほど役に立たないかを示すために、厳密さに欠けていると批判された公表情報を記録して いる。このような優れた(そしてあまり良くない)実践から得られた教訓は、附属書 A を相互に参照することで本文に組 み込まれている。このようにして、この科学的意見書(Scientific Opinion)は、それにもかかわらずアクセス可能なすべ ての裏付けとなるデータで読者を圧倒することなく、本文の議論を明確に抽出し、効果的に伝えることを目的としている。

さらに、付属書 B には、疫学研究におけるばく露評価のためのツールとしての労働安全衛生戦略におけるヒト生物 学的モニタリング(HBM)の役割をさらに調査し、農薬への職業上ばく露による潜在的な健康リスクの評価に貢献する ために、2015年に EFSA が委託したプロジェクトの主な成果の要約が含まれている(Bevan 6、2017年)。

# 2. 農薬に関する疫学研究の一般的枠組み

ここでは、農薬に関する疫学研究の基本的な要素を紹介し、他のタイプの研究との対比を行う。詳細については、疫 学の一般的な教科書を勧める(Rothman ら、2009 年)。

# 2.1. 研究デザイン

疫学は、いつ、どこで、どのようにして疾患が発生したかを確認するために、ヒトまたは他の標的種の集団における健 康影響の分布と決定要因を研究する。これは観察による研究や介入研究(すなわち臨床試験)®によって行うことができ、 潜在的なリスク因子へのばく露が異なる研究グループを比較する。どちらのタイプの研究も、実験室よりも管理の行き届 いていない自然環境で実施される。

<sup>&</sup>lt;sup>8</sup> In this opinion, 'human data' includes observational studies, also called epidemiological studies, where the researcher is observing natural relationships between factors and health outcomes without acting upon study participants. Vigilance data also fall under this concept. In contrast, intervention studies (also referred to as experimental studies) are outside the scope of this Opinion, and their main feature is that the researcher intercedes as part of the study design.

<sup>8</sup> この見解では、「ヒトデータ」には、皮学研究とも呼ばれる観察研究が含まれ、研究者は研究参加者に影響を与えることなく、因子と健康影響との 間の自然な関係を観察している。警板データやまた、この概念に該当する。これに対して、介入研究(実験研究ともいう)は本意見の対象外であ り、研究者が研究デザインの一部として介入することが大きな特徴である。

groups subject to differing exposure to a potential risk factor. Both types of studies are carried out in a natural setting, which is a less controlled environment than laboratories.

Information on cases of disease occurring in a natural setting can also be systematically recorded in the form of case reports or case series of exposed individuals only. Although case series/reports do not compare study groups according to differing exposure, they may provide useful information, particularly on acute effects following high exposures, which makes them potentially relevant for hazard identification.

In randomised clinical trials, the exposure of interest is randomly allocated to subjects and, whenever possible, these subjects are blinded to their treatment, thereby eliminating potential bias due to their knowledge about their exposure to a particular treatment. This is why they are called intervention studies. Observational epidemiological studies differ from clinical intervention studies in that the exposure of interest is not randomly assigned to the subjects enrolled and participants are often not blinded to their exposure. This is why they are called observational. As a result, randomised clinical trials rank higher in terms of design as they provide unbiased estimates of average treatment effects.

The lack of random assignment of exposure in observational studies represents a key challenge, as other risk factors that are associated with the occurrence of disease may be unevenly distributed between those exposed and non-exposed. This means that known confounders need to be measured and accounted for. However, there is always the possibility that unknown or unmeasured confounders are left unaccounted for, although unknown confounders cannot be addressed. Furthermore, the fact that study participants are often unaware of their current or past exposure or may not recall these accurately in observational studies (e.g. second-hand smoke, dietary intake or occupational hazards) may result in biased estimates of exposure if it is based on self-report. As an example, it is not unlikely that when cancer cases and controls are asked whether they have previously been exposed to a pesticide the cancer cases may report their exposure differently from controls, even in cases where the past exposures did not differ between the two groups.

Traditionally, designs of observational epidemiological studies are classified as either ecological, cross-sectional, case-control or cohort studies. This approach is based on the quality of exposure assessment and the ability to assess directionality from exposure to outcome. These differences largely determine the quality of the study (Rothman and Greenland, 1998; Pearce, 2012).

- Ecological studies are observational studies where either exposure, outcome or both are measured on a group but not at individual level and the correlation between the two is then examined. Most often, exposure is measured on a group level while the use of health registries often allows for extraction of health outcomes on an individual level (cancer, mortality). These studies are often used when direct exposure assessment is difficult to achieve and in cases where large contrast in exposures are needed (comparing levels between different countries or occupations). Given the lack of exposure and/or outcome on an individual level, these studies are useful for hypothesis generation but results generally need to be followed up using more rigorous design in either humans or use of experimental animals.
- In cross-sectional studies, exposure and health status are assessed at the same time, and prevalence rates (or incidence over a limited recent time) in groups varying in exposure are compared. In such studies, the temporal relationship between exposure and disease cannot be established since the current exposure may not be the relevant time window that leads to development of the disease. The inclusion of prevalent cases is a major drawback of (most) cross-sectional studies, particularly for chronic long-term diseases. Cross-sectional studies may nevertheless be useful for risk assessment if exposure and effect occur more or less simultaneously or if exposure does not change over time.
- · Case-control studies examine the association between estimates of past exposures among individuals that already have been diagnosed with the outcome of interest (e.g. cases) to a control group of subjects from the same population without such outcome. In populationbased incident case-control studies, cases are obtained from a well-defined population, with controls selected from members of the population who are disease free at the time a case is incident. The advantages of case-control studies are that they require less sample sizes, time and resources compared to prospective studies and often they are the only viable option when studying rare outcomes such as some types of cancer. In case-control studies, past exposure is most often not assessed based on 'direct' measurement but rather through less certain measurements such as a recall captured through interviewer or self-administered

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#### Epidemiological studies and pesticides

自然環境で発生した疾病の事例に関する情報は、ばく露者のみを対象とした症例報告や症例シリーズという形で体 系的に記録することも可能である。症例報告や症例シリーズは、ばく露の違いによって研究グループを比較するもので はないが、有用な情報、特に高濃度ばく露後の急性影響に関する情報を提供することができ、ハザードの特定に役立 つ可能性がある。

無作為化臨床試験では、対象となるばく露が被験者に無作為に割り付けられ、可能な限り被験者は治療法を盲検 化し、それによって特定の治療法へのばく露に関する知識に起因する潜在的なバイアスを排除する。これが介入研究 と呼ばれる理由である。観察による疫学研究は臨床介入研究とは異なり、対象となるばく露が登録された被験者にラン ダムに割り付けられておらず、参加者はばく露について盲検化されていないことが多い。これが観察的研究と呼ばれる 理由である。その結果、無作為化臨床試験は平均的な治療効果のバイアスのない推定値を提供するため、計画の点 で上位にランクされている。

観察による研究におけるばく露の無作為割り付けがないことは、疾患の発生に関連する他のリスク因子がばく露者と 非ばく露者の間で不均等に分布している可能性があるため、重要な課題となる。これは、既知の交絡因子を測定して 説明する必要があることを意味する。しかし、未知の交絡因子は対処できないが、未知の交絡因子または測定されてい ない交絡因子が考慮されずに放置されている可能性が常にある。さらに、観察による研究で研究参加者が現在または 過去のばく露を知らないことが多い、またはこれらを正確に記憶していないことがある(例えば、副流煙、食事摂取量、 または職業上のハザード)という事実は、自己報告に基づいている場合、ばく露の推定値に偏りが生じる可能性がある。 例えば、がん症例と対照者が過去に農薬にばく露されたことがあるかどうかを尋ねられたとき、過去のばく露が両群間 で差がなかった場合でも、がん症例は対照者とは異なるばく露を報告する可能性は低くない。

伝統的に、観察による疫学研究計画は、生態学的研究、横断研究、症例対照研究、コホート研究のいずれかに分 類される。このアプローチは、ばく露評価の質とばく露から結果への方向性を評価する能力に基づいている。これらの 違いは、研究の質を大きく左右する(Rothman 及び Greenland、1998年; Pearce、2012年)。

- 生態学的研究は観察研究であり、ばく露、影響(結果)、またはその両方を個人レベルではなく集団レベルで測 定し、両者の相関関係を調べるものである。多くの場合、ばく露は集団レベルで測定されるが、健康登録を利用 することで、個人レベルでの健康影響(がん、死亡率)を抽出することができる。これらの研究は、直接のばく露 評価が困難な場合や、ばく露量の大きな対照が必要な場合(異なる国や職業間のレベルの比較)によく利用さ れる。個人レベルでのばく露及び/または影響がないことを考えると、これらの研究は仮説を立てるのに有用で あるが、一般的には、ヒトまたは実験動物を用いたより厳密な計画で結果をフォローアップ(追跡調査)する必要 がある。
- 横断研究では、ばく露と健康状態が同時に評価され、ばく露の程度が異なる群における有病率(または最近の 限られた時間における罹患率)が比較される。このような研究では、現在のばく露が疾患の発症につながる関連 時間枠ではないかもしれないので、ばく露と疾患の間の時間的関係は確立できない。有病率の高い症例を含 めることは、(ほとんどの)横断研究の大きな欠点であり、特に慢性的な長期疾患の場合には注意が必要である。 それでも、ばく露と影響が多かれ少なかれ同時に発生している場合や、ばく露が経時的に変化しない場合には、 横断研究はリスク評価に有用であるかもしれない。
- ・症例対照研究では、すでに対象となる疾患(例:症例)と診断されている個人の過去のばく露の推定値と、その ような疾患のない同一集団の対照との間の関連を調べるものである。集団ベースの症例対照研究では、症例は 十分に整備された集団から得られ、対照は症例が発生した時点で病気にかかっていない集団のメンバーから 選ばれる。症例対照研究の利点は、前向き研究に比べてサンプル数、時間、供給源が少なくて済むことであり、 ある種のがんのようなまれな疾患を研究する場合には、症例対照研究が唯一の実行可能な選択肢となることが 多い。症例対照研究では、ほとんどの場合、過去のばく露は「直接的な」測定に基づいて評価されるのではなく、 質問者または自己記入式のアンケートや職務記述書の肩書きや職務歴などの代用手段によって得られた想起 など、より確実性の低い測定を介して評価される。症例対照研究は適切なばく露評価を可能にするかもしれな いが、これらの研究はばく露を推定する際に想起バイアスに陥りやすい。その他の課題としては、適切な対照の EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

questionnaires or proxies such as job descriptions titles or task histories. Although case-control studies may allow for proper exposure assessment, these studies are prone to recall-bias when estimating exposure. Other challenges include the selection of appropriate controls; as well as the need for appropriate confounder control.

· In cohort studies, the population under investigation consists of individuals who are at risk of developing a specific disease or health outcome at some point in the future. At baseline and at later follow-ups (prospective cohort studies) relevant exposures, confounding factors and health outcomes are assessed. After an appropriate follow-up period, the frequency of occurrence of the disease is compared among those differently exposed to the previously assessed risk factor of interest. Cohort studies are therefore by design prospective as the assessment of exposure to the risk factor and covariates of interest are measured before the health outcome has occurred. Thus, they can provide better evidence for causal associations compared to the other designs mentioned above. In some cases, cohort studies may be based on estimates of past exposure. Such retrospective exposure assessment is less precise than direct measure and prone to recall bias. As a result, the quality of evidence from cohort studies varies according to the actual method used to assess exposure and the level of detail by which information on covariates were collected. Cohort studies are particularly useful for the study of relatively common outcomes. If sufficiently powered in terms of size, they can also be used to appropriately address relatively rare exposures and health outcomes. Prospective cohort studies are also essential to study different critical exposure windows. An example of this is longitudinal birth cohorts that follow children at regular intervals until adult age. Cohort studies may require a long observation period when outcomes have a long latency prior to onset of disease. Thus, such studies are both complex and expensive to conduct and are prone to loss of follow-up.

# 2.2. Population and sample size

A key strength of epidemiological studies is that they study diseases in the very population about which conclusions are to be drawn, rather than a proxy species. However, only rarely will it be possible to study the whole population. Instead, a sample will be drawn from the reference population for the purpose of the study. As a result, the observed effect size in the study population may differ from that in the population if the former does not accurately reflect the latter. However, observations made in a non-representative sample may still be valid within that sample but care should then be made when extrapolating findings to the general population.

Having decided how to select individuals for the study, it is also necessary to decide how many participants should minimally be enrolled. The sample size of a study should be large enough to warrant sufficient statistical power. The standard power (also called sensitivity) is 80%, which means the ability of a study to detect an effect of a given magnitude when that effect actually exists in the target population; in other words, there is 80% probability of drawing the right conclusion from the results of the analyses and a corresponding probability of 20% or drawing the wrong conclusion and missing a true effect. Power analysis is often used to calculate the minimum sample size required to likely detect an effect of a given size. Small samples are likely to constitute an unrepresentative sample. The statistical power is also closely related to risk inflation, which needs to be given special attention when interpreting statistically significant results from small or underpowered studies (see Annex D).

Epidemiological studies, like toxicological studies in laboratory animals, are often designed to examine multiple endpoints unlike clinical trials that are designed and conducted to test one single hypothesis, e.g. efficacy of a medical treatment. To put this in context, for laboratory animal toxicology test protocols, OECD guidance for pesticides may prescribe a minimum number of animals to be enrolled in each treatment group. This does not guarantee adequate power for any of the multitude of other endpoints being tested in the same study. It is thus important to appropriately consider the power of a study when conducting both epidemiology and laboratory studies.

# 2.3. Exposure

The quality of the exposure measurements influences the ability of a study to correctly ascertain the causal relationship between the (dose of) exposure and a given adverse health outcome.

In toxicological studies in laboratory animals, the 'treatment regime' i.e. dose, frequency, duration and route are well defined beforehand and its implementation can be verified. This often allows

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選択及び適切な交絡因子管理の必要性が挙げられる。

・コホート研究では、調査対象となる集団は、将来のある時点で特定の疾患や健康影響を発症するリスクがある 個人で構成されている。ベースライン時及びその後の追跡調査(前向きコホート研究)では、関連するばく露、 交絡因子及び健康影響が評価される。適切な追跡期間の後、以前に評価された対象となるリスク因子に異なる ばく露を受けた人々の間で、疾患の発生頻度が比較される。したがって、コホート研究は計画としては前向きで あり、対象となるリスク因子や共変量へのばく露の評価は健康影響が発生する前に測定される。したがって、コ ホート研究は、上記の他の計画と比較して、因果関係のより良いエビデンスを提供することができる。場合によっ ては、コホート研究が過去のばく露の推定値に基づいていることもある。このような回顧的ばく露評価は、直接測 定に比べて精度が低く、想起バイアスがかかりやすい。その結果、コホート研究から得られるエビデンスの質は、 ばく露を評価するために実際に使用された方法や共変量に関する情報が収集された詳細のレベルによって異 なる。コホート研究は、比較的一般的な健康影響に関する研究に特に有用である。規模の点で十分な検出力 があれば、比較的まれなばく露と健康影響に適切に対処するためにも利用できる。前向きコホート研究は、異な る臨界ばく露枠を研究するためにも不可欠である。その例として、成人になるまで一定間隔で子供を追跡する 縦断的出生コホート研究がある。コホート研究では、疾患発症前の潜伏期間が長い場合、長い観察期間を必要 とすることがある。このような研究は、実施するには複雑で費用がかかり、追跡調査の損失が生じやすい。

# 2.2. 母集団とサンプルサイズ

疫学研究の主な強みは、代理種ではなく、結論を出すべき集団の中で病気を研究することである。しかし、全集団を 調査できることは稀であり、その代わりに研究の目的のために参照母集団からサンプルが引き出される。その結果、前 者が後者を正確に反映していない場合、研究母集団で観察された効果量は、母集団で観察された効果量と異なる可 能性がある。しかし、非代表的なサンプルで行われた観察は、そのサンプル内ではまだ有効であるかもしれないが、結 果を一般集団に外挿する際には注意が必要である。

研究のために対象個人をどのように選択するかを決定した後、最小で何人の参加者を登録すべきかを決定すること も必要である。研究のサンプルサイズは、十分な統計的検出力を保証するのに十分な大きさでなければならない。標 準検出力(感度とも呼ばれる)は80%で、これは、ある研究が、その効果が対象集団に実際に存在するときに、ある大き さの効果を検出する能力を意味する。言い換えれば、解析の結果から正しい結論を導き出せる確率は80%で、それに 対応する確率は20%で、間違った結論を導き出して真の効果を見逃してしまう確率である。検出力解析は、与えられた サイズの効果を検出するのに必要な最小サンプルサイズを計算するためによく使用される。小規模サンプルは、非代 表的サンプルを構成する可能性が高い。統計的検出力はリスク・インフレーション(risk inflation)と密接に関係してお り、小規模または検出力不足の研究から統計的に有意な結果を解釈する際には特別な注意を払う必要がある(付属書 Dを参照)。

疫学研究は、実験動物を用いた毒性学的研究と同様に、多くの場合は複数のエンドポイントを調査するように計画さ れているが、臨床試験は単一の仮説、例えば治療の有効性などを検証するために計画され、実施される。この点では、 実験動物の毒性試験プロトコールについては、OECDの農薬に関するガイダンスでは、各投与群に登録する動物の 最小数が規定されているので、同じ研究で試験される他の多数のエンドポイントのいずれに対しても、十分な検出力を 保証することはできない。したがって、疫学研究と実験室研究の両方を実施する際には、研究の検出力を適切に考慮 することが重要である。

# 2.3. ばく露

ばく露測定の質は、ばく露(用量)と特定の毒性影響との間の因果関係を正確に確認する研究の能力に影響を与える。

実験動物を用いた毒性学的試験では、用量、頻度、期間、経路などの「試験実施計画」が事前に十分に定められており、その実施状況を確認することができる。これにより、例えば90日間の研究では、飼料中に存在する化学物質の目

expression of exposure in terms of external dose administered daily via oral route for example in a 90day study, by multiplying the amount of feed ingested every day by a study animal with the intended (and verified) concentration of the chemical present in the feed. Also, in the future, the internal exposure has to be determined in the pivotal studies.

In the case of pesticides, estimating exposure in a human observational setting is difficult as the dose, its frequency and duration over time and the route of exposure are not controlled and not even well known.

Measuring the intensity, frequency and duration of exposure is often necessary for investigating meaningful associations. Exposure may involve a high dose over a relatively short period of time, or a low-level prolonged dose over a period from weeks to years. While the effects of acute, high-dose pesticide exposure may appear within hours or days, the effects of chronic, low-dose exposures may not appear until years later. Also, a disease may require a minimal level of exposure but increase in probability with longer exposure.

There may be differences in absorption and metabolism via different routes (dermal, inhalation and oral). While dermal or inhalation are often the routes exposure occurs in occupational settings, ingestion (food, water) may be the major route of pesticide exposure for the general population. Pharmacokinetic differences among individuals may result in differing systemic or tissue/organ doses even where the absorbed external doses may appear similar.

#### 2.4. Health outcomes

The term health outcome refers to a disease state, event, behaviour or condition associated with health that is under investigation. Health outcomes are those clinical events (usually represented as diagnosis codes, i.e. International Classification of Diseases (ICD) 10) or outcomes (i.e. death) that are the focus of the research. Use of health outcomes requires a well-defined case definition, a system to report and record the cases and a measure to express the frequency of these events.

A well-defined case definition is necessary to ensure that cases are consistently diagnosed, regardless of where, when and by whom they were identified and thus avoid misclassification. A case definition involves a standard set of criteria, which can be a combination of clinical symptoms/signs, sometimes supplemented by confirmatory diagnostic tests with their known sensitivity and specificity. The sensitivity of the whole testing procedure (i.e. the probability that a person with an adverse health condition is truly diagnosed) must be known to estimate the true prevalence or incidence.

The clinical criteria may also involve other characteristics (e.g. age, occupation) that are associated with increased disease risk. At the same time, appropriately measured and defined phenotypes or hard clinical outcomes add validity to the results.

Disease registries contain clinical information of patients on diagnosis, treatment and outcome. These registries periodically update patient information and can thus provide useful data for epidemiological research. Mortality, cancer and other nation-wide health registries generally meet the case-definition requirements and provide (almost) exhaustive data on the incident cases within a population. These health outcomes are recorded and classified in national health statistics databases, which depend on accepted diagnostic criteria that are evolving and differ from one authority to another. This may confound attempts to pool data usefully for societal benefit. Registry data present many opportunities for meaningful analysis, but the degree of data completeness and validity may challenge making appropriate inferences. Also, changes in coding conventions over the lifetime of the database may have an impact on retrospective database research.

Although the disease status is typically expressed as a dichotomous variable, it may also be measured as an ordinal variable (e.g. severe, moderate, mild or no disease) or as a quantitative variable for example by measuring molecular biomarkers of toxic response in target organs or physiological measures such as blood pressure or serum concentration of lipids or specific proteins.

The completeness of the data capture and its consistency are key contributors to the reliability of the study. Harmonisation of diagnostic criteria, data storage and utility would bring benefits to the quality of epidemiological studies.

A surrogate endpoint is used as substitute for a well-defined disease endpoint, an outcome measure, commonly a laboratory measurement (biomarker of response). These measures are considered to be on the causal pathway for the clinical outcome. In contrast to overt clinical disease, such biological markers of health may allow to detect subtle, subclinical toxicodynamic processes. For such outcomes, detailed analytical protocols for quantification should be specified to enable comparison or replication across laboratories. The use of AOPs can highlight differences in case definitions.

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標とする(そして確認された)濃度と、試験動物が毎日摂取した飼料の量を掛け合わせることで、経口経路を介して毎 日投与された外部ばく露量を把握することが可能になる。また、将来的には、重要な試験で内部ばく露量を決定しなけ ればならない。

農薬の場合、ヒトの観察環境においてはばく露濃度、ばく露経路、ばく露期間が管理されておらず曖昧のため正確 なばく露量を推定することは困難である。

意味のある関連性を調べるためには、ばく露の強度、頻度、期間を測定することが必要であることが多い。ばく露に は、比較的短期間の高濃度ばく露もあれば、数週間から数年にわたる低レベルの長期ばく露もある。急性の高用量の 農薬ばく露の影響は数時間から数日以内に現れるかもしれないが、慢性の低用量ばく露の影響は数年後にならないと 現れないかもしれない。また、病気によっては最小限のばく露で発現することもあろうが、ばく露期間が長くなればその 確率は高くなる。

異なるばく露経路(経皮、吸入、経口)では、吸収と代謝に違いが生じる可能性がある。経皮または吸入が職業上の 環境でばく露される経路であることが多いが、一般集団では経口摂取(食品、水)が農薬ばく露の主要な経路である。ヒ トにおける薬物動態には個人差が存在するため、吸収された外部ばく露量が類似している場合でも、異なる全身ばく 露または組織/器官ばく露をもたらす可能性がある。

# 2.4. 健康影響

健康影響という用語は、調査中の健康に関連する疾病状態、事象、行動、または状態を指す。健康影響とは、研究 の焦点となる臨床事象(通常は診断コード、すなわち国際疾病分類(ICD)10)または健康影響(すなわち死亡)として 表現されるものである。健康影響データを使用する際には、十分に詳細な症例定義、症例を報告し記録するシステム、 そしてこれらの事象の頻度を示す尺度が必要である。

明確な症例の定義は、どこで、いつ、誰によって診断されたかを問わず、一貫して診断されることを保証し、誤分類を 避けるのに必要である。症例定義には標準的な基準が必要であり、それは臨床症状や徴候の組み合わせであり、時に は感度と特異性が知られている診断検査によって補完されることもある。真の有病率または罹患率を推定するためには、 検査手順全体の検出感度(すなわち、健康状態の悪い人が本当に不健康と診断される確率)を認識する必要がある。

また、臨床基準には、疾患リスクの増加と関連する他の特性(例えば、年齢、職業)も含まれている。同時に、適切に 測定・定義された表現型あるいは困難な臨床結果は、調査結果の妥当性を高める。

疾患登録には、診断、治療、結果に関する患者の臨床情報が含まれている。これらの登録は定期的に患者情報を 更新しているため、疫学研究に有用なデータを提供することができる。死亡率、がん、その他の全国的な健康登録は、 一般的に症例定義の要件を満たしており、母集団内の偶発的な症例に関する(ほぼ)網羅的なデータを提供している。 これらの健康影響は、国民健康統計データベースに記録され、分類されているものの、内容的にまだ改善の余地が 多々あり、また、国ごとに異なる許容診断基準に依存していることも問題である。これは、社会的利益のために有効なデ ータを集積する試みを混乱させる可能性がある。登録データは有意義な解析を可能にするが、データの完全性と妥当 性の程度によっては、適切な推論を行うことを困難にするかもしれない。また、データベースの存続期間中におけるコ ーディング規約の変更は、後ろ向きデータベース研究に影響を与える可能性がある。

疾患状態は一般的に二分変数として表現されるが、順序変数(例えば、重度、中等度、軽度、無疾患)あるいは定量 的変数(例えば、標的臓器における毒性反応の分子バイオマーカーや血圧、脂質、特定タンパク質血清濃度などの生 理学的測定値)として測定されることもある。

データ収集の完全性とその一貫性は、研究の信頼性に大きく寄与する。診断基準、データ保存、有用性の調和は、 疫学研究の質に利益をもたらすであろう。

代替エンドポイント(surrogate endpoint)は、十分に定義された疾患エンドポイント、健康影響指標、一般的な臨床 検査値(反応のバイオマーカー)等の代替として使用される。これらの指標は、臨床事象の原因経路上にあると考えら れる。明白な臨床疾患とは対照的に、このような健康状態の生物学的マーカーは、微妙な不顕性の毒性力学的プロセ スを検出することができるかもしれない。このような健康影響のために、詳細な定量分析プロトコールは、研究室間での www.efsa.europa.eu/efsalourna

Although surrogate outcomes may offer additional information, the suitability of the surrogate outcome examined needs to be carefully assessed. In particular, the validity of surrogate outcomes may represent a major limitation to their use (la Cour et al., 2010). Surrogate endpoints that have not been validated should thus be avoided.

When the health status is captured in other ways, such as from self-completed questionnaires or telephone interviews, from local records (medical or administrative databases) or through clinical examination only, these should be validated to demonstrate that they reflect the underlying case definition.

#### 2.5. Statistical analysis and reporting

Reporting in detail materials, methods and results, and conducting appropriate statistical analyses are key steps to ensure quality of epidemiological studies. Regarding statistical analysis, one can distinguish between descriptive statistics and modelling of exposure–health outcome relationship.

#### 2.5.1. Descriptive statistics

Descriptive statistics aim to summarise the important characteristics of the study groups, such as exposure measures, health outcomes, possible confounding factors and other relevant factors. The descriptive statistics often include frequency tables and measures of central tendency (e.g. means and medians) and dispersion (e.g. variance and interquartile range) of the parameters or variables studied.

#### 2.5.2. Modelling exposure-health outcome relationship

Modelling of the exposure–health relationship aims to assess the possible relationship between the exposure and the health outcome under consideration. In particular, it can evaluate how this relationship may depend on dose and mode of exposure and other possible intervening factors.

Statistical tests determine the probability that the observations found in scientific studies may have occurred as a result of chance. This is done by summarising the results from individual observations and evaluating whether these summary estimates differ significantly between, e.g. exposed and non-exposed groups, after taking into consideration random errors in the data.

For dichotomous outcomes, the statistical analysis compares study groups by assessing whether there is a difference in disease frequency between the exposed and control populations. This is usually done using a relative measure. The relative risk (RR) in cohort studies estimates the relative magnitude of an association between exposure and disease comparing those that are exposed (or those that have a higher exposure level) with those that are not exposed (or those that have a lower exposure level). It indicates the likelihood of developing the disease in the exposed group relative to those who are not (or less) exposed. An odds ratio (OR), generally an outcome measure in case-control and cross-sectional studies, represents the ratio of the odds of exposure between cases and controls (or diseased and non-diseased individuals in a cross-sectional study) and is often the relative measure used in statistical testing. Different levels or doses of exposure can be compared in order to see if there is a dose-response relationship. For continuous outcome measures, mean or median change in the outcome are often examined across different level of exposure; either through analyses of variance or through other parametric statistics.

While the statistical analysis will show that observed differences are significantly different or not significantly different, both merit careful reflection (Greenland et al., 2016).

**Interpretation of the absence of statistically significant difference.** Failure to reject the null hypothesis does not necessarily mean that no association is present because the study may not have sufficient power to detect it. The power depends on the following factors:

- sample size: with small sample sizes, statistical significance is more difficult to detect, even if true;
- variability in individual response or characteristics, either by chance or by non-random factors: the larger the variability, the more difficult to demonstrate statistical significance;
- effect size or the magnitude of the observed difference between groups: the smaller the size of the effect, the more difficult to demonstrate statistical significance.

**Interpretation of statistically significant difference.** Statistical significance means that the observed difference is not likely due to chance alone. However, such a result still merits careful consideration.

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比較や再現を可能にするために特定されるべきである。AOP の使用は、症例定義における違いを強調することができる。

代替健康影響(surrogate outcomes)は付加的な情報を提供するかもしれないが、検査された代替健康影響の適 合性は慎重に評価される必要がある。特に、代替健康影響の妥当性は、その主な使用制限となりうる(la Cour ら、 2010 年)。したがって、妥当性が確認されていない代替エンドポイントの採択は避けるべきである。

健康状態が他の方法により、例えば自己記入式のアンケートや電話インタビュー、地域の記録(医療や行政のデー タベース)から得られた場合、あるいは臨床検査のみで収集された場合、これらは基礎となる症例の定義を正確に反映 していることを実証するために検証されるべきである。

# 2.5. 統計的解析と報告

疫学研究の質を保証するためには、資料、方法、結果を詳細に報告し、適切な統計解析を行うことが重要である。統 計解析については、記述的統計及びばく露ー健康影響の関係のモデル化に分けることができる。

# 2.5.1. 記述的統計

記述的統計は、ばく露尺度、健康影響、可能性のある交絡因子やその他の関連因子など、研究対象グループの重要な特徴を要約することを目的としている。記述的統計には、しばしば頻度表と調査したパラメータまたは変数の中心傾向(平均値や中央値など)及びばらつき度(分散や四分位数範囲など)の測定が含まれる。

# 2.5.2. ばく露ー健康影響の関係のモデル化

ばく露ー健康の関係のモデル化は、検討中のばく露と健康影響との間に考えられる関係を評価することを目的とし ている。特に、この関係によってばく露の量や様式、その他の介入因子にどのように依存しているかを評価することがで きる。

統計的検定は、科学的研究で発見された結果が偶然の結果として起こった可能性があるかどうかを判定する。これ は、個々の所見からの結果を要約し、データのランダムエラーを考慮した後、これらの要約推定値が、例えば、ばく露 群と非ばく露群の間で有意に異なるかどうかを評価することによって行われる。

二分された調査結果については、統計解析によりばく露群と対照群との間で疾患頻度に差があるかどうかを検索す る。これは通常、相対的な尺度を用いて行われる。コホート研究における相対リスク(RR)は、ばく露群(または高ばく露 群)と非ばく露群(または低ばく露群)を比較して、ばく露と疾患との関連性の相対的な大きさを推定する。これは、ばく 露群では、非ばく露群(または低ばく露群)と比較して、病気を発症する可能性が高いことを示唆している。オッズ比 (OR)は、一般的に症例対照研究や横断研究における健康影響の指標であり、症例と対照(または横断研究では罹患 者と非罹患者)の間のばく露のオッズ比を表し、しばしば統計的検査で使用される相対的な尺度である。用量反応関 係については、異なるレベルまたは用量のばく露を比較することによって確認できる。連続的な健康影響測定の場合、 結果の平均値や中央値の変化は、分散分析やその他のパラメトリック統計を用いて、異なるばく露レベルにまたがって 検討されることが多い。

統計解析は、観察された変化に統計学的有意差があるか否かを確認することであるが、いずれの結果においても慎重な再検討が必要である(Greenlandら、2016年)。

統計的に有意差がないことの解釈。帰無仮説を棄却できなかったからといって、必ずしも関連性がないということで はなく、関連性の有無はその研究の検出力が十分か否かに起因する。検出力は以下の要因に依存する。

- ・標本サイズ:標本サイズが小さいと、たとえ真であっても統計的な有意差を検出するのは困難である。
- 偶然性または非ランダムな要因による個々の反応や特性のばらつき:ばらつきが大きいほど、統計的な有意性 を示すのは難しい。
- 効果の大きさ、またはグループ間の観察された差の大きさ:効果の大きさが小さければ小さいほど、統計的有意
   性を示すのは困難である。

- Biological relevance, Rejection of the null hypothesis does not necessarily mean that the association is biologically meaningful, nor does it mean that the relationship is causal (Skelly, 2011). The key issue is whether the magnitude of the observed difference (or 'effect size') is large enough to be considered biologically relevant. Thus, an association that is statistically significant may be or may be not biologically relevant and vice versa. While epidemiological results that are statistically significant may be dismissed as 'not biologically relevant', non-statistically significant results are seldom determined to be 'biologically relevant'. Increasingly, researchers and regulators are looking beyond statistical significance for evidence of a 'minimal biologically important difference' for commonly used outcomes measures. Factoring biological significance relevance into study design and power calculations, and reporting results in terms of biological as well as statistical significance will become increasingly important for risk assessment (Skelly, 2011). This is the subject of an EFSA Scientific Committee guidance document outlining generic issues and criteria to be taken into account when considering biological relevance (EFSA Scientific Committee, 2017a); also a framework is being developed to consider biological relevance at three main stages related to the process of dealing with evidence (EFSA Scientific Committee, 2017b).
- Random error. Evaluation of statistical precision involves consideration of random error within the study. Random error is the part of the study that cannot be predicted because that part is attributable to chance. Statistical tests determine the probability that the observations found in scientific studies have occurred as a result of chance. In general, as the number of study participants increases, precision (often expressed as standard error) of the estimate of central tendency (e.g. the mean) is increased and the ability to detect a statistically significant difference, if there is a real difference between study groups, i.e. the study's power, is enhanced. However, there is always a possibility, at least in theory, that the results observed are due to chance only and that no true differences exist between the compared groups (Skelly, 2011). Very often this value is set at 5% (significance level).
- <u>Multiple testing</u>. As mentioned previously when discussing sample size, modelling of the exposure-health relationship is in principle hypothesis-driven, i.e. it is to be stated beforehand in the study objectives what will be tested. However, in reality, epidemiological studies (and toxicological studies in laboratory animals) often explore a number of different health outcomes in relation to the same exposure. If many statistical tests are conducted, some 5% of them will be statistically significant by chance. Such testing of multiple endpoints (hypotheses) increases the risk of false positive results and this can be controlled for by use of Bonferroni, Sidak or Benjamini–Hochberg corrections or other suitable methods. But this is often omitted. Thus, when researchers carry out many statistical tests on the same set of data, they can conclude that there are real differences where in fact there are none. Therefore, it is important to consider large number of statistical results as preliminary indications that require further validation. The EFSA opinion on statistical significance and biological significance notes that the assumptions derived from a statistical analysis should be related to the study design (EFSA, 2011b).
- Effect size magnification. An additional source of bias, albeit one that is lesser known, is that which may result from small sample sizes and the consequent low statistical power. This lesser known type of bias is 'effect size magnification' which can result from low powered studies. While it is generally widely known that small, low-powered studies can result in false negatives since the study power is inadequate to reliably detect a meaningful effect size, it is less well known that these studies can result in inflation of effect sizes if those estimated effects pass a statistical threshold (e.g. the common p < 0.05 threshold used to judge statistical significance). This effect –also known as effect size magnification is a phenomenon by which a 'discovered' association (i.e. one that has passed a given threshold of statistical significance) from a study with suboptimal power to make that discovery will produce an observed effect size that is artificially and systematically inflated. This is because smaller, low-powered studies are more likely to be affected by random variation among individuals than larger ones. Mathematically, conditional on a result passing some predetermined threshold of statistical significance, the estimated effect size is a biased estimate of the true effect size, with the magnitude of this bias inversely related to power of the study.

As an example, if a trial were run thousands of times, there will be a broad distribution of observed effect sizes, with smaller trials systematically producing a wider variation in observed effect sizes than larger trials, but the median of these estimated effect sizes is close to the true

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統計的に有意な差の解釈。統計的有意差とは、観察された差が偶然性だけによるものではないことを意味する。しか し、そのような結果はまだ慎重に検討する必要性がある。

- ・ 生物学的関連性:帰無仮説の否定は、必ずしも関連が生物学的に意味のあるものであることを意味するわけではなく、関連が因果関係にあることを意味するわけでもない(Skelly, 2011 年)。重要な問題は、観察された差の大きさ(または「効果の大きさ」)が、生物学的に関連性があると考えられるほど大きいかどうかということである。このように、統計的に有意な関連性は、生物学的に関連性があるかもしれないし、ないかもしれないし、その逆もある。統計的に有意な度学的結果は「生物学的に関連性がない」として却下されるかもしれないが、統計的に有意でない結果が「生物学的に関連性がある」と判断されることはめったにない。研究者や規制当局は、一般的に使用されている健康影響の指標について、統計的有意性を超えて「生物学的に重要な差が最小である」というエビデンスを求めているケースが増えている。生物学的有意性の関連性を研究デザインや検出力の計算に配慮した上で、統計的有意性と同様に生物学的有意性の観点から結果を報告することは、リスク評価においてますます重要になってくるであろう(Skelly, 2011 年)。これは、生物学的関連性を考慮する際に考慮すべき一般的な問題と基準を概説した EFSA Scientific Committee のガイダンス文書の対象となっている(EFSA Scientific Committee, 2017年a);また、エビデンスを扱うプロセスに関連した三つの主要な段階で生物学的関連性を考慮するためのフレームワークが開発されている(EFSA Scientific Committee, 2017年b)。
- ・偶発誤差(ランダムエラー):統計的精度の評価には、研究内の偶然誤差を考慮する必要がある。偶発誤差とは、研究における予見できない部分であり、その部分が偶然性に起因する。統計的検定は、科学的研究で発見された結果が偶然性の結果として発生した確率を決定する。一般的に、研究参加者の数が増えると、中心傾向(例えば平均値)の推定値の精度(標準誤差として表現されることが多い)が上がり、研究グループ間に実際の差がある場合、統計的に有意な差を検出する能力が向上する。しかし、少なくとも理論的には、観察された結果が偶然に起因するものであり、比較されたグループ間に真の違いが存在しないという可能性が常にある(Skelly、2011 年)。多くの場合、この値は 5%(有意水準)に設定される。
- 多重検定:サンプルサイズについて議論する際に前述したように、ばく露ー健康影響の関係のモデル化は、原則として仮説主導型であり、予め何を検索するかを研究目的に明記しておく必要がある。しかしながら、実際には、疫学的研究(及び実験動物を用いた毒性学的研究)では、多くの場合、同じばく露に関連して多くの異なる健康影響を調査している。多くの統計的検定が実施された場合、そのうちの5%程度は偶然にも統計的に有意な結果が得られることがある。このような複数のエンドポイント(仮説)の検定は、偽陽性結果のリスクを高めるが、これはBonferroni、Sidak、あるいはBenjamini-Hochbergの補正や、他の適切な方法を使用することでコントロールすることができる。しかし、これはしばしば省略される。このように、研究者が同じデータセットを対象に多くの統計的検定を行った場合、実際には何も変化はないのにも拘わらず差があるように結論づけられてしまうことがある。したがって、多くの統計結果は、さらなる検証を必要とする予備的な指標と考えることが重要である。統計的有意性と生物学的有意性に関する EFSA の見解は、統計解析から導き出される仮定は、研究デザインに関連して採択すべきであることに注意を促している(EFSA、2011年b)。
- · <u>効果量の拡大:</u>あまり知られていないとはいえ、バイアス追加の原因は、サンプルサイズが小さく、結果として統計 的検出力が低いことに起因する可能性がある。このあまり知られていないバイアスの種類として知られているのは、 低検出力研究から生じる「効果量の拡大」である。小規模で低検出力の研究では、研究の検出力が意味のある効 果量を確実に検出するには不十分であるため、偽陰性が生じる可能性があることは一般的に知られているが、推 定された効果が統計的閾値(例えば、統計的有意性の判定に使用される一般的な p<0.05 閾値)を通過した場合 に、これらの研究が効果量の誇張(インフレ)をもたらす可能性があることはあまり知られていない。この効果は、効 果量の拡大としても知られているが、これは、「発見された」関連性(すなわち、統計的有意性のある閾値を通過し たもの)を有効化することを目的とした最適下限の検出力を伴う研究から得られる現象であり、観察された効果量が、 人工的かつ系統的に誇張されることを意味する。これは、小規模で検出力の低い研究は、大規模な研究よりも個 人間のランダムな変動(ばらつき)の影響を受けやすいからである。数学的には、結果が統計的に有意であるという

effect size. However, in a small and low powered study, only a small proportion of observed effects will pass any given (high) statistical threshold of significance and these will be only the ones with the greatest of effect sizes. Thus, when these smaller, low powered studies with areater random variation do indeed find a significance-triggered association as a result of passing a given statistical threshold, they are more likely to overestimate the size of that effect. What this means is that research findings of small and significant studies are biased in favour of finding inflated effects. In general, the lower the background (or control or natural) rate, the lower the effect size of interest, and the lower the power of the study, the greater the tendency towards and magnitude of inflated effect sizes.

It is important to note, however, that this phenomenon is only present when a 'pre-screening' for statistical significance is done. The bottom line is that if it is desired to estimate a given quantity such as an OR or RR, 'pre-screening' a series of effect sizes for statistical significance will result in an effect size that is systematically biased away from the null (larger than the true effect size). To the extent that regulators, decision-makers, and others are acting in this way looking for statistically significant results in what might be considered a sea of comparisons and then using those that cross a given threshold of statistical significance to evaluate and judge the magnitude of the effect – will likely result in an exaggerated sense of the magnitude of the hypothesised association. Additional details and several effect size simulations are provided in Annex D of this document.

Confounding occurs when the relationship between the exposure and disease is to some extent attributable to the effect of another risk factor, i.e. the confounder. There are several traditionally recognised requirements for a risk factor to actually act as a confounder as described by McNamee (2003) and illustrated below. The factor must:

- be a cause of the disease, or a surrogate measure of the cause, in unexposed people; factors satisfying this condition are called 'risk factors';
- be correlated, positively or negatively, with exposure in the study populations independently from the presence of the disease. If the study population is classified into exposed and unexposed groups, this means that the factor has a different distribution (prevalence) in the two aroups:
- not be an intermediate step in the causal pathway between the exposure and the disease

Confounding can result in an over- or underestimation of the relationship between exposure and disease and occurs because the effects of the two risk factors have not been separated or 'disentangled'. In fact, if strong enough, confounding can also reverse an apparent association. For instance, because agriculture exposures cover many different exposure categories, farmers are likely to be more highly exposed than the general population to a wide array of risk factors, including biological agents (soil organisms, livestock, farm animals), pollen, dust, sunlight and ozone amongst others, which may act as potential confounding factors.

A number of procedures are available for controlling confounding, both in the design phase of the study or in the analytical phase. For large studies, control in the design phase is often preferable. In the design phase, the epidemiological researcher can limit the study population to individuals that share a characteristic which the researcher wishes to control. This is known as 'restriction' and in fact removes the potential effect of confounding caused by the characteristic which is now eliminated. A second method in the design phase through which the researcher can control confounding is by 'matching'. Here, the researcher matches individuals based on the confounding variable which ensures that this is evenly distributed between the two comparison groups.

Beyond the design phase, at the analysis stage, control for confounding can be done by means of either stratification or statistical modelling. One means of control is by stratification in which the association is measured separately, under each of the confounding variables (e.g. males and females, ethnicity or age group). The separate estimates can be 'brought together' statistically - when appropriate - to produce a common OR, RR or other effect size measure by weighting the estimates measured in each stratum (e.g. using Mantel-Haenszel approaches). This can be done at the cost of reducing the sample size for the analysis. Although relatively easy to perform, there can be difficulties associated with the inability of this stratification to deal with multiple confounders simultaneously. For these situations, control can be achieved through statistical modelling (e.g. multiple logistic regression).

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あらかじめ決められた閾値を通過することを条件に、推定された効果量は真の効果量の偏った推定値となり、この 偏りの大きさは研究の検出力に反比例している。例えば、ある試験を何千回も実施した場合、観察された効果量に は広い分布があり、小規模な試験では大規模な試験よりも観察された効果量のばらつきが大きくなるが、これらの 推定効果量の中央値は真の効果量に近いものになる。しかし、小規模で低検出力の研究では、観察された効果 のうち、任意の(高い)統計的閾値を通過するのはごく一部であり、これらは最大の効果量を持つものだけである。 したがって、ランダム変動が大きい小規模で検出力の低い研究では、与えられた統計的閾値を通過した場合、実 際に有意性を重視することにより引き起こされる関連性については、その効果量を過大評価する可能性が高くなる。 このことが意味するものは、小規模研究における有意な研究結果は、効果を誇張して発見することに有利になるよ うに偏っているということである。一般的に、バックグラウンド(または対照または無処置)の割合が低く、対象となる 効果量が小さく、研究の検出力が低いほど、誇張効果量の増大傾向がみられる。

しかし、この現象は、統計的有意性のための「事前スクリーニング」が行われた場合にのみ存在することに注意する ことが重要である。要するに、オッズ比(OR)や相対リスク(RR)のような与えられた量を推定したい場合、統計的有 意性のために一連の効果量を「事前スクリーニング」すると、無効値から系統的に偏った(真の効果量よりも大きい) 効果量が得られるということである。規制当局、政策決定者、その他の人々がこの方法で行動している範囲では、 限りない比較と考えられるものの中から統計的に有意な結果を探し、効果の大きさを評価し判断するために統計的 有意性のある閾値を超えたものを使用しているため、仮定された関連の大きさを誇張した感覚になる可能性が高 い。追加の詳細といくつかの効果量のシミュレーションは、本書の付属書 D で提供されている。

**交絡**は、ばく露と疾病との関係が他のリスク因子の影響、すなわち交絡因子の影響にある程度起因している場合に 発生する。リスク因子が実際に交絡因子として作用するためには、McNamee(2003年)により提示(以下に図示)され、 従来から認識されているいくつかの要件がある。その因子は次のようなものでなければならない。

- ・ 被曝していない人に疾患を引き起こす原因、または原因の代替指標となること。この条件を満たす因子は「リスク 因子」と呼ばれる。
- 病気の存在とは無関係に、調査集団のばく露と正または負の相関があること。調査集団がばく露群と非ばく露群に 分類されている場合、その因子が2つの群で異なる分布(有病率)を持っていることを意味する。
- ばく露と疾患の間の因果関係の経路に中間段階はない。

交絡は、ばく露と疾病の関係を過大または過小に評価する結果となり、2 つのリスク因子の影響が分離されていなか ったり、「解放」されていなかったりするために起こる。実際には、十分に強固な場合、交絡はまた、見かけ上の関連性 を逆転させることもある。例えば、農業上ばく露は多くの異なるばく露カテゴリーがあるため、農業従事者は、生物学的 要因(土壤生物、家畜、農場動物)、花粉、粉塵、日光、オゾンなど、潜在的な交絡因子として作用する可能性のあるも のを含む、多種多様なリスク因子に一般集団よりも多くばく露されている可能性が高い。

交絡を制御するために、研究の計画段階または解析段階の両方で、多くの手順が利用可能である。大規模な研究 では、計画段階でのコントロールが好ましいことが多い。計画段階では、疫学研究者は、研究者がコントロールしたい 特徴を共有する個人に研究集団を限定することができる。これは「限定」として知られており、実際には、その特性によ って引き起こされる交絡の潜在的な影響を取り除くことができる。研究者が交絡をコントロールするための計画段階での 2つ目の方法は、「マッチング」によるものである。ここでは、研究者は交絡変数に基づいて個人をマッチングさせ、交絡 変数が2つの比較グループ間で均等に分布するようにする。

計画段階を超えて、解析段階では、層別化または統計的モデリングのいずれかの方法で交絡をコントロールすること ができる。コントロールの1つの手段は、交絡変数(例えば、男性と女性、民族、または年齢グループ)のそれぞれの下 で、関連性が別々に測定される層別化によるものである。別々の推定値は、各層で測定された推定値を重み付けする ことによって、共通のオッズ比(OR)、相対リスク(RR)、または他の効果量を生成するために(必要に応じて)統計学的 に「集積する」ことができる(例えば、Mantel-Haenszel アプローチを使用する)。これは、分析のサンプルサイズを小さ くする代償として行うことができる。比較的簡単に実行できるが、この層別化が複数の交絡因子を同時に扱うことができ ないことに起因する困難が生ずるかもしれない。このような状況では、統計的なモデル化(例えば、多重ロジスティック EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

Regardless of the approaches available for control of confounding in the design and analysis phases of the study described above, it is important – prior to any epidemiological studies being initiated in the field – that careful consideration be given to confounders because researchers cannot control for a variable which they have not considered in the design or for which they have not collected data.

Epidemiological studies – published or not – are often criticised for ignoring potential confounders that may possibly either falsely implicate or inappropriately negate a given risk factor. Despite these critiques, rarely is an argument presented on the likely size of the impact of the bias from such possible confounding. It should be emphasised that a confounder must be a relatively strong risk factor for the disease to be strongly associated with the exposure of interest to create a substantial distortion in the risk estimate. It is not sufficient to simply raise the possibility of confounding; one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be and how important that impact might be to the interpretation of findings. It is important to consider the magnitude of the association as measured by the RR, OR, risk ratio, regression coefficient, etc. since strong relative risks are unlikely to be due to unmeasured confounding, while weak associations may be due to residual confounding by variables that the investigator did not measure or control in the analysis (US-EPA, 2010b).

Effect modification. Effects of pesticides, and other chemicals, on human health can hardly be expected to be identical across all individuals. For example, the effect that any given active substance might have on adult healthy subjects may not be the same as that it may have on infants, elderly, or pregnant women. Thus, some subsets of the population are more likely to develop a disease when exposed to a chemical because of an increased sensitivity. For this, the term 'vulnerable subpopulation' has been used, which means children, pregnant women, the elderly, individuals with a history of serious illness and other subpopulations identified as being subject to special health risks from exposure to environmental chemicals (i.e. because of genetic polymorphisms of drug-metabolising enzymes, transporters or biological targets). The average effect measures the effect of an exposure averaged over all subpopulations. However, there may be heterogeneity in the strength of an association between various subpopulations. For example, the magnitude of the association between exposure to chemical A and health outcome B may be stronger in children than in healthy adults, and absent in those wearing protective clothing at the time of exposure or in those of different genotype. If heterogeneity is truly present, then any single summary measure of an overall association would be deficient and possibly misleading. The presence of heterogeneity is assessed by testing for the presence of statistically significant interaction between the factor and the effect in the various subpopulations. But, in practice, this requires large sample size.

Investigating the effect in subpopulations defined by relevant factors may advance knowledge on the effect on human health of the risk factor of interest.

#### 2.6. Study validity

When either a statistically significant association or no such significant association between, for example, pesticide exposures and a health outcome is observed, there is a need to also evaluate the validity of a research study, assessing factors that might distort the true association and/or influence its interpretation. These imperfections relate to systematic sources of error that result in a (systematically) incorrect estimate of the association between exposure and disease. In addition, the results from a single study takes on increased validity when it is replicated in independent investigations conducted on other populations of individuals at risk of developing the disease.

**Temporal sequence.** Any claim of causation must involve the cause preceding in time the presumed effect. Rothman (2002) considered temporality as the only criterion that is truly causal, such that lack of temporality rules out causality. While the temporal sequence of an epidemiological association implies the necessity for the exposure to precede the outcome (effect) in time, measurement of the exposure is not required to precede measurement of the outcome. This requirement is easier met in prospective study designs (i.e. cohort studies), than when exposure is assessed retrospectively (case-control studies) or assessed at the same time than the outcome (cross-sectional studies). However, also in prospective studies, the time sequence for cause and effect and the temporal direction might be difficult to ascertain if a disease developed slowly and initial forms of disease were difficult to measure (Höfler, 2005).

The generalisability of the result from the population under study to a broader population should also be considered for study validity. While the random error discussed previously is considered a precision problem and is affected by sampling variability, **bias** is considered a validity issue. More

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#### 回帰)によってコントロールを達成することができる。

上述の研究の計画と解析の段階で交絡をコントロールするために利用可能なアプローチにかかわらず、研究者が計 画で考慮しなかった変数や、データを収集しなかった変数をコントロールすることができないため、この分野で疫学研 究を開始する前に、交絡因子を慎重に考慮することが重要である。

度学研究は、公表されているかどうかにかかわらず、特定のリスク因子を誤って暗示したり、不適切に否定したりする 可能性のある潜在的な交絡因子を無視しているとして、しばしば批判される。このような批判にもかかわらず、そのよう な可能性のある交絡因子によるバイアスの影響の大きさについての議論が提示されることはほとんどない。交絡因子は、 リスク推定値に実質的な歪みを生じさせるためには、対象となるばく露に強く関連した疾患の比較的強いリスク因子で なければならないことを強調しなければならない。単に交絡の可能性を提起するだけでは十分ではなく、リスク因子が なぜ交絡因子になりやすいのか、その影響がどのようなものなのか、そしてその影響が結果の解釈にとってどれほど重 要なのかを説明する説得力のある議論をしなければならない。強い相対リスクは測定されていない交絡因子によるもの である可能性が低いのに対し、弱い交絡因子は、研究者が解析で測定または管理していない変数による残存交絡因 子によるものである可能性があるため、相対リスク(RR)、オッズ比(OR)、リスク比、回帰係数などで測定される交絡の 大きさを考慮することが重要である(US-EPA、2010 年 b)。

**効果修飾。**ドトの健康に対する農薬及びその他の化学物質の影響は、すべての個人で同一であるとは考えにくい。 例えば、ある特定の有効成分が成人の健康な被験者に及ぼす影響は、乳児、高齢者、妊婦に及ぼす影響と同じでは ない可能性がある。このように、ある化学物質にばく露された場合、ある集団の一部(サブセット)が感受性が高いことか ら疾患を発症する可能性が高くなる。このため、「脆弱な小集団」という用語が使用されているが、これは子供、妊婦、高 齢者、重病歴のある人に加え、環境化学物質へのばく露による特別な健康リスク(薬物代謝酵素、トランスポーター、ま たは生物学的標的の遺伝的多型のため)の対象となると同定された小集団を含む。平均効果とは、あるばく露の影響 をすべての小集団で平均化したものである。しかし、様々な小集団間の関連の強さには不均一性があるかもしれない。 例えば、化学物質 A へのばく露と健康影響 B との間の関連の程度は、健康な成人よりも子供の方が強く、また、ばく露 時に防護服を着用している人や遺伝子型の異なる人には同様な影響は見られないかもしれない。もし不均一性が本当 に存在するのであれば、全体的な関連性を示す単一の要約尺度は意味をなさず、誤解を招く可能性がある。不均質 性の存在は、様々な小集団における因子と効果の間に統計的に有意な相互作用があるかどうかを検定することによっ て評価される。しかし、実際には、これは大きな標本サイズを必要とする。

関連因子によって定義された小集団での効果を調査することは、対象となるリスク因子のとトの健康への影響につい ての知識を前進させるかもしれない。

# **2.6.** 研究の妥当性

例えば、農薬ばく露と健康影響の間に統計的に有意な関連が観察された場合、またはそのような有意な関連が観察 されなかった場合には、真の関連を歪めたり、その解釈に影響を及ぼす可能性のある要因を評価して、調査研究の妥 当性も評価する必要がある。これらの不完全性は、ばく露と疾病の間の関連性を(系統的に)誤って推定することになる 系統的な誤差の原因に関係している。さらに、単一の研究から得られた結果は、病気を発症するリスクのある他の集団 で実施された独立した調査で再現された場合、より高い妥当性を持つことになる。

時間的シーケンス(順序)。因果関係の断定は、推定される効果に先行する原因を時間的に関与させなければならない。Rothman(2002年)は、時間性を真に因果関係がある唯一の基準と考え、時間性の欠如は因果関係を排除する。疫学的関連の時間的順序は、時間的にはばく露が結果(効果)に先行する必要性を示唆しているが、ばく露の測定が結果の測定に先行する必要はない。この要件は、ばく露が後ろ向きに評価される場合(症例対照研究)や、結果と同時に評価される場合(横断研究)よりも、前向き研究の計画(すなわちコホート研究)では容易に満たされる。しかし、前向き研究においても、疾患の発症が遅かったり、初期の疾患形態が測定しにくかったりすると、原因と結果の時間的な順序や時間的な方向性を確認することが困難になることがある(Höfler、2005年)。

研究の妥当性については、研究対象集団からより広い集団への結果の一般化可能性も考慮しなければならない。 www.efsa.europa.eu/efsajourna EFSA Journal 2017;15(10):5007

specifically, bias issues generally involve methodological imperfections in study design or study analysis that affect whether the correct population parameter is being estimated. The main types of bias include selection bias, information bias (including recall bias and interviewer/observer bias) and confounding. An additional potential source of bias is effect size magnification, which has already been mentioned.

Selection bias concerns a systematic error relating to validity that occurs as a result of the procedures and methods used to select subjects into the study, the way that subjects are lost from the study or otherwise influence continuing study participation.

Typically, such a bias occurs in a case-control study when inclusion (or exclusion) of study subjects on the basis of disease is somehow related to the prior exposure status being studied. One example might be the tendency for initial publicity or media attention to a suspected association between an exposure and a health outcome to result in preferential diagnosis of those that had been exposed compared to those that had not. Selection bias can also occur in cohort studies if the exposed and unexposed groups are not truly comparable as when, for example, those that are lost from the study (loss to follow-up, withdrawn or non-response) are different in status to those who remain. Selection bias can also occur in cross-sectional studies due to selective survival: only those that have survived are included in the study. These types of bias can generally be dealt with by careful design and conduct of a study (see also Sections 4, 6 and 8).

The 'healthy worker effect' (HWE) is a commonly recognised selection bias that illustrates a specific bias that can occur in occupational epidemiology studies: workers tend to be healthier than individuals from the general population overall since they need to be employable in a workforce and can thus often have a more favourable outcome status than a population-based sample obtained from the general population. Such a HWE bias can result in observed associations that are masked or lessened compared to the true effect and thus can lead to the appearance of lower mortality or morbidity rates for workers exposed to chemicals or other deleterious substances.

**Information bias** concerns a systematic error when there are systematic differences in the way information regarding exposure or the health outcome are obtained from the different study groups that result in incorrect or otherwise erroneous information being obtained or measured with respect to one or more covariates being measured in the study. Information bias results in misclassification which in turn leads to incorrect categorisation with respect to either exposure or disease status and thus the potential for bias in any resulting epidemiological effect size measure such as an OR or RR.

Misclassification of exposure status can result from imprecise, inadequate or incorrect measurements; from a subject's incorrect self-report; or from incorrect coding of exposure data.

Misclassification of disease status can, for example, arise from laboratory error, from detection bias, from incorrect or inconsistent coding of the disease status in the database, or from incorrect recall. Recall bias is a type of information bias that concerns a systematic error when the reporting of disease status is different, depending on the exposure status (or vice versa). Interviewer bias is another kind of information bias that occurs where interviewers are aware of the exposure status of individuals and may probe for answers on disease status differentially – whether intended or not – between exposure groups. This can be a particularly pernicious form of misclassification – at least for case-control studies - since a diseased subject may be more likely to recall an exposure that occurred at an earlier time period than a non-diseased subject. This will lead to a bias away from null value (of no relation between exposure and disease) in any effect measure.

Importantly, such misclassifications as described above can be 'differential' or 'non-differential' and these relate to (i) the degree to which a person that is truly exposed (or diseased) is correctly classified as being truly exposed or diseased and (ii) the degree to which an individual who is truly not exposed (or diseased) is correctly classified in that way. The former is known as 'sensitivity' while the latter is referred to as 'specificity' and both of these play a role in determining the existence and possible direction of bias. Differential misclassification means that misclassification has occurred in a way that depends on the values of other variables, while non-differential misclassification refers to misclassifications that do not depend on the value of other variables.

What is important from an epidemiological perspective is that misclassification biases - either differential or non-differential - depend on the sensitivity and specificity of the study's methods used to categorise such exposures and can have a predictable effect on the direction of bias under certain (limited) conditions: this ability to characterise the direction of the bias based on knowledge of the study methods and analyses can be useful to the regulatory decision-maker since it allows the decision maker to determine whether the epidemiological effect sizes being considered (e.g. OR, RR) are likely underestimates or overestimates of the true effect size. While it is commonly assumed by some that

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#### Epidemiological studies and pesticides

前述したランダムエラーは精度の問題と考えられ、サンプリング変動の影響を受けるが、バイアスは妥当性の問題と考 えられている。より具体的には、バイアスの問題は一般的に、正しい母集団パラメータが推定されているかどうかに影響 を与える研究デザインまたは研究分析における方法論的な不完全性を伴う。バイアスの主なタイプには、選択バイアス、 情報バイアス(想起バイアス,質問者/オブザーバーバイアスを含む)、交絡因子がある。追加の潜在的なバイアスの 発生源は、すでに述べた効果量の規模である。

選択バイアスは、被験者を研究に参加させるために使用された手順や方法、被験者が研究から外れる方法や、そう でなければ研究への継続的な参加に影響を与える結果として発生する妥当性に関する系統的な誤差に関係している。

典型的には、このようなバイアスは、症例対照研究において、疾患に基づいて被験者を含める(または除外する)こと が、研究対象となる前のばく露状態と何らかの形で関連している場合に発生する。一例としては、ばく露と健康影響との 間に関連性が疑われることに対する初期の広報やメディアの注目が、ばく露を受けた人はばく露を受けていない人に 比べて優先的に診断される傾向があるかもしれない。選択バイアスはまた、コホート研究においても、例えば、研究から 外れた人(追跡調査に参加できなくなった人、離脱した人、無回答の人)と残された人の状態が異なる場合のように、ば く露群と非ばく露群が真に比較可能でない場合に発生しうる。また、横断研究では、生存者のみを研究対象とする選択 的生存により、選択バイアスが生じることがある。このようなタイプのバイアスは、一般的に研究の慎重な計画と実施によ って対処できる(第4節、第6節、第8節も参照)。

「健康労働者効果」(HWE)は、一般的に認識されている選択バイアスであり、職域疫学研究で起こりうる特定のバイ アスを示すものである:労働者は、労働力として雇用される必要があるため、一般集団からの個人よりも健康である傾向 があり、そのため、一般集団から得られた集団ベースのサンプルよりも好ましい健康状態を持つことが多い。このような HWE バイアスは、観察された関連性が真の効果に比べて隠されたり、軽減されたりすることがあり、その結果、化学物 質やその他の有害物質にばく露された労働者の死亡率や罹患率が低く見えることがある。

情報バイアスとは、ばく露または健康影響に関する情報が異なる研究グループから得られる方法に系統的な違いが あり、その結果、研究で測定される1つ以上の共変量に関して不正確な情報が得られたり、測定されたりする場合の系 統的な誤差のことである。情報のバイアスは、結果として、ばく露または疾病状態のいずれかに関して誤った分類につ ながり、ORやRRのような疫学的効果の大きさの尺度にバイアスが生じる可能性がある。

ばく露状態の誤分類は、不正確、不十分、または不正確な測定値、被験者の不正確な自己申告、またはばく露デー タの不正確なコーディングに起因する可能性がある。

疾患状態の誤分類は、例えば、検査室のエラー、検出バイアス、データベース内の疾患状態の不正確な、または一 貫性のないコーディング、あるいは不正確な想起から生じることがある。想起バイアスは情報バイアスの一種であり、ば く露状態(またはその逆)に応じて疾患状態の報告が異なる場合の系統的な誤りに関係している。質問者・バイアスは、 質問者が個人のばく露状況を認識している場合に発生するもう一つの情報バイアスで、ばく露グループ間で意図して いるかどうかに関わらず、ばく露グループ間で異なる疾患状況に関する回答を求めてしまうことがある。なぜなら、病気 の被験者は、病気ではない被験者に比べて、より早い時期に発生したばく露を思い出す可能性が高いからである。こ れは、何らかの効果測定において帰無値(ばく露と疾病の間に関係がないという)から遠ざかるバイアスにつながる。

重要なことに、上述のような誤分類は、「差がある(differential)」場合と「差がない(non-differential)」場合があるこ とである。これらは、(i)真にばく露されている(または病気にかかっている)人が、真にばく露されている、または病気に かかっていると正しく分類される度合いと、(ii)真にばく露されていない(または病気にかかっていない)人が、そのよう に正しく分類される度合いに関係している。前者は「感度」として知られているが、後者は「特異性」と呼ばれ、これらの 両方がバイアスの存在と可能性のある方向性を決定する役割を果たしている。差異的誤分類とは、他の変数の値に依 存する方法で誤分類が発生したことを意味し、非差異的誤分類とは、他の変数の値に依存しない誤分類を意味する。

疫学的観点から重要なことは、誤分類バイアス(差異的か非差異的か)は、そのようなばく露を分類するために使用さ れた研究方法の感度と特異性に依存し、特定の(限定された)条件の下でバイアスの方向に予測可能な影響を及ぼす ことができるということである。すなわち、研究の方法や解析の知識に基づくバイアスの方向性を特性評価する能力は、 考慮される疫学的効果量(OR、RR など)が真の効果量の過小評価か過大評価かを政策決定者が判断することができ www.efsa.europa.eu/efsajourna

non-differential misclassification bias produces predictable biases towards the null (and thus systematically under-predicts the effect size), this is not necessarily the case. Also, the sometimes common assumption in epidemiology studies that misclassification is non-differential (which is sometimes also paired with the assumption that non-differential misclassification bias is always towards the null) is not always justified (e.g. see Jurek et al., 2005).

When unmeasured confounders are thought to affect the results, researchers should conduct sensitivity analyses to estimate the range of impacts and the resulting range of adjusted effect measures (US-EPA, 2010b). Quantitative sensitivity (or bias) analyses are, however, not typically conducted in many epidemiological studies, with most researchers instead describing various potential biases qualitatively in the form of a narrative in the discussion section of a paper.

It is often advisable that the epidemiological investigator performs sensitivity analysis to estimate the impact of biases, such as exposure misclassification or selection bias, by known but unmeasured risk factors or to demonstrate the potential effects that a missing or unaccounted for confounder may have on the observed effect sizes (see Lash et al., 2009; Gustafson and McCandless, 2010). Sensitivity analyses should be incorporated in the list of criteria for reviewing epidemiological data for risk assessment purposes.

#### 3. Key limitations of the available epidemiological studies on pesticides

# 3.1. Limitations identified by the authors of the EFSA external scientific report

The EFSA External scientific report (Ntzani et al., 2013; summarised in Annex A) identified a plethora of epidemiological studies which investigate diverse health outcomes. In an effort to systematically appraise the epidemiological evidence, a number of methodological limitations were highlighted. In the presence of these limitations, robust conclusions could not be drawn, but outcomes for which supportive evidence from epidemiology existed were highlighted for future investigation. The main limitations identified included (Ntzani et al., 2013):

- · Lack of prospective studies and frequent use of study designs that are prone to bias (casecontrol and cross-sectional studies). In addition, many of the studies assessed appeared to be insufficiently powered.
- · Lack of detailed exposure assessment, at least compared to many other fields within epidemiology. The information on specific pesticide exposure and co-exposures was often lacking, and appropriate biomarkers were seldom used. Instead, many studies relied on broad definition of exposure assessed through questionnaires (often not validated).
- · Deficiencies in outcome assessment (broad outcome definitions and use of self-reported outcomes or surrogate outcomes).
- Deficiencies in reporting and analysis (interpretation of effect estimates, confounder control and multiple testing).
- Selective reporting, publication bias and other biases (e.g. conflict of interest).

The observed heterogeneity in the results within each studied outcome was often large. However, heterogeneity is not always a result of biases and may be genuine and consideration of a priori defined subgroup analysis and meta-regression should be part of evidence synthesis efforts. Occupational studies, which are of particular importance to pesticide exposure, are also vulnerable to the healthy worker effect, a bias resulting in lower morbidity and mortality rates within the workforce than in the general population. The healthy worker effect tends to decline with increasing duration of employment and length of follow-up.

Studies with sufficient statistical power, detailed definition of pesticide exposure, data for many health outcomes and transparent reporting are rare, apart from the Agricultural Health Study (AHS) and other similarly designed studies. It is important to note that several of these methodological limitations have not been limited to pesticide exposure studies and, most importantly, are not specific in epidemiology and have been observed in other specific fields including in animal studies (Tsilidis et al., 2013).

Given the wide range of pesticides with various definitions found in the EFSA External scientific report, it is difficult to harmonise this information across studies. Although heterogeneity of findings across studies can be as informative as homogeneity, information needs to be harmonised such that replication can be assessed and summary effect sizes be calculated. This does not mean that if there is

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るから、規制当局の政策決定に有用である。非差異的な誤分類バイアスが帰無値への予測可能なバイアスをもたらす (したがって、体系的に効果量を過小予測する)と一般的に想定されていますが、これは必ずしもそうではない。また、 誤分類は非差異的であるという疫学研究で時々みられる一般的な仮定(これは、非差異的な誤分類バイアスが常に帰 無(null)に向かっているという仮定と対になっていることもある)は、必ずしも正当化されているわけではない(例えば、 Jurekら、2005 年を参照)。

測定されていない交絡因子が結果に影響を与えると考えられる場合、研究者は感度分析を実施して、影響の範囲と その結果として生じる調整された効果測定値の範囲を推定すべきである(US-EPA、2010 年 b)。しかし、定量的感度 (またはバイアス)分析は、多くの疫学研究では一般的には行われておらず、ほとんどの研究者は、論文の考察で様々 な潜在的なバイアスを定性的に記述している。

疫学研究者は既知ではあるが測定されていないリスク因子によるばく露の誤分類や選択バイアスなどのバイアスの 影響を推定したり、見落としや考慮されていない交絡因子が観察された効果の大きさに及ぼす影響を示すために感度 分析を実施することが推奨されている(Lash ら、2009 年;Gustafson 及び McCandless、2010 年)。感度分析は、リ スク評価目的で疫学データをレビューする際の基準リストに組み込まれるべきである。

# 3. 農薬に関する利用可能な疫学研究の主な限界事項 3.1. EFSA 外部科学研究報告書の著者が指摘した限界

EFSA 外部科学報告書(Ntzani ら、2013 年;付属書 A に要約)では、多様な健康影響を調査する疫学的研究が 多数報告されている。疫学的証拠を体系的に評価する努力の中で、多くの方法論的限界が強調された。これらの限界 の存在下では、確固たる結論を導き出すことはできなかったが、疫学からの裏付けとなるエビデンスが存在する結果は、 今後の調査のために強調された。識別された主な限界は以下の通りである(Ntzaniら、2013年)。

- ・ 前向き研究の欠如と、バイアスがかかりやすい研究デザイン(症例対照研究と横断研究)の頻繁な使用。さら に、評価された研究の多くは、十分な検出力を持っていないようにみえる。
- 少なくとも疫学の他の多くの分野の疫学と比較して、詳細なばく露評価が欠如している。特定の農薬ばく露と混 合ばく露に関する情報は、多くの場合、不足しており、適切なバイオマーカーはほとんど使用されていない。代 わりに、多くの研究では、アンケート調査(多くの場合、妥当性が確認されていない)によって評価されたばく露 の大まかな定義に頼っていた。
- ・ 結果評価の不備(おおまかな結果の定義、自己申告性の健康影響または代替健康影響の使用)。
- 報告と解析の不備(効果推定値の解釈、交絡因子のコントロール、多重検定)。
- 選択的な報告・出版バイアスと、その他のバイアス(例:利害の対立)。

各研究結果の中で観察された結果の不均一性は、しばしば大きなものであった。しかし、不均一性は常にバイアス の結果であるとは限らず、本物である可能性があり、先験的に定義されたサブグループ解析やメタ回帰を考慮すること は、エビデンス統合の努力の一環であるべきである。農薬ばく露に関して特に重要な職域研究もまた、健康労働者効 果の影響を受けやすく、このバイアスによって労働者の罹患率と死亡率が一般集団よりも低くなっている。健康労働者 効果は、雇用期間と追跡調査の期間が長くなるにつれて低下する傾向がある。

十分な統計力を持ち、農薬ばく露の詳細な定義、多くの健康影響に関するデータ及び明白性な報告を備えている 研究は、農業健康調査(Agricultural Health Study: AHS)や他の同様の研究を除けば、稀である。これらの方法論 的限界のいくつかは、農薬ばく露研究に限定されたものではなく、最も重要なことは、疫学的には特異的なものではな く、動物試験を含む他の特異的な研究分野でも観察されていることに注意することが重要である(Tsilidis ら、2013 年)。

EFSA の外部科学報告書には様々に定義された広範囲の農薬が記載されているが、この情報を研究間で調和させ ることは困難である。研究間での結果の不均一性は、同質性と同じくらい有益な情報になるが、情報は、反復を評価し たり、要約効果量を計算したりできるように調和されている必要がある。これは、真の不均一性がある場合、異なる研究 をプールすることができないことを意味するものではない。単一の研究からは限られた結論を導き出すことができるのみ である。それにもかかわらず、報告書では、さらなる検討と調査に値する農薬と健康影響との間の多くの関連性が強調 EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

genuine heterogeneity the different studies cannot be pooled. Limited conclusions can be made from a single study. Nonetheless, the report highlighted a number of associations between pesticides and health effects that merit further consideration and investigation. Of interest is the fact that a considerable proportion of the published literature focused on pesticides no longer approved for use in the EU and in most developed countries e.g. studies focusing solely on DDT and its metabolites constituted almost 10% of the eligible studies (Ntzani et al., 2013). These may still be appropriate since they may persist as pesticide residues or because they continue to be used in developing countries. Also, the report focused on epidemiological evidence in relation to any health outcome across an approximately 5-year window. Although the report is valuable in describing the field of epidemiological assessment of pesticide-health associations, it is not able to answer specific diseasepesticide questions thoroughly. A more in-depth analysis of specific disease endpoints associated with pesticides exposure is needed, where this information is available, and studies published earlier than the time window covered by the EFSA External scientific report should be also included.

#### 3.2. Limitations in study designs

For ethical reasons, randomised controlled trials are not allowed to test the safety of low dose pesticide exposure in the EU. Therefore, information on potential adverse health consequences in humans has to be extracted using observational studies.

For diseases with long-latency periods, measurement of exposure at one time point may not accurately reflect the long-term exposure which is needed to develop such diseases. This is particularly important for non-persistent pesticides, whose levels in biological samples are not constant but vary quite often. Thus, those studies that claim an association between a single measurement in urine samples and a long latency outcome should be carefully interpreted.

Among the 795 studies reviewed in the Ntzani report, 38% were case-control studies and 32% cross-sectional studies. As a result, evidence on potential adverse health consequences of pesticide exposure is largely based on studies that lack prospective design at least for outcomes that have long latency periods. For the cross-sectional studies, directionality cannot be assessed and observed associations may often reflect reverse causation (is the disease caused by the exposure, or does the disease influence the exposure?). Although reverse causation is a potential problem of cross-sectional studies in many fields of epidemiology, in pesticide epidemiology, it is less of an issue, because in most situations it is unlikely that a disease will cause exposure to pesticides.

Although case-control studies are frequently used for rare outcomes, such as several cancers, their main limitation is that they are prone to recall bias and they have to rely on retrospective assessment of exposure. However, they can still provide useful information, especially for rare outcomes. It is important to examine whether results from case-control and prospective studies converge. This was, for example, the case amongst studies that were conducted to examine associations between intake of trans-fatty acids and cardiovascular disease (EFSA, 2004), where both case-control and prospective studies consistently reported positive associations. The effect estimates between the two study designs were systematically different with prospective studies reporting more modest effect sizes but both study designs reached similar conclusions. As for pesticides, similar values have been observed for the magnitude of association between Parkinson's disease and pesticide exposure irrespective of the study design (reviewed in Hernández et al., 2016).

#### 3.3. Relevance of study populations

Because the environmentally relevant doses of pesticides to which individuals are exposed are lower than those required to induce observed toxicity in animal models, the associated toxic effects need to be understood in the context of differences of susceptibility of subpopulations. Potentially vulnerable groups are at an increased risk against exposure to low levels of pesticides than healthy individuals, sometimes during sensitive windows of exposure. This is the case of genetic susceptibility, which represents a critical factor for risk assessment that should be accounted for (Gómez-Martín et al., 2015). Genetic susceptibility largely depends on functional genetic polymorphisms affecting toxicokinetics (e.g. genes encoding xenobiotic metabolising enzymes and membrane transporters) and/or toxicodynamics (e.g. different receptor gene polymorphisms). This genetic variability should be considered on the basis of a plausible scientific hypothesis.

While different disorders, particularly neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis) have been linked to exposures to environmental factors (e.g.

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されている。興味深いのは、公表されている文献のかなりの割合が EU 及びほとんどの先進国で使用が認可されてい ない農薬に焦点を当てているという事実である(例えば、DDTとその代謝物のみに焦点を当てた研究は、対象となる研 究の10%近くを占めている(Ntzaniら、2013年)。これらの研究は、残留農薬として残留している可能性があることや、 開発途上国で使用され続けていることから、まだ適切であるかもしれない。また、報告書では、約5年の期間にわたる あらゆる健康影響に関連した疫学的証拠に焦点を当てている。この報告書は、農薬-健康影響の関連性の疫学的評 価の分野を記述する上で有用なものではあるが、特定の疾患と農薬の問題に完全に答えることはできない。農薬ばく 露に関連する疾患エンドポイントのより詳細な解析が必要であり、そのような情報が入手できる場合には、EFSA の外 部科学研究報告書でカバーされている期間よりも前に発表された研究も含めるべきである。

# 3.2. 研究デザインの限界

倫理的な理由から、EU では低用量の農薬ばく露の安全性を試験するための無作為化比較試験は認められていな い。したがって、ヒトにおける潜在的な有害健康影響に関する情報は、観察による研究を用いて抽出する必要がある。

潜伏期間の長い疾患では、ある時点でのばく露量を測定しても、そのような疾患の発症に必要な長期ばく露量を正 確に再現できない可能性がある。これは、生物学的サンプル中の濃度が一定ではなく、頻繁に変動する非持続性農薬 の場合に特に重要である。したがって、尿サンプル中の単一の測定値と長期の潜伏期間の結果との間に関連性がある と断定する研究は、慎重に解釈されるべきである。

Ntzani の報告書で検討された 795 件の研究のうち、38%が症例対照研究であり、32%が横断研究であった。その 結果、農薬ばく露による潜在的な有害健康影響のエビデンスは、少なくとも潜伏期間が長い結果については、前向き な計画を欠いた研究に大部分が基づいている。横断研究では方向性を評価することができず、観察された関連性はし ばしば逆因果関係(病気はばく露によって引き起こされたのか、それとも病気がばく露に影響を与えたのか)をもたらす 可能性がある。逆因果関係は多くの疫学の分野で横断研究の潜在的な問題であるが、農薬疫学では、ほとんどの場 合、病気が農薬へのばく露を引き起こすことはほとんどないため、問題にはならない。

いくつかのがんなどのまれな転帰に対しては症例対照研究が頻繁に用いられるが、その主な限界は、想起バイアス がかかりやすく、ばく露の後ろ向き評価に頼らなければならないことである。しかしながら、特にまれな転帰に対しては 有用な情報を提供することはできる。症例対照研究と前向き研究の結果が一致するかどうかを調べることが重要である。 例えば、トランス脂肪酸の摂取量と心血管疾患との関連を調べるために実施された研究(EFSA, 2004 年)では、症例 対照研究と前向き研究の両方で一貫して正の関連が報告されている。2 つの研究デザイン間の効果推定値は控えめ な効果量が報告された前向き研究とは系統的に異なるが、どちらの研究デザインも同様の結論に達した。農薬に関し ては、研究デザインにかかわらず、パーキンソン病と農薬ばく露との間の関連の大きさについては、同様の値が観察さ れている(レビューは Hernández ら、2016 年)。

# **3.3.** 研究対象の妥当性

個人がばく露される農薬の環境的に適切な用量は、動物モデルで観察された毒性を誘発するのに必要な用量よりも 低いため、関連する毒性影響は、小集団の感受性の違いとの関連で理解する必要がある。潜在的に脆弱な集団は、 健康な個人よりも低用量の農薬へのばく露に対してリスクが高く、時にはばく露の敏感な時期にばく露されることもある。 これは遺伝的感受性の場合であり、これはリスク評価のために説明されるべき重要な要因である(Gómez-Martín ら、 2015年)。遺伝的感受性は、毒物動態に影響を与える機能的な遺伝的多型(例えば、異物代謝酵素及び膜トランスポ ーターをコードする遺伝子)及び/または毒力学に影響を与える機能的な遺伝的多型(例えば、異なる受容体遺伝子) 多型)に大きく依存する。この遺伝的多様性は、妥当な科学的仮説に基づいて考慮されるべきである。

さまざまな障害、特に神経変性疾患(パーキンソン病、アルツハイマー病、筋萎縮性側索硬化症)は、環境因子(例 えば農薬)へのばく露と結び付けられてきたが、多くの場合、病気の遺伝子構造は考慮されていない。特定の集団では、 特定の遺伝子変異の有病率は 5-10%に達し、時には症例の 20%を超えることもある(Gibson ら、2017 年)ので、農薬 ばく露とこれらの疾患の関連性は、研究対象となる集団内の遺伝的構造によって大きく影響を受ける可能性がある。こ EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

pesticides), in many instances the genetic architecture of the disorder has not been taken into account. The prevalence of specific gene mutations may reach 5–10% and sometimes over 20% of cases in certain populations (Gibson et al., 2017), so that the links of these diseases to pesticide exposure may be heavily influenced by genetic structure within populations under study. Given the small effect sizes for many of these disorders, the underlying effects of specific genes not accounted for in the study design may modify the disease risk estimates. Hence, associations with pesticide exposure may need to be evaluated in the light of common genetic influences known to be associated with a spectrum of neurodegenerative diseases. However, genetic variation by itself does not predispose people for an increased pesticide exposure.

A subgroup of population of special interest is represented by children, because their metabolism, physiology, diet and exposure patterns to environmental chemicals differ from those of adults and can make them more susceptibile to their harmful effects. The window(s) of biologic susceptibility remain unknown for the most part, and would be expected to vary by mechanism. Gender-based susceptibility also merits consideration in case of pesticide-related reproductive toxicity and endocrine disruption. Those subgroups are currently considered during the risk assessment process but may deserve more attention to provide additional protection.

#### 3.4. Challenges in exposure assessment

The main limitations of epidemiological studies conducted on pesticides derive from uncertainty in exposure assessment. Limitations include the fact that most currently approved pesticides tend to have short elimination half-lives and that their use involves application of various formulations depending on the crop and season. As a result, accurate assessment needs to capture intermittent long-term exposure of these non-persistent chemicals as well as being able to quantify exposure to individual pesticides.

Numerous studies have assessed internal exposure by measuring urinary non-active metabolites common for a large group of pesticides (for example, dialkyl phosphates for organophosphates, 3-phenoxybenzoic acid for pyrethroids or 6-chloronicotinic acid for neonicotinoids). These data should not be utilised to infer any risk because: (a) a fraction of these metabolites might reflect direct exposure through ingestion of preformed metabolites from food and other sources, rather than ingestion of the parent compound and (b) the potency of the different parent pesticides can vary by orders of magnitude. Thereby, HBM data based on those urine metabolites can be unhelpful unless they are paired with other data indicating the actual pesticide exposure.

Ideally exposure should be quantified on an individual level using biomarkers of internal dose. As most available biomarkers reflect short term (few hours or days) exposure and given the cost and difficulty of collecting multiple samples over time, many studies quantify exposure in terms of external dose. Quantitative estimation of external dose needs to account for both frequency and duration of exposure and should preferably be done on an individual but not group level. Often external exposure is quantified using proxy measures such as:

- subject- or relative-reported jobs, job titles, tasks or other lifestyle habits which are being
  associated with the potential exposure to or actual use of pesticides in general;
- handling of a specific product or set of products and potential exposure to these as documented through existing pesticide records or diaries or estimated from crops grown;
- environmental data: environmental pesticide monitoring, e.g. in water, distance from and/or duration of residence in a particular geographical area considered to be a site of exposure.

In many cases, these proxy measures are recorded with use of questionnaires, which can be either interviewer-administered or based on self-report. However, questionnaire data often rely on individual recall and knowledge and are thus potentially subject to both recall bias and bias introduced by the interviewer or study subjects. These sources of bias can to some extent be quantified if the questionnaires are validated against biomarkers (that is, to what extent do individual questions predict biomarker concentrations in a sub-sample of participants). If the exposure is assessed retrospectively the accuracy of the recall is for obvious reasons more likely to be compromised and impossible to validate. When exposure is based on records, similar difficulties may occur due to, e.g. incomplete or inaccurate records.

In many previous studies, duration of exposure is often used as a surrogate of cumulative exposure, assuming that exposure is uniform and continuous over time (e.g. the employment period) but this assumption must be challenged for pesticides. Although for some chemicals the exposure patterns may be fairly constant, exposures for the large number of pesticides available in the market

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れらの疾患の多くの効果量が小さいことを考えると、研究デザインでは考慮されていない特定の遺伝子の根本的な効 果が、疾患リスクの推定値を修正する可能性がある。したがって、農薬ばく露との関連は、一連の神経変性疾患に関連 することが知られている一般的な遺伝的変異に照らして評価する必要があるかもしれない。しかし、遺伝的変異はそれ 自体が人々を農薬ばく露の増加に向かわせるものではない。

特に注目すべきサブグループは子供であり、彼らの代謝、生理、食生活、環境化学物質へのばく露パターンは成人 とは異なり、有害な影響を受けやすくなるからである。生物学的感受性の窓口はほとんどの場合不明のままであり、メカ ニズムによって異なると予想される。性別に基づく感受性は、農薬に関連した生殖毒性や内分泌かく乱の場合にも考 慮する必要がある。これらのサブグループは現在リスクアセスメントの過程で考慮されているが、追加的な保護を提供 するためには、より注意を払う必要があるかもしれない。

# 3.4. ばく露評価における課題

農薬に関する疫学研究の主な限界は、ばく露評価の不確実性に由来する。現在認可されているほとんどの農薬は、 消失半減期が短い傾向があり、作物や季節に応じて様々な製剤を散布しなければならないという事実もその限界に含 まれている。その結果、正確な評価には、これらの非難分解性化学物質の間欠的な長期ばく露を把握し、個々の農薬 へのばく露を定量化する必要がある。

多くの研究では、大規模な農薬群に共通する尿中の非活性代謝物(例えば、有機リン酸塩の場合はジアルキルリン 酸塩、ビレスロイドの場合は3・フェノキシ安息香酸、ネオニコチノイドの場合は6・クロロニコチン酸)を測定することで内 部ばく露を評価している。これらのデータを、以下の理由からリスクを推測するために利用すべきではない。(a)これら の代謝物の一部は、親化合物を摂取するのではなく、食品やその他の供給源から事前に生成された代謝物を摂取す ることで直接ばく露を反映する可能性があり、(b)異なる親化合物農薬の効果は桁違いに異なる可能性があるからであ る。そのため、これらの尿中代謝物に基づくHBMデータは、実際の農薬ばく露量を示す他のデータと組み合わせな い限り、役に立たない可能性がある。

理想的には、内部ばく露量を示すバイオマーカーを使用して個人レベルでばく露量を定量化する必要がある。利用 可能なバイオマーカーのほとんどは短期間(数時間または数日)のばく露を対象としており、長期にわたって複数のサ ンプルを収集するコストと困難さを考えると、多くの研究では外部ばく露量として定量化されている。外部ばく露量の定 量的な推定には、ばく露の頻度と期間の両方を考慮する必要があり、グループレベルではなく個人レベルで行うことが 望ましい。多くの場合、外部ばく露は以下のような代理指標を用いて定量化される。

- 一般的な農薬への潜在的ばく露または実際の使用に関連する、対象者または親族が報告した仕事、職種、作業、 その他のライフスタイルの習慣。
- 特定の製品または製品群の取り扱いと、既存の農薬の記録や日誌を通じて文書化された、または栽培された作物から推定された、これらへの潜在的なばく露。
- 環境データ:環境農薬モニタリング、例えば水中、ばく露場所と考えられる特定の地理学上の地域からの距離及び /または居住期間。

多くの場合、これらの代理指標測定は、質問者によるものでも、自己申告に基づくものでもよいアンケート調査を用い て記録される。しかし、アンケートデータはしばしば個人の想起と知識に依存しており、想起バイアスや質問者や被験者 によってもたらされるバイアスの影響を受ける可能性がある。これらのバイアスの原因は、アンケートがバイオマーカーに 対して検証されていれば、ある程度定量化することができる(つまり、個々の質問が参加者のサンプルにおけるバイオマ ーカー濃度をどの程度予測しているか)。ばく露が後ろ向きに評価された場合、明らかな理由により、想起の精度が損 なわれ、検証が不可能になる可能性が高くなる。ばく露が記録に基づいて評価される場合も、例えば記録が不完全で あったり、不正確であったりすることで、同様の困難が生じる可能性がある。

これまでの多くの研究では、ばく露の持続時間が累積ばく露の代用として使用されることが多く、ばく露は時間的に 均一で連続的であると仮定している(例えば、雇用期間)が、農薬の場合はこの仮定に疑問を呈しなければならない。 一部の化学物質ではばく露パターンはかなり一定であるかもしれないが、市場に出回っている多数の農薬のばく露は、 www.efsa.europa.eu/efsalourna EFSA Journal 2017;15(10):5007

will vary with season, by personal protective equipment (PPE) and by work practices, and in many cases, uses are not highly repetitive. At an individual level, exposures can vary on a daily and even hourly basis, and often involve several pesticides. This temporal variability can result in particularly high variation in systemic exposures for pesticides with short biological half-lives and considerable uncertainty in extrapolating single or few measurements to individual exposures over a longer term. Hence, many repeated measurements over time may be required to improve exposure estimates.

#### 3.5. Inappropriate or non-validated surrogates of health outcomes

Self-reported health outcomes are frequently used in epidemiological research because of the difficulty of verifying responses in studies with large samples and limited funds, among other reasons. Although a number of studies have examined agreement between self-reported outcomes and medical records, the lack of verification of such metrics can lead to misclassification, particularly in large population-based studies, which may detract from reliability of the associations found.

Reliance on clinically manifested outcomes can increase the likelihood that individuals who have progressed along the toxicodynamic continuum from exposure to disease but have not yet reached an overt clinical disease state will be misclassified as not having the disease (Nachman et al., 2011). Thereby, delay in onset of clinical symptoms following exposure may cause underreporting where clinical assessment alone is used at an inappropriate point in time.

In the case of carcinogenesis, there are some examples where subclinical outcomes have been assessed as preneoplastic lesions with potential to progress to neoplastic conditions. This is the case of monoclonal gammopathy of undetermined significance (MGUS), which has been associated with pesticide exposure in the AHS (Landgren et al., 2009), as this condition has a 1% average annual risk of progression to malignant multiple myeloma (Zingone and Kuehl, 2011). However, it is difficult to predict if and when an MGUS will progress to multiple myeloma. Since there are studies indicating that pesticide exposure may be associated with the risk of precancerous lesions in animal research, a combined epidemiological analysis of both preneoplastic and neoplastic outcomes may increase the power of such an analysis.

Surrogate outcomes may seem an attractive alternative to clinically relevant outcomes since there may be various surrogates for the same disease and they may occur sooner and/or be easier to assess, thereby shortening the time to diagnosis. A valid surrogate endpoint must, however, be predictive of the causal relationship and accurately predict the outcome of interest. In addition, these surrogates should be relevant to the mode of action of a pesticide such that they should be anchored to established toxicological endpoints to support their predictivity. Although surrogate markers may correlate with an outcome, they may not capture the effect of a factor on the outcome. This may be because the surrogate may not be causally or strongly related to the clinical outcome, but only a concomitant factor, and thus may not be predictive of the clinical outcome. The validity of surrogate outcomes may thus represent a major limitation to their use (la Cour et al., 2010).

However, concerns arise as to whether critical regulatory decisions can be made based on epidemiological studies that did not directly measure the adverse health outcome but valid surrogates instead. The use of surrogates as replacement endpoints should be considered only when there is substantial evidence to establish their reliability in predicting clinical meaningful effects.

#### 3.6. Statistical analyses and interpretation of results

The statistical analyses and the interpretation of scientific findings that appear in the epidemiological literature on the relationship between pesticides and health outcomes do not substantially deviate from those reported in other fields of epidemiological research. Therefore, the advantages and limitations of epidemiological studies presented in Section 2.5 also apply to the epidemiological studies on pesticides.

The few distinctive features of the epidemiological studies on pesticides include the following: (a) sparse use of appropriate statistical analyses in the presence of measurement errors when assessing exposure to pesticides and (b) paucity of information on other important factors that may affect the exposure-health outcome relationship. These features are expanded on in the following paragraphs.

a) Statistical analyses in the presence of measurement errors

The difficulties inherent in correctly measuring exposure are frequent in many areas of epidemiological research, such as nutritional epidemiology and environmental epidemiology. It is not

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季節や個人用保護具(PPE)、作業習慣によって異なり、多くの場合、使用の反復性は高くない。個人のレベルでは、 ばく露量は日ごと、時間ごとに異なることがあり、多くの場合、複数の農薬が関与している。この時間的変動性は、生物 学的半減期が短い農薬の全身ばく露において特に高い変動性をもたらし、長期にわたって個人のばく露に単一または 少数の測定値を外挿する際にかなりの不確実性をもたらす可能性がある。したがって、ばく露の推定値を改善するため には、時間をかけて多くの測定を繰り返すことが必要になるかもしれない。

# 3.5. 不適切な、あるいは検証されていない健康影響のサロゲート

疫学研究では自己申告による健康影響が頻繁に用いられているが、その理由は、大規模なサンプルや限られた資 金を用いた研究では、回答を検証することが困難であることなどが挙げられる。多くの研究では、自己報告された影響 と医療記録との一致について調べられているが、このような指標の検証が不足しているために、特に大規模な集団ベ ースの研究では、誤分類につながる可能性があり、発見された関連性の信頼性を損なう可能性がある。

臨床的に明らかになった結果に依存すると、ばく露から疾患への毒力学的連続して進行したが、まだ明らかな臨床 的疾患状態に達していない人が、疾患を持っていないと誤分類される可能性が高くなる可能性がある(Nachman ら、 2011 年)。そのため、ばく露後の臨床症状の発現が遅れると、不適切な時期に臨床評価だけが用いられた場合、過小 申告の原因となることがある。

発がん性の場合、無症状であるが、腫瘍性状態に進行する可能性のある前腫瘍性病変として評価されている例があ る。これは、AHS における農薬ばく露と関連している有効性が未決定である単クローン性ガンマグロブリン血症 (MGUS)の例であり(Landgren 6、2009 年)、この状態は悪性多発性骨髄腫に進行するリスクが年平均 1%である (Zingone 及び Kuehl、2011)。しかし、MGUS が悪性多発性骨髄腫に進行するかどうか、いつ、どのように進行する かを予測することは困難である。動物実験では、農薬ばく露が前がん病変のリスクと関連している可能性があることを示 す研究があるので、前がん病変とがん病変の両方の転帰を組み合わせた疫学的解析を行うことで、そのような解析の 検出力を高めることができるかもしれない。

代替健康影響は、臨床的に関連する転帰に代わる注目される選択肢である。なぜならば、同じ疾患の様々な代替健康影響が存在し、それらの健康影響はより早く発生したり、評価が容易であったりして、診断までの時間を短縮できるからである。しかし、有効な代替エンドボイントは、因果関係を予測し、対象となる健康影響を正確に予測するものでなければならない。さらに、これらの代替指標は農薬の作用機序に関連していなければならず、その予測性を裏付けるために、確立された毒物学的エンドポイントに固定されていなければならない。代替指標は健康影響と相関があるかもしれないが、健康影響に対する要因の効果を捉えていないかもしれない。これは、代替指標が臨床健康影響の因果関係または強く関連しているのではなく、付随する要因に過ぎないため、臨床健康影響の予測にはならない可能性があるからである。このように、代替健康影響の妥当性は、サロゲートを使用する上での大きな制限となりうる(la Cour 6、2010年)。

しかし、有害健康影響を直接測定したのではなく、代わりに有効な代替指標を用いた疫学研究に基づいて、規制上 の政策決定を下すことができるかどうかについては、懸念がある。代替エンドポイントとしての代替指標の使用は、臨床 的に意味のある影響を予測する上での信頼性を立証する十分なエビデンスがある場合にのみ検討されるべきである。

#### 3.6. 統計解析と結果の解釈

農薬と健康影響との関係に関する疫学的文献にみられる統計解析と科学的知見の解釈は、他の分野で報告された 疫学研究と大きく異なるものではない。したがって、2.5 節で示した疫学研究の利点と限界は、農薬に関する疫学研究 にも適用される。

農薬の疫学研究のいくつかの特徴は以下の通りである。(a) 農薬へのばく露を評価する際の測定誤差の存在下で の適切な統計解析を行うことが少ないこと、(b) ばく露ー健康影響の関係に影響を及ぼす可能性のある他の重要な因 子に関する情報が不十分なことである。これらの特徴については、次の段落で詳しく説明する。

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a)測定誤差のある統計解析

easy to gauge the short- and long-term exposure outside controlled laboratory experimental settings. In large populations, individuals are exposed to a variety of different agents in a variety of different forms for varying durations and with varying intensities.

Unlike nutritional or environmental epidemiology, however, pesticide epidemiology has so far made little use of statistical analyses that would appropriately incorporate measurement errors, despite their wide availability and sizable literature on the topic. A direct consequence of this is that the inferential conclusions may not have been as accurate and as precise as they could have been if these statistical methods were utilised (Bengtson et al., 2016; Dionisio et al., 2016; Spiegelman, 2016).

#### b) Information on other important factors of interest

Identifying and measuring the other relevant factors that might affect an outcome of interest is a recurrent and crucial issue in all fields of science. For example, knowing that a drug effectively cures a disease on average may not suffice if such drug is indeed harmful to children or pregnant women. Whether or not age, pregnancy and other characteristics affect the efficacy of a drug is an essential piece of information to doctors, patients, drug manufacturers and drug-approval agencies alike.

Pesticide epidemiology provides an opportunity for careful identification, accurate measuring and thorough assessment of possible relevant factors and their role in the exposure-health outcome relationship. Most often, relevant factors have been screened as potential confounders. When confounding effects were detected, these needed to be adjusted for in the statistical analyses. This has left room for further investigations that would shed light on this important issue by reconsidering data that have already been collected and that may be collected in future studies. The statistical methods in the pesticide literature have been mainly restricted to standard applications of basic regression analyses, such as binary probability and hazard regression models. Potentially useful analytical approaches, such as propensity score matching, mediation analyses, and causal inference, would be helpful for pesticide epidemiology (Imbens and Rubin, 2015).

#### 4. Proposals for refinement to future epidemiological studies for pesticide risk assessment

This section is aimed at addressing methods for assessment of available pesticide epidemiological studies and proposals for improvement of such studies to be useful for regulatory purposes.

When considering the potential regulatory use of epidemiological data, many of the existing epidemiological studies on pesticides exposure and health effects suffer from a range of methodological limitations or deficiencies which limit their value in the assessment of individual active substances. Epidemiological studies on pesticides exposure and health effects would ideally generate semi-quantitative data or be able to have greater relevance to quantitative risk assessment with respect to the output from prediction models. This would allow epidemiological results to be expressed in terms more comparable to the quantitative risk assessments, which are more typically used in evaluating the risks of pesticides. The question arises how such epidemiological data could be considered for risk assessment when judged in comparison to the predictive models. A precisely measured quantitative dose-response relationship is presently rarely attainable as a result of current pesticide epidemiological studies.

The quality, reliability and relevance of the epidemiological evidence in relation to pesticide exposure and health effects can be enhanced by improving (a) the quality of each individual study and (b) the assessment of the combined evidence accrued from all available studies.

# 4.1. Assessing and reporting the guality of epidemiological studies

The quality and relevance of epidemiological research should be considered when selecting epidemiological studies from the literature for use in risk assessment. The quality of this research can be enhanced by (US-EPA, 2012; Hernández et al., 2016);

- a) an adequate assessment of exposure, preferentially biomarker concentrations at individual level reported in a way which will allow for a dose-response assessment;
- b) a reasonably valid and reliable outcome assessment (well-defined clinical entities or validated surrogates);
- c) an adequate accounting for potentially confounding variables (including exposure to multiple chemicals);
- d) the conduct and reporting of subgroup analysis (e.g. stratification by gender, age, ethnicity). 24

ばく露量を正確に測定するための困難さは、栄養疫学や環境疫学などの疫学研究の多くの分野で頻繁にみられる。 制御された実験室での実験環境の外で、短期及び長期のばく露を評価することが容易ではない。大規模集団では、個 人は様々な期間、様々な強度で様々な形で様々な薬剤にばく露されている。

しかし、栄養疫学や環境疫学とは異なり、農薬疫学では、測定誤差を適切に考慮した統計解析が広く利用可能であ り、このテーマに関する膨大な文献があるにもかかわらず、これまでのところほとんど利用されていない。その直接的な 結果として、これらの統計的手法が利用されていたとしても、推論的結論が正確で、精密なものではなかった (Bengtsonら、2016年; Dionisioら、2016年; Spiegelman、2016年)。

b)その他の重要な関係因子に関する情報

対象となる結果に影響を与える可能性のある他の関連因子を特定して測定することは、科学のすべての分野で繰り 返し起こる重要な問題である。例えば、ある薬が平均的に病気を効果的に治すということを知っていても、その薬が子 供や妊婦に有害であるかもしれない。年齢、妊娠、その他の特性が薬の有効性に影響を与えるかどうかは、医師、患者、 製薬会社、医薬品承認機関にとって重要な情報である。

農薬疫学は、可能性のある関連因子を慎重に特定し、正確に測定し、徹底的に評価し、ばく露と健康結果の関係に おけるそれらの役割を評価する機会を提供している。最も多くの場合、関連因子は潜在的な交絡因子としてスクリーニ ングされている。交絡因子の影響が検出された場合には、統計解析で補正する必要があった。このことは、すでに収集 されたデータや今後の研究で収集される可能性のあるデータを再検討することで、この重要な問題を明らかにするため の更なる調査の余地を残している。農薬の文献における統計的手法は、主に二項確率やハザード回帰モデルなどの 基本的な回帰分析の標準的な応用に限定されてきた。潜在的に有用な解析アプローチ、例えば、傾向スコアマッチン グ、媒介分析、因果推論などは、農薬疫学に役立つであろう(Imbens 及び Rubin、2015 年)。

# 4. 農薬リスク評価のための将来の疫学研究への再検討案

このセクションでは、利用可能な農薬の疫学的研究の評価方法と、規制目的に役立つようにそのような研究を改善 するための提案を取り上げることを目的としている。

疫学的データの潜在的な規制上の利用の可能性を検討する際に、農薬ばく露と健康影響に関する既存の疫学的 研究の多くは、個々の有効成分の評価においてその価値を制限する様々な方法論的限界や不完全性に悩まされてい る。農薬ばく露と健康影響に関する疫学研究は、半定量的なデータを生成するか、予測モデルのアウトプットに関して 定量的なリスク評価との関連性を高めることが理想的である。これにより、疫学的な結果は、農薬のリスク評価に一般的 に用いられる定量的なリスク評価に匹敵する言葉で表現できるようになる。このような疫学データを予測モデルと比較し てリスク評価を行う際に、どのように考えればよいのかという疑問が生じる。現行の農薬疫学研究の結果では、正確に測 定された定量的な用量反応関係はほとんど達成されていない。

農薬ば<露と健康影響に関連した疫学的証拠の質、信頼性、妥当性は、(a)個々の研究の質及び(b)利用可能な すべての研究から得られた複合的なエビデンスの評価を改善させることによって向上させることができる。

# 4.1 疫学研究の質の評価と報告

リスク評価に使用するために文献から疫学研究を選択する際には、疫学研究の質と関連性を考慮する必要がある。 この研究の質は、次のようにして高めることができる(US-EPA、2012年:Hernándezら、2016年)。

a) ばく露の適切な評価、特に個人レベルでのバイオマーカー濃度が用量反応評価を可能にする方法で報告されて いること。

b) 合理的に有効で信頼性の高い健康影響評価(よく観察された臨床症状または有効な代替指標)。

c)交絡変数(複数の化学物質へのばく露を含む)を適切に考慮していること。

d)サブグループ解析の実施と報告(例えば、性別、年齢、民族による層別化)。

生物医学的研究が多様な限界の対象となり、その限界に苦しむことは広く受け入れられている。農薬に関する疫学 EESA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

EFSA Journal 2017;15(10):5007

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www.efsa.europa.eu/efsajournal

It is widely accepted that biomedical research is subject to and suffers from diverse limitations. An assessment of weaknesses in the design, conduct and analysis of epidemiology research studies on pesticides is essential to identify potentially misleading results and identify reliable data.

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Guidelines and checklists help individuals meet certain standards by providing sets of rules or principles that guide towards the best behaviour in a particular area. Several tools and guidelines have been developed to aid the assessment of epidemiological evidence; however, there is no specific tool for assessing studies on pesticides. Although these studies have special considerations around exposure assessment that require specific attention, standard epidemiological instruments for critical appraisal of existing studies may apply. Existing reporting guidelines usually specify a minimum set of information needed for a complete and clear account of what was done and what was found during a research study focusing on aspects that might have introduced bias into the research (Simera et al., 2010).

A number of tools were specifically designed for quality appraisal of observational epidemiological studies, such as the Newcastle–Ottawa scale (NOS) and the Research Triangle Institute (RTI) item bank. The latter is a practical and validated tool which consists of a checklist of 29 questions for evaluating the risk of bias and precision of epidemiological studies of chemical exposures. In addition, the Biomonitoring, Environmental Epidemiology, and Short–Lived Chemicals (BEES-C) instrument was developed to evaluate the quality of epidemiological research that use biomonitoring to assess short-lived chemicals (LaKind et al., 2015), but it can also be used for persistent chemicals and environmental measures as its main elements are cross-cutting and are more broadly applicable. Two earlier efforts to develop evaluative schemes focused on epidemiology research on environmental chemical exposures and neurodevelopment (Amler et al., 2006; Youngstrom et al., 2011).

Regarding quality of reporting, the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) Network, officially launched in June 2008, is an international initiative that promotes transparent and accurate reporting of health research studies. It currently lists over 90 reporting guidelines with some of them being specific for observational epidemiological studies (e.g. Strengthening the Reporting of OBservational studies in Epidemiology (STROBE)). The STROBE statement includes recommendations on what should be included in an accurate and complete report of an observational study including cross-sectional, case-control and cohort studies using a checklist of 22 items that relate to the title, abstract, introduction, methods, results and discussion sections of articles (von Elm et al., 2007). The STROBE statement has been endorsed by a growing number of biomedical journals which refer to it in their instructions for authors. Table 1 presents a summary of the main features that STROBE proposes to be taking into account when assessing the quality of reporting epidemiological studies. Extensions to STROBE are available including the STROBE Extension to Genetic Association studies (STREGA) initiative and the STROBE-ME statement for assessment of molecular epidemiology studies. Since the STROBE checklist mentions only in a general way exposure and health outcomes, the PPR Panel recommends that an extension of the STROBE statement be developed, for inclusion in the EOUATOR network library, specifically relevant to the area of pesticide exposure and health outcomes. This would greatly assist researchers and regulatory bodies in the critical evaluation of study guality.

Table 1: Main features of the STROBE tool to assess quality of reporting of epidemiological studies

STROBE Statement Items		
Factor	Item	Recommendation
Title and Abstract		
	1	<ul> <li>a) Indicate the study's design with a commonly used term in the title of the abstract</li> <li>b) Provide in the abstract an informative and balanced summary of what was done and what was found</li> </ul>
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up and data collection

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研究の計画、実施、解析における弱点の評価は、誤解を招く可能性のある結果を特定し、信頼性の高いデータを特定 するために不可欠である。

ガイドラインやチェックリストは、特定の分野での最良の行動に向けて導く一連のルールや原則を提供することで、個人が特定の基準を満たすのを助けるものである。疫学的証拠の評価を助けとなるためにいくつかのツールやガイドラインが開発されているが、農薬に関する研究を評価するための特定のツールは存在しない。これらの研究には、特定の注意を必要とするばく露評価に関する特別な考慮事項があるが、既存の研究を批判的に評価するための標準的な疫学的手法を適用できる可能性がある。現行の報告ガイドラインは通常、研究にバイアスをもたらした可能性のある側面に焦点を当て、調査研究中に何が行われ、何が発見されたかを完全かつ明確に説明するために必要な最低限の情報を規定している(Simera6, 2010年)。

観察による疫学研究の品質評価のために、Newcastle-Ottawa スケール(NOS)や Research Triangle Institute (RTI)のアイテムバンクなど、多くのツールが特別に計画されている。後者は、化学物質ばく露の疫学研究のバイアス のリスクと精度を評価するための 29 の質問のチェックリストからなる実用的で検証済みのツールである。また、バイオモ ニタリング、環境疫学、短命化学物質(BEES-C)は、バイオモニタリングを用いて短命化学物質を評価する疫学研究 の質を評価するために開発されたが(LaKind ら、2015 年)、主な要素が横断であり、より広範囲に適用可能であるた め、難分解性化学物質や環境対策にも利用できる。評価スキームの開発に向けた 2 つの先行研究は、環境化学物質 ばく露と神経発達に関する疫学研究に焦点を当てたものである(Amler ら、2006 年; Youngstrom ら、2011 年)。

報告の質に関しては、2008 年 6 月に発足した EQUATOR ネットワーク(Enhancing the QUAlity and Transparency Of health Research (EQUATOR) Network)は、健康調査研究の明白性と正確な報告を促進する 国際的な取り組みである。現在、90 以上の報告ガイドラインがリストアップされており、その中には観察による疫学研究 に特化したものもある(例:Strengthening the Reporting of OBservational studies in Epidemiology (STROBE))。STROBE ステートメントには、論文のタイトル、要約、緒言、方法、結果、考察のセクションに関連する 22 項目のチェックリストを使用して、横断、症例対照、コホート研究を含む観察による研究の正確で完全な報告に何を 含めるべきかについての勧告が含まれている(von Elm ら、2007 年)。STROBE ステートメントは、著者への指示書の 中で言及されている生物医学雑誌の数が増えてきており、支持されている。表 1 は、疫学研究の報告の質を評価する 際に STROBE が考慮すべき主な特徴をまとめたものである。STROBE の拡張機能として、STROBE Extension to Genetic Association studies (STREGA) や分子疫学研究の評価のための STROBE・ME ステートメントがある。 STROBE チェックリストではばく露と健康の影響について一般的にしか言及されていないため、PPR パネルは、農薬 ばく露と健康影響の分野に特化した EQUATOR ネットワークライブラリに含めるための STROBE ステートメントの拡張 版を開発することを推奨する。これは、研究者や規制機関が研究の質を批判的に評価する際に大いに役立つであろう。

#### 表 1:疫学研究報告の質を評価するための STROBE ツールの主な特徴

ファクター	項目	推奨
タイトルと概要		
	1	a) 要約のタイトルに一般的に使用される用語を用いて、研究の計画を示す。 b) 何が行われ、何が発見されたかについて、有益でパランスのとれた要約を記載する
序章		
背景・根拠	2	報告された調査の科学的背景と根拠を説明する。
目的	3	事前に決められた仮説を含めた具体的な目的を述べる。
方法		
研究デザイン	4	研究デザインの重要な要素を論文の前の方で提示する。
設定	5	募集期間、ばく露期間、追跡調査期間、データ収集期間を含めて、環境、場所、関連す 日付を記述する。
参加者	6	a) コホート研究-参加資格の基準、参加者の選択の情報源と方法を示す。フォローア プの方法を記述する。 症例対照研究-適格性の基準及び症例の確認及び対照の選択の情報源と方法を示す。



Factor	Item	Recommendation
Participants	6	<ul> <li>a) Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study – Give the eligibility criteria, and the sources and methods of cases ascertainment and control selection. Give the rationale for the choice of cases and controls</li> <li>Cross-sectional study – Give eligibility criteria, and the sources and methods of selection of participants</li> <li>b) Cohort study – For matched studies, give matching criteria and the number of exposed and unexposed</li> <li>Case-control study – For matched studies, giving matching criteria and the number of controls per case</li> </ul>
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurements	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	<ul> <li>a) Describe all statistical methods, including those used to control for confounding</li> <li>b) Describe any methods used to examine subgroups and interactions</li> <li>c) Explain how missing data were addressed</li> <li>d) Cohort study – If applicable, explain how loss to follow-up was addressed Case-control study – If applicable, explain how matching of cases and controls was addressed</li> <li>Cross-sectional study – If applicable, describe analytical methods taking account of sampling strategy</li> <li>e) Describe any sensitivity analyses</li> </ul>
Results		
Participants	13*	<ul> <li>a) Report numbers of individuals at each stage of study – e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up and analysed</li> <li>b) Give reasons for non-participation at each stage</li> <li>c) Consider use of a flow diagram</li> </ul>
Descriptive data	14*	<ul> <li>a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders</li> <li>b) Indicate number of participants with missing data for each variable of interest</li> <li>c) Cohort study – Summarise follow-up time (e.g. average and total amount)</li> </ul>
Outcome data	15*	Cohort study – Report numbers of outcome events or summary measures over time Case-control study – Report numbers in each exposure category, or summary measures of exposure Cross-sectional study – Report numbers of outcome events or summary measures
Main results	16	<ul> <li>a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included</li> <li>b) Report category boundaries when continuous variables were categorised</li> <li>c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period</li> </ul>

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		症例及び対照の選択の根拠を示す。 横断研究-参加資格の基準及び参加者の選択の情報源と方法を示す。 b) コホート研究-マッチさせた研究については、マッチさせた基準とばく露者と未ばく
		露者の数を示す。 症例対照研究-マッチさせた研究の場合、マッチさせた基準と症例ごとの対照の数を 示す。
変数	7	すべての転帰、ばく露、予測因子、潜在的交絡因子及び効果修飾因子を明確に示す。該当 する場合は診断基準を示す
データソース/測 定	8*	対象となる各変数について、データの出所と評価 (測定) 方法の詳細を述べる。複数のク ループがある場合は、評価方法の類似性を記述する。
バイアス	9	バイアスの原因となる可能性のあるものに対処するための対応を記述する。
研究サイズ	10	研究規模がどのようにして決定されたか説明する。
量的変数	11	分析において量的変数がどのように扱われたかを説明する。該当する場合は、どのグル ープ分けが選択されたか、またその理由を説明する。
統計的手法	12	<ul> <li>a) 交絡因子をコントロールするために使用したものを含め、すべての統計的方法を記述 する。</li> </ul>
		b)サブグループと相互作用を調べるために使用された方法を記述する。
		c)不足しているデータにどのように対処したかを説明する。
		d) コホート研究-該当する場合、追跡調査までの期間の損失がどのように対処されたか
		を説明する。
		症例対照研究-該当する場合、症例と対照のマッチングがどのように対処されたかを 説明する。
		武円9 G。 横断研究-該当する場合は、サンプリング戦略を考慮した分析方法を記述する。
		e) 感度分析を記述する
結果		
参加者	13*	<ul> <li>a)研究の各段階における個人の数を報告する一例えば、適格性の可能性がある、適格性の審査を受けた、適格性があると判断された、研究に参加した、追跡調査を完了した、 分析をうけた、など。</li> <li>b)各段階で不参加の理由を述べる。</li> </ul>
		c) フロー図の使用を検討する。 
記述データ	14*	<ul> <li>a)研究参加者の特徴(例:人口学的、臨床的、社会的)及びばく露及び潜在的交絡因子に関する情報を提供する。</li> </ul>
		に因りる情報を提供りる。 b)対象となる各変数について、データが欠落している参加者の数を示す。
		c)コホート研究一追跡調査期間の要約(平均値や総量など)。
アウトカムデータ	15*	コホート研究-時間経過に伴うアウトカムの数または要約評価尺度の報告。
		症例対照研究-各ばく露カテゴリーの数値、またはばく露の要約評価尺度を報告する。
		横断研究-アウトカムの数または要約評価尺度の報告。
主な結果	16	a)未調整推定値及び該当する場合は交絡因子調整済み推定値とその精度を示す(例:95%
		信頼区間)。どの交絡因子が調整されたか及びそれらが含まれている理由を明確にす
		b)連続変数が分類された場合のカテゴリー境界を報告する。
		c) 関連性がある場合、相対リスクの推定値を意味のある期間の絶対リスクに変換することを検討する。
その他の分析	17	実施したその他の分析(サブグループと相互作用の分析、感度分析など)を報告する。



STROBE Statement Items			
Factor	Item	Recommendation	
Other analyses	17	Report other analyses done – e.g. analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information	n		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

\*: Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Selective reporting can occur because non-significant results or unappealing significant results may not be published. Investigators should avoid the selective reporting of significant results and high-risk estimates. In this regard, standardisation of reporting of epidemiological studies could help to reduce or avoid selective reporting. The STROBE statement and similar efforts are useful tools for this purpose. Although some epidemiological research will remain exploratory and *post hoc* in nature, this should be clarified in the publications and selective reporting minimised, so that epidemiological findings could be interpreted in the most appropriate perspective (Kavvoura et al., 2007).

Preregistration of studies and prepublication of protocols are the measures taken by some Journal editors and Ethics Committees to reduce reporting bias and publication bias in clinical trials on pharmaceuticals. Although a similar proposal has been suggested for observational epidemiological studies in order to be conducted as transparently as possible to reduce reporting bias and publication bias, there is no consensus among epidemiologists (Pearce, 2011; Rushton, 2011). In contrast, a number of initiatives have been undertaken by professional societies to foster good epidemiological practice. This is the case, for example, of the International Epidemiological Association (IEA, 2007) or the Dutch Society for Epidemiology on responsible epidemiologic Research Practice (DSE, 2017).

Data quality assessment of formal epidemiological studies is based solely on the methodological features of each individual study rather than on the results, regardless of whether they provide evidence for or against an exposure/outcome association. However, for risk assessment, it is important to assess not only the quality of study methods but also the quality of the information they provide. Indeed, good studies may be dismissed during the formal quality assessment by the poor reporting of the information.

#### 4.2. Study design

Well conducted prospective studies with appropriate exposure assessment provide the most reliable information and are less prone to biases. When prospective studies are available, results from studies of less robust design can give additional support. In the absence of prospective studies the results from cross-sectional and case-control studies should be considered but interpreted with caution. However, it is acknowledged that a well-designed case-control study may be superior to a less well designed cohort study. Analytical approaches should be congruent with the study design, and assumptions that the statistical methods required should be carefully evaluated.

Ideally observational studies for long-term diseases should be prospective and designed such that the temporal separation between the exposure and the health outcome is appropriate with respect to the time it takes to develop the disease. For outcomes such as cancer or cardiovascular diseases, which often have a long latency period (> 10 years), exposure should be assessed more than once prior to the outcome assessment. For other outcomes with a shorter latency period, such as immune function disturbances, the appropriate temporal separation may be in the range of days or weeks and a single exposure assessment may be adequate. In short, the ideal design of a study depends on the latency period for the outcome under consideration. The expected latency period the determines both the length of follow-up and the frequency for which the exposure has to be quantified.

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主要な結果	18	研究目的を参考にして、主要な結果を要約する。
限界事項	19	潜在的なバイアスや不正確さの原因を考慮に入れて、研究の限界について議論する。 落 在的なバイアスの方向性と大きさについて議論する。
解釈	20	目的、限界、分析の重複、類似研究の結果、関連エビデンスなどを考慮して、結果の全体 的な解釈を慎重に行う。
一般化可能性	21	研究結果の一般化可能性(外的妥当性)を考察する。
その他の情報		
資金調達	22	本研究の資金源と資金提供者の役割、また、該当する場合には、本論文の基礎となった 原著研究の資金源を述べる。

\*:症例対照研究では症例と対照について、また、コホート研究及び横断研究では、該当する場合には、ばく露群と非ばく露群につい て、別々に情報を提供する。

選択的報告は、有意性のない結果や注目されない有意な結果が公表されないことで起こる可能性がある。研究者は、 有意な結果やリスクの高い推定値の選択的報告を避けるべきである。この点では、疫学研究の報告の標準化は、選択 的な報告を減らすか回避するのに役立つであろう。STROBE 声明や同様の取り組みは、この目的のための有用なツ ールである。疫学研究の中には探索的でその場限りの性質を持つ研究もあるだろうが、これは出版物に明記されるべ きであり、選択的報告は最小限に抑えられるべきであり、そうすれば疫学研究の結果は最も適切な観点から解釈される (Kavvoura 6、2007 年)。

研究の事前登録とプロトコールの事前発表は、医薬品の臨床試験における報告バイアスと出版バイアスを減らすた めに、いくつかの雑誌の編集者と倫理委員会によって取られている措置である。観察による疫学研究についても、でき るだけ明白性をもって報告バイアスや出版バイアスを減らすことを実施するために、同様の提案がなされているが、疫 学者の間ではコンセンサスが得られていない(Pearce、2011年; Rushton、2011年)。これとは対照的に、優れた疫学 的実践を促進するための多くの取り組みが専門学会によって実施されてきた。例えば、国際疫学協会(IEA、2007年) やオランダ疫学協会の responsible epidemiologic Research Practice (DSE、2017年)などがその例である。

正式な疫学研究のデータの質の評価は、ばく露/影響の関連性についてのエビデンスを提供するか否かに関わらず、結果よりも個々の研究の方法論的特徴のみに基づいている。しかし、リスク評価においては、研究方法の質だけでなく、研究が提供する情報の質も評価することが重要である。実際、優れた研究であっても、正式な品質評価の際に、 情報の報告が不十分なために却下されてしまうことがある。

# 4.2. 研究デザイン

適切なばく露評価を行い、十分に実施された前向き研究は、最も信頼性の高い情報を提供し、バイアスがかかりにく い。前向き研究が利用可能な場合には、計画があまり強固でない研究の結果が追加的な裏付けとなることがある。前 向き研究がない場合は、横断研究や症例対照研究の結果を考慮すべきであるが、慎重に解釈すべきである。しかし、 適切に計画された症例対照研究は、あまり適切に計画されていないコホート研究よりも優れている可能性があることは 認められている。解析的アプローチは研究デザインに合致したものでなければならず、必要とされる統計的手法の仮 定は慎重に評価されるべきである。

長期疾患の観察による研究は前向きであることが理想的であり、ばく露と健康影響との間の時間区分は、疾患の発症に要する時間に関して適切であるように計画されるべきである。がんや心血管疾患のように潜伏期間が長い(10年以上)ことが多い健康影響については、健康影響の評価に先立って複数回のばく露評価を行うべきである。免疫機能障害のような潜伏期間の短い他の健康影響では、適切な時間区分は数日から数週間の範囲であり、1回のばく露評価で十分であろう。要するに、研究の理想的な計画は、検討されている健康影響の潜伏期間に依存する。予測される潜伏期間は、追跡調査の長さとばく露量が定量化されなければならない頻度の両方を決定する。

#### 4.3. Study populations

The EU population, which exceeds 500 million people, can be assumed to be fairly heterogeneous and so expected to include a number of more sensitive individuals that may be affected at lower doses of pesticide exposure. To address this, in stratified sampling, the target population is divided into subgroups following some key population characteristics (e.g. sex, age, geographic distribution, ethnicity or genetic variation) and a random sample is taken within each subgroup. This allows subgrouplations to be represented in a balanced manner in the study population.

Vulnerable populations should then be examined in epidemiological studies either through subgroup or sensitivity analysis. However, such analyses need to be defined *a priori*. In case of ad hoc subgroup sensitivity analysis, the statistical thresholds should be adjusted accordingly and the replication of results should follow. Evidence of vulnerable subpopulations would ideally involve prospective studies that include assessment of biomarkers of exposure, subclinical endpoints and disease incidence over time.

It may be impossible to find a threshold of a toxic-induced increase in disease in the population because a large number of people are in a preclinical state and would be sensitive to the low end of the dose-response curve. For that to be evident, the epidemiology data would need to characterise the relationship between chemical exposure and risk of disease in a broad cross-section of the population (or look at precursor lesions or key events) and allow a robust examination of a low-dose slope.

On the basis of the degree of evidence relevant to a vulnerable subpopulation, consideration should be given to whether dose-response assessment will focus on the population as a whole or will involve separate assessments for the general population and susceptible subgroups. If it is the population as a whole, the traditional approach is to address variability with uncertainty factors; it may also be possible to analyse the effect of variability on risk by evaluating how the risk distribution of the disease shifts in response to the toxicant. In essence, the risk distribution based on a subclinical biomarker is an expression of toxicodynamic variability that can be captured in dose-response assessment.

The alternative approach is to address vulnerable subpopulations as separate from the general population and assign them unique potencies via dose–response modelling specific to the groups that might be based on actual dose–response data for the groups, on adjustments for specific toxicokinetic or toxicodynamic factors, or on more generic adjustment or uncertainty factors. For a pesticide, if it is known that a particular age group, disease (or disease-related end-point), genetic variant or co-exposure creates unique vulnerability, efforts should be made to estimate the potency differences relative to the general population and on that basis to consider developing separate potency values or basing a single value on the most sensitive group or on the overall population with adjustments for vulnerable groups.

#### 4.4. Improvement of exposure assessment

The difficulties often associated with pesticide exposure assessment in epidemiological studies have been highlighted above. The description of pesticide exposure (in particular quantitative information on exposure to individual pesticides) is generally reported in insufficient detail for regulatory purposes and this limitation is difficult to overcome, especially for diseases with a long latency period (e.g. many cancers and neurodegenerative disorders).

It is noteworthy that the methods necessary to conduct exposure monitoring are to be submitted by the applicant in the dossier. The regulation requirements do ask for validated methods that can be used for determining exposure. The Commission Regulation (EU) No 283/2013, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of PPP on the market, addresses information on methods of analysis required to support both pre-approval studies and post-approval monitoring. In this context, the post-approval requirements are the most relevant and the regulation literally states:

 $^{\rm V4.2.}$  Methods for post-approval control and monitoring purposes – Methods, with a full description, shall be submitted for:

 a) the determination of all components included in the monitoring residue definition as submitted in accordance with the provisions of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin;

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# 4.3. 研究対象集団

EUの人口は5億人を超えており、かなり雑多であるため、低用量の農薬ばく露で影響を受ける可能性のある、より 感受性の高い個人が多数含まれていることが予想される。これに対処するために、層別サンプリングでは、いくつかの 主要な集団特性(性別、年齢、地理的分布、民族性、遺伝的変動など)に従って対象集団をサブグループに分割し、 各サブグループ内で無作為にサンプルを採取する。これにより、調査母集団の中でサブグループをバランスよく表現す ることができる。

次に、脆弱な集団は、小集団解析または感度分析のいずれかを用いて疫学研究で調査されるべきである。しかし、 そのような解析は事前に行う必要がある。事後的なサブグループ感度分析の場合、統計的閾値はそれに応じて調整さ れるべきで、結果の再現性はそれに従うべきである。脆弱な小集団のエビデンスは、理想的には、ばく露のバイオマー カー、前駆症状、経時的な疾患発生率の評価を含む前向き研究を必要とする。

多くの人が前臨床状態にあり、用量反応曲線の下端に敏感に反応してしまうため、集団における毒性による疾病の 増加の閾値を特定することは不可能かもしれない。このことを明らかにするためには、疫学データが、集団の広範な横 断面における化学物質ばく露と疾病リスクの関係を特徴づけ(あるいは前兆病変や重要事象を調べ)、低用量の傾きを しっかりと検討する必要がある。

脆弱な小集団に関連するエビデンスの程度に基づいて、用量反応評価が集団全体に焦点を当てるのか、一般集団 と影響を受けやすいサブグループに分けて評価するのかを検討すべきである。集団全体を対象とする場合、従来のア プローチでは、不確実性因子を用いて変動性に対処することになるが、毒性物質に反応して疾患のリスク分布がどのよ うに変化するかを評価することで、変動性のリスクへの影響を解析することも可能である。要するに、不顕性バイオマー カーに基づくリスク分布は、用量反応評価で捉えることができる毒力学的変動の表現である。

別のアプローチとしては、脆弱な小集団を一般集団とは別個のものとして扱い、その集団の実際の用量反応データ、 特定の薬物動態または毒物動態学的要因の調整、またはより一般的な補正や不確実性の要因に基づいた用量反応 モデルを用いて、その集団に固有の効果を割り当てることである。農薬については、特定の年齢層、疾患(または疾患 関連のエンドポイント)、遺伝的変異、または共ばく露が独特の脆弱性を生んでいることが分かっている場合には、一般 集団に対する効果の差を推定するよう努めるべきであり、それに基づいて、別個の効果を開発するか、または最も感受 性の高い集団または脆弱性のある集団に対する補正を加えた全体の集団に対する単一の効果をベースにすることを 検討すべきである。

# 4.4. ばく露評価の改善

疫学研究における農薬ばく露評価の困難さは、上記したように強調されている。農薬ばく露の記述(特に個々の農薬 へのばく露に関する定量的な情報)は、一般的に規制目的のためには十分な詳細が報告されておらず、特に潜伏期 間の長い疾患(多くのがんや神経変性疾患など)では、この限界を克服するのは困難である。

ばく露モニタリングを実施するために必要な方法は、申請者が申請書類の中で提出しなければならないことは注目 に値する。この規則の要求事項は、ばく露量の判定に使用できる有効な方法を要求している。欧州議会及び理事会の PPPの上市に関する規則(EC)No 1107/2009に基づき、有効成分のデータ要求を定めた欧州委員会規則(EU)No 283/2013 では、承認前の試験と承認後のモニタリングの両方をサポートするために必要な分析方法に関する情報が 記載されている。この文脈では、承認後の要求が最も有用性が高く、規則には実際に次の通りに記載されている。

- <sup>4.2.</sup> 承認後の管理及びモニタリング目的のための方法-方法は、以下の目的で提出されなければならない。
- a)加盟国が確立された最大残留レベル(MRL)への準拠を決定できるようにするために、6.7.1 項の規定に従って 提出されたモニタリング残留物の定義に含まれるすべての成分の決定;これは、植物及び動物由来の食品及び飼料に含まれる、または上述の残留物を対象とする。

b)7.4.2項の規定に従って提出された土壌及び水の残留基準をモニタリングする目的のための含有全成分の測定。c)申請者が、作業者、労働者、住民または居合わせただけの者(bystanders)のばく露が無視できる程度であることを示さない場合には、散布中または散布後に生成された有効成分及び関連する分解生成物の大気中の分析。



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- b) the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2;
- c) the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible;
- d) the analysis in body fluids and tissues for active substances and relevant metabolites.

As far as practicable these methods shall employ the simplest approach, involve the minimum cost, and require commonly available equipment. The specificity of the methods shall be determined and reported. It shall enable all components included in the monitoring residue definition to be determined. Validated confirmatory methods shall be submitted if appropriate. The linearity, recovery and precision (repeatability) of methods shall be determined and reported.

Data shall be generated at the LOQ and either the likely residue levels or ten times the LOQ. The LOO shall be determined and reported for each component included in the monitoring residue definition. For residues in or on food and feed of plant and animal origin and residues in drinking water, the reproducibility of the method shall be determined by means of an independent laboratory validation (ILV) and reported'.

From this, it can be concluded that the requirements exist, but are somewhat less stringent for human biomonitoring than for monitoring of residues in food and feed.

Failure to use these existing methods restricts the potential for the use of epidemiological evidence in the regulation of specific pesticides. It is therefore important that those contemplating future studies carefully consider approaches to be used to avoid misclassification of exposure, and to conduct appropriate detailed exposure assessments for specific pesticides, which allow for sound doseresponse analyses, and demonstrate the validity of the methods used.

A given exposure may have a different health impact depending on the period in the lifespan when exposure takes place. Greater attention needs to be paid to exposures occurring during periods of potential susceptibility for disease development by ensuring that the exposure assessment adequately addresses such critical times. This may be particularly relevant for studies involving neurodevelopment, obesity or allergic responses, which are complex multistage developmental processes that occur either prenatally or in the early post-natal life. For this reason, measurement of the exposure at one single time period may not properly characterise relevant exposures for all health effects of the environmental factors, and thus, the possibility arises of needing to measure the exposure at several critical periods of biological vulnerability to environmental factors. It is particularly challenging to construct an assessment of historical exposures which may deviate from current exposures, in both the range of chemicals and intensity of exposure and also co-exposure to other substances which are not included in the scope of study.

There are advantages and disadvantages to all methods of measuring pesticide exposure, and specific study designs and aims should be carefully considered to inform a specific optimal approach.

Exposure assessment can be improved at the *individual* level in observational research by using:

a) **Personal exposure monitoring:** This can be used to document exposures as readings measure pesticide concentration at the point of contact. Personal exposure monitors have been costly and burdensome for study participants. However, technological advances have recently driven personal exposure monitoring for airborne exposures to inexpensive, easy to use devices and these are suitable for population research. Personal exposure monitors that are specific to pesticide exposure could involve sensors to measure airborne concentrations, 'skin' patches to measure dermal concentrations, indoor home monitors that capture dust to measure other means of exposure. These mobile technology advances can be employed to provide observational studies with detailed and robust exposure assessments. Such equipment is now increasingly being adapted to serve large-scale population research and to capture data from large cohort studies. These coupled with other technological advances, such as real time data transfers via mobile phones and mobile phone applications to capture lifestyle and other habits, could bring next generation observational studies far more detailed and robust exposure assessments compared to current evidence. However, the generation of huge volumes of data can pose organisational, statistical and technical challenges, particularly with extended follow-up times. Ethics and personal data protection issue should be taken into account, and local regulations may prevent extensive use of such technologies. However, use of such personal monitors only provides information for one of the different potential routes of exposure.

b) Biomarkers of exposure (human biomonitoring (HBM)). An alternative and/or complementary approach is to ascertain the internal dose, which is the result of exposure via different routes (dermal, 29

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d) 体液中及び組織中の有効成分及び関連代謝物の分析。

これらの方法は、可能な限り、最も簡単な方法を採用し、最小限のコストで、一般的に利用可能な機器を必要とする ものとする。分析の特異性を確認し、報告されなければならない。それにより、モニタリング残留物の定義に含まれるす べての成分を測定できるようにしなければならない。必要に応じて、検証された測定法を提出しなければならない。方 法の直線性、回収率、精度(再現性)が測定され、報告されなければならない。

データは、LOQと残留可能性の高いレベル、またはLOQの10倍のいずれかで生成されなければならない。LOQ は、モニタリング残留物の定義に含まれる各成分について決定され、報告されなければならない。植物や動物由来の 食品や飼料中の残留物や飲料水中の残留物については、方法の再現性は、独立した試験施設のバリデーション(ILV) によって確認され、報告されなければならない。

このことから、要求事項は存在するが、食品や飼料中の残留物のモニタリングよりもヒトのバイオモニタリングの方が やや厳しくないと結論づけることができる。

これらの既存の方法を使用しないと、特定の農薬の規制における疫学的証拠の使用の可能性が制限される。したが って、今後の研究を検討する際には、ばく露の誤分類を避けるために使用する手法を慎重に検討することが重要であ り、当該農薬について適切で詳細なばく露評価を行い、適切な用量反応分析を可能にし、使用された方法の妥当性を 証明することが重要である。

ばく露は、ばく露される生涯の期間に応じて、異なる健康影響を及ぼす可能性がある。ばく露評価がそのような重要 な時期に適切に対応していることを確実にすることで、疾患発症の可能性がある時期のばく露には、より大きな注意を 払う必要がある。これは、神経発達、肥満、アレルギー反応など、出生前または出生後早期に発生する複雑な多段階 の発達過程を伴う研究に特に関連していると考えられる。このため、単一の期間におけるばく露の測定では、環境因子 のすべての健康影響に対する関連ばく露を適切に特徴付けることができない可能性があり、したがって、環境因子に 対するいくつかの重要な生物学的に脆弱な期間におけるばく露を測定する必要がある可能性が生じてくる。化学物質 の範囲とばく露の強度及び研究範囲ではない他の物質とのばく露も含めた中で、現在のばく露とは異なる可能性があ る過去のばく露の評価を構築することは、特に困難である。

農薬ばく露を測定するすべての方法には長所と短所があり、特定の研究計画と目的は、特定の最適なアプローチを 通知するために慎重に考慮されるべきである。

観察による研究では、個人レベルのばく露評価は以下の方法を用いて改善することができる。

a) 個人のばく露モニタリング:これは、測定装置を接触時での農薬濃度を記録するために使用することができる。個 人用ばく露モニターは、研究参加者にとって高価で負担が大きいものであった。しかし、技術の進歩により、最近では 大気中の浮游物質ばく露のための個人ばく露モニターが安価で使いやすい装置になり、これらは集団研究に適してい る。農薬ばく露に特化した個人ばく露モニターには、空気中の濃度を測定するためのセンサー、経皮濃度を測定する ための「皮膚」パッチ、他のばく露手段を測定するための塵埃を捕らえる屋内家庭用モニターなどがある。これらのモバ イル技術の進歩は、詳細で強固なばく露評価がなされた観察による研究を提供するために利用することができる。この ような機器は現在、大規模な集団研究や大規模なコホート研究からのデータを収集するために、ますます適応が増え ている。これらは、ライフスタイルやその他の習慣を捉えるための携帯電話や携帯電話アプリケーションを介したリアル タイムのデータ転送などの他の技術的進歩と相まって、次世代の観察による研究では、現在のエビデンスと比較して、 はるかに詳細で強固なばく露評価が可能になるだろう。しかし、膨大な量のデータを生成することは、組織的、統計的、 技術的な課題、特に追跡調査時間の延長をもたらす可能性がある。倫理や個人データ保護の問題を考慮しなければ ならず、地域の規制により、このような技術の大規模な使用が妨げられる可能性がある。しかし、このような個人モニター の使用は、異なる潜在的なばく露経路のうちの1つの情報を提供するにすぎない。

b) ばく露のバイオマーカー(ヒト・バイオモニタリング(HBM))。代替的及び/または補完的なアプローチとして、異 なる経路(経皮、吸入及び経口のばく露)を介したばく露の結果である内部ばく露量の確認がある。これらのバイオマー カーは、農薬への総体的なばく露を評価し、累積的なリスク評価に情報を提供する上で重要な役割を果たす可能性が ある。バイオモニタリングには、検討対象の化学物質(親化合物または代謝物)またはその病態生理学的影響のマーカ ー(付加生成物など)の生体試料中の濃度を測定することが必要である。しかし、課題としては、生体試料中の濃度測

inhalation and dietary exposure). These biomarkers have the potential to play an important role in assessing aggregate exposure to pesticides and informing cumulative risk assessment. Biomonitoring requires measurements in biological samples of concentrations of chemical under consideration (parent or metabolites) or markers of pathophysiologic effects thereof (such as adducts). However, challenges may include uncertainties relating to extrapolation of measured concentrations in biological samples to relevant doses.

Although biomonitoring has the potential to provide robust estimates of absorbed doses of xenobiotics, modern pesticides and their metabolites are eliminated from the body relatively quickly, with excretion half-lives typically measured in a few days (Oulhote and Bouchard, 2013). Consequently, use of biomarkers is both resource intensive and intrusive. The process is even more intrusive when it has to be conducted repeatedly on large numbers of individuals to monitor exposures over long durations.

Nevertheless, because of the potential to provide accurate integrated estimates of absorbed doses, biological monitoring of pesticides and their metabolites can be usefully employed to calibrate other approaches of exposure assessment. A good example of such an approach is that used by the Agricultural Health Study (Thomas et al., 2010; Coble et al., 2011; Hines et al., 2011). Also, HBM methods can be used with other forms of exposure assessment for the construction of long exposure histories.

Biomonitoring improves the precision in characterisation of exposure and allows the investigation of changes in exposure that occur at environmentally relevant exposure concentrations. Data collected in large-scale biomonitoring studies can be useful in setting reference ranges to assist in exposure classification in further epidemiological studies. Biomonitoring data also provide critical information for conducting improved risk assessment and help to identify subpopulations at special risk for adverse outcomes.

Biobanks, as repositories of biological samples, can be exploited to assess biomarkers of exposure with the aim of investigating early exposure–late effect relationships. That is, whether exposures occurring during early life are critical for disease development later in life (e.g. neurobehavioral impairment, children tumours, immunotoxic disorders, etc.) and to retrospectively assess health risks according to current health guidelines.

The results of measurements of metabolite levels in human matrices, e.g. urine, blood or hair do not provide the complete story with respect to the actual received dose. Additional assessment, possibly employing physiological-based toxicokinetic (PBTK) approaches, may be required to estimate the total systemic or tissue/organ doses. A PBTK model is a physiologically based compartmental model used to characterise toxicokinetic behaviour of a chemical, in particular for predicting the fate of chemicals in humans. Data on blood flow rates, metabolic and other processes that the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBTK model. PBTK models cannot be used only to translate external exposures into an internal (target) dose in the body, but also to infer external exposures from biomonitoring data. Furthermore, PBTK models need to be validated.

Toxicokinetic processes (ADME) determine the 'internal concentration' of an active substance reaching the target and help to relate this concentration/dose to the observed toxicity effect. Studies have been prescribed by the current regulations, but it would be beneficial to survey all the evidence, be it from *in vitro*, animal or human studies, about toxicokinetic behaviour of an active substance. Further discussion on quality assurance issues and factors to consider in relation to HBM studies is present in the report of the EFSA outsourced project (Bevan et al., 2017).

Exposure assessment can also be improved at the population level in observational research by using:

a) Larger epidemiological studies that make use of novel technologies and big data availability, such as **registry data** or data derived from large databases (including administrative databases) on health effects and pesticide usage, could provide more robust findings that might eventually be used for informed decision-making and regulation. Much effort needs to concentrate around the use of registered data which may contain records of pesticide use by different populations, such as farmers or other professional users that are required to maintain.<sup>9</sup> Such data could be further linked to

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定値を関連する用量に外挿することに伴う不確実性が含まれる場合がある。

バイオモニタリングは異物の吸収量を確実に推定できる可能性があるが、現代の農薬とその代謝物は比較的迅速に 体内から排泄され、排泄半減期は通常数日で測定される(Oulhote 及び Bouchard、2013 年)。そのため、バイオマ ーカーの使用はそのため、バイオマーカーの使用は、資源を必要とし、かつ煩わしいものとなります。長期にわたるばく 露を監視するために、多数の人を対象に繰り返し実施しなければならない場合には、このプロセスはさらに煩わしいも のとなる。

とはいえ、吸収量の正確な積算値を提供できる可能性があるため、農薬とその代謝物の生物学的モニタリングは、ば く露評価の他のアプローチを調整するために有効に利用できる。このようなアプローチの例は、農業健康調査 (Agricultural Health Study)で使用されているものである(Thomas ら、2010 年;Coble ら、2011 年;Hines ら、 2011 年)。また、長いばく露履歴を構築するために、他の形態のばく露評価と組み合わせて HBM の手法を使用する こともできる。

バイオモニタリングは、ばく露の特性評価の精度を向上させ、環境に関連するばく露濃度で発生するばく露の変化を 調査することを可能にする。大規模なバイオモニタリング研究で収集されたデータは、今後の疫学研究におけるばく露 の分類を支援するための基準範囲を設定するのに有用である。また、バイオモニタリングデータは、リスク評価の改訂を 実施するための重要な情報を提供し、有害な健康影響に対して特別なリスクのある小集団を特定するのに役立つ。

生体試料他(Biobanks)は、生物試料の保管場所として、早期ばく露と遅発影響の関係を調査する目的で、ばく露 のバイオマーカーを評価するために利用することができる。すなわち、生涯初期に発生したばく露が、生涯後期におけ る疾患(神経行動障害、小児腫瘍、免疫毒性障害など)の発症に重要であるかどうかを調べ、現在の健康ガイドライン に沿って健康リスクを後ろ向きに評価することができる。

尿、血液、毛髪などのヒトの試料中の代謝物レベルの測定結果だけは、実際に受けたばく露量を完全に把握できな い。全身または組織・器官の総ばく露量を推定するためには、場合によっては生理学的毒物動態学(PBTK)アプロー チを用いた追加評価が必要となる。PBTK モデルは、化学物質の毒物動態を特徴づけるために使用される生理学的 なコンパートメントモデルであり、特にヒトにおける化学物質の運命を予測するために使用される。各コンパートメント内 で化学物質が受ける血流速度、代謝及びその他のプロセスに関するデータは、PBTK モデルのマス-バランス・フレ ームワークを構築するために使用される。PBTK モデルは、外部ばく露を体内の内部(標的)ばく露量に変換するだけ でなく、バイオモニタリングデータから外部ばく露を推測するためにも使用することができる。さらに、PBTK モデルは検 証される必要がある。

毒物動態プロセス(ADME)は、標的に到達した有効成分の「内部濃度」を決定し、この濃度/用量を観察された毒 性効果と関連付けるのに役立つ。現行の規制でも試験実施が規定されているが、in vitro 試験、動物試験、ヒト試験な ど、有効成分の毒物動態に関するすべてのエビデンスを調査することは有益であろう。品質保証の問題や HBM 試験 に関連して考慮すべき要素についての更なる議論は、EFSA が委託したプロジェクトの報告書(Bevan 6、2017 年)に 記載されている。

ばく露評価は、観察による研究における集団レベルでも、以下のような方法で改善することができる。

a)健康影響や農薬使用に関する登録データや大規模データベース(行政データベースを含む)から得られたデー タなど、新しい技術やビッグデータを利用した大規模な疫学研究は、より確かな知見が得られ、最終的には情報に基づ いた政策決定や規制に利用できる可能性がある。このようなデータは、維持管理が義務付けられている農家やその他 の専門的ユーザーなど、異なる集団による農薬使用の記録が含まれている可能性がある登録データの利用を中心に、 多くの努力が必要である<sup>9</sup>。このようなデータは、電子的健康記録(上記参照)にさらにリンクされ、これまでにないサンプ ルサイズ及びばく露とその後の病気に関する情報を持つ研究を提供し、最終的にはこれまで回答のなかった強固な問

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<sup>&</sup>lt;sup>9</sup> Regulation 1107/2009 Article 67 states: Record-keeping 1. Producers, suppliers, distributors, importers, and exporters of plant protection products shall keep records of the plant protection products they produce, import, export, store or place on the market for at least 5 years. Professional users of plant protection products shall, for at least 3 years, keep records of the plant protection products shall, for at least 3 years, keep records of the plant protection products they use, containing the name of the plant protection product, the time and the dose of application, the area and the crop where the plant protection product was used. They shall make the relevant information contained in these records available to the competent authority on request. Third parties such as the drinking water industry, retailers or residents, may request access to this information by addressing the competent authority. The competent authorities shall provide access to such information in accordance with applicable national or Community law.

<sup>9</sup> 規則1107/2009第67条は次のように述べている。記録の保持 1. 植物保護製剤(農薬)の必定産者、供給者、流通業者、輸入業者及び輸出業者 は、自らが生産、輸入、輸出、保管又は市場に出した植物保護製剤(農薬)の記録を少なくとも5年間保管しなければならない。植物保護製剤(農 業)の専門的使用者は、使用した植物防疫製品(農業)の記録を少なくとも3年間(除物保護製剤(農薬)の名称、適用時間及び適用量、植物保 護製剤(農薬)が使用された地域及び作物を記載して保管しなければならない。これらの記録に含まれる関連情報は、要求があれば所轄官庁に 提供しなければならない。飲料水製造業界、小売業者又は住民などの第三者は、所轄官庁に運絡することにより、この情報へのアクセスを要求す ることができる。所轄官庁に、適用される国内法または共同体法に従って、当該情報へのアクセスを提供するものとする。

electronic health records (*vide supra*) and provide studies with unprecedented sample size and information on exposure and subsequent disease and will eventually be able to answer robustly previously unanswered questions. At the same time, information on active substances needs to be better captured in these registries and large databases. Dietary pesticide residue exposure can be estimated more accurately by using spraying journal data in combination with supervised residue trials. This method has the advantage of including more comprehensive and robust source data, more complete coverage of used pesticides and more reliable and precise estimates of residues below standard limit of quantification (LOQ) (Larsson et al., 2017).

b) Novel sophisticated approaches to geographical information systems (GIS) and small area studies might also serve as an additional way to provide estimates of residential exposures. Exposure indices based on GIS (i.e. residential proximity to agricultural fields and crop surface with influence around houses), when validated, may represent a useful complementary tool to biomonitoring and have been used to assess exposure to pesticides with short biological half-lives (Cornelis et al., 2009). As some such exposures maybe influenced by wind direction, amongst other factors, this should be taken into account through a special analysis of outcomes to make best of use of the approach. Also, these indices could be more representative, albeit non-specific, measures of cumulative exposure to non-persistent pesticides for long periods of time than biomonitoring data (González-Alzaga et al., 2015).

As already discussed, to be useful for the regulatory risk assessments of individual compounds epidemiological exposure assessments should provide information on specific pesticides. However, epidemiological studies which include more generic exposure assessments also have the potential to identify general risk factors and suggest inferences of causal associations in relevant human populations. Such observations may be important both informing overall regulatory policies, and for identification of matters for further epidemiological research.

Recent advances in modern technologies make it possible to estimate pesticide exposures to an unprecedented extent using novel analytical strategies:

a) The development of the so called **-omic techniques**, such as metabolomics and adductomics, also presents intriguing possibilities for improving exposure assessment through measurement of a wide range of molecules, from xenobiotics and metabolites recorded over time in biological matrices (blood, saliva, urine, hair, nails, etc.), to covalent complexes with DNA and proteins (adductomics) and understanding biological pathways. These methodologies could be used in conjunction with other tools. There is also both interest and the recognition that further work is required before such techniques can be applied in regulatory toxicology. The use of the exposome (the totality of exposures received by an individual during life) might be better defined by using 'omics' technologies and biomarkers appropriate for human biomonitoring. Nevertheless, important limitations have to be acknowledged because of the lack of validation of these methodologies and their cost, which limits their use at large scale.

b) Environmental exposures are traditionally assessed following 'one-exposure-one-health-effect' approach. In contrast, the **exposume** encompass the totality of human environmental exposures from conception onward complementing the genetics knowledge to characterise better the environmental exposures but also other external and or internal environmental factors, such as infections, physical activity, diet, stress and internal biological factors (metabolic factors, gut microflora, inflammation and oxidative stress). A complete exposure would have to integrate many external and internal exposures from different sources continuously over the life course. However, a truly complete exposome will likely never be measured. Although all these domains of the exposome need to be captured by using different approaches than the traditional ones, it is envisaged that no single tool will be enough to this end.

The more holistic approach of exposure is not intended to replace the traditional 'one-exposureone-health-effect' approach of current epidemiological studies. However, it would improve our understanding of the predictors, risk factors and protective factors of complex, multifactorial chronic diseases. The exposome offers a framework that describes and integrates, holistically, the environmental influences or exposures over a lifetime (Nieuwenhuijsen, 2015).

Collaborative research and integration of epidemiological or exploratory studies forming large consortia are needed to validate these potential biomarkers and eventually lead to improved exposure assessment. The incorporation of the exposome paradigm into traditional biomonitoring approaches offers a means to improve exposure assessment. Exposome-wide association studies (EWAS) allow to measurement of thousands of chemicals in blood from healthy and diseased people, test for disease

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題に答えることができるようになるだろう。同時に、これらの登録や大規模なデータベースでは、有効成分に関する情報 をよりよく把握する必要がある。食品中の残留農薬ばく露は、監督下の残留試験と組み合わせて散布日誌データを使 用することで、より正確に推定することができる。この方法は、より包括的で強固な源データを含み、使用された農薬を より完全にカバーし、標準的な定量限界(LOQ)以下の残留物のより信頼性が高く正確な推定値を得ることができると いう利点がある(Larsson 6、2017年)。

b) 地理的情報システム(GIS) や小地域調査への新しい高度なアプローチも、住居ばく露の推定値を提供する追加 的な方法として役立つかもしれない。GIS に基づくばく露指標(すなわち、農業用地への住居の近接性や住居周辺へ の影響のある農地面積)は、検証された場合、バイオモニタリングを補完する有用なツールとなる可能性があり、生物学 的半減期の短い農薬へのばく露を評価するために使用されてきた(Cornelis 6、2009 年)。このようなばく露の中には、 他の要因の中でも特に風向によって影響を受けるものがあるため、このアプローチを最大限に活用するためには、結 果の特別な分析を通じて、この点を考慮に入れる必要がある。また、これらの指標は、特定の指標ではないにせよ、生 物モニタリングデータよりも、長期にわたる非残留性農薬への累積ばく露のより代表的な指標となり得る(González-Alzaga 6、2015 年)。

すでに議論したように、個々の化合物の規制リスク評価に有用であるためには、疫学的ばく露評価は特定の農薬に 関する情報を提供すべきである。しかし、より一般的なばく露評価を含む疫学的研究は、一般的なリスク因子を特定し、 関連するとト集団における因果関係の推論を示唆する可能性もある。このような観察結果は、全体的な規制政策の情 報提供と、さらなる疫学的研究のための事項の特定の両方に重要であるかもしれない。

現代技術の最近の進歩により、新しい分析方法を用いて農薬ばく露を前例のない範囲で推定することが可能になった。

a)メタボロミクス(metabolomics)やアダクトミクス(adductomics)などのいわゆる**オミクス技術**の発展は、生物学的 マトリックス(血液、唾液、尿、毛髪、爪など)中に経時的に記録された異物や代謝物から、DNA やタンパク質との共有 結合体(アダクトミクス)や生物学的経路の理解に至るまで、幅広い分子の測定を通じてばく露評価を改善するための 魅力のある可能性を提示している。これらの方法論は、他のツールと組み合わせて使用することができる。また、このよ うな技術を規制毒性学に応用するには、さらなる研究が必要であるという認識と関心がある。エクスポソーム(exposome) (一生の間に個人が受けたばく露の全体)の利用は、「オミックス」技術ととトのバイオモニタリングに適したバイオマーカ ーを使用することで、より良い結果を得ることができるかもしれない。それにもかかわらず、これらの方法論の検証が不 足していることと、大規模での使用を制限するコストのため、重要な制限が認められなければならない。

b)環境ばく露は従来「一回のばく露に一回の健康影響」というアプローチで評価されてきた。これに対して、エクスポ ソームは、受胎以降のヒト環境ばく露の全体を網羅しており、遺伝学の知識を補完することで、疾患の病因における環 境要因をよりよく特徴づけることができる。このように、エクスポソームには、生涯にわたる化学的ばく露だけでなく、感染 症、身体活動、食事、ストレス、内部生物学的因子(代謝因子、腸内フローラ、炎症、酸化ストレス)などの外部環境因 子や内部環境因子も含まれている。完全なエクスポソームを構築するためには、生涯にわたって継続的に異なる源か らの多くの外部ばく露と内部ばく露を統合しなければならない。しかし、真に完全なエクスポソームを測定することは不 可能である。エクスポソームのこれらすべての領域を従来のものとは異なるアプローチで捉える必要があるが、この目的 のためには単一のツールでは十分ではないと考えられている。

ばく露のより総合的なアプローチは、現在の疫学研究における従来の「一回のばく露-一回の健康影響」アプロー チに取って代わることを意図したものではない。しかし、それは複雑で多因子性の慢性疾患の予測因子、リスク因子、 保護因子についての理解を向上させるものである。エクスポソームは、生涯にわたる環境の影響やばく露を総合的に 記述し、統合する枠組みを提供している(Nieuwenhuijsen、2015年)。

これらの潜在的なバイオマーカーを検証し、最終的にはばく露評価の改善につなげるためには、共同研究や、大規 模なコンソーシアムを形成する疫学研究や探索的研究の統合が必要である。エクスポソームパラダイムを従来のバイオ モニタリング手法に組み込むことは、ばく露評価を改善する手段となる。エクスポソーム拡大関連研究(EWAS)は、健 康な人と病気の人の血液中の何千もの化学物質を測定し、病気との関連性を検査し、ばく露源を特定し、作用機序を 確立し、因果関係を明らかにするために、その後の調査で対象とすることができるばく露の有用なバイオマーカーを特

associations and identify useful biomarkers of exposure that can be targeted in subsequent investigations to locate exposure sources, establish mechanisms of action and confirm causality (Rappaport, 2012). After identifying these key chemicals and verifying their disease associations in independent samples of cases and controls, the chemicals can be used as biomarkers of exposures or disease progression in targeted analyses of blood from large populations.

In relation to the exposome concept, the -omics technologies have the potential to measure profiles or signatures of the biological response to the cumulative exposure to complex chemical mixtures. An important advance would be to identify a unique biological matrix where the exposome could be characterised without assessing each individual exposure separately in a given biological sample. The untargeted nature of omics data will capture biological responses to exposure in a more holistic way and will provide mechanistic information supporting exposure-related health effects. Importantly, omics tools could shed light on how diverse exposures act on common pathways to cause the same health outcomes.

While improved exposure assessment increases the power to detect associations, in any individual study it is necessary to maximise the overall power of the study by optimising the balance between the resource used for conducting an exposure assessment for each subject and the total number of subjects.

#### 4.5. Health outcomes

For pesticides, the health outcomes are broad as these chemicals have not shown a particular effect in relation to just one single disease area. For each health outcome, multiple definitions may exist in the literature with a varying degree of validation and unknown reproducibility across different databases, which are limited by the lack of generalisability. A proper definition of a health outcome is critical to the validity and reproducibility of observational epidemiological studies, and the consistency and clarity of these definitions need to be considered across studies. While prospective observational studies have explicit outcome definitions, inclusion and exclusion criteria and standardised data collection, retrospective studies usually rely on identification of health outcomes based largely on coded data, and classification and coding of diseases may change over time. Detailed description of the actual codes used to define key health outcomes and the results of any validation efforts are valuable to future research efforts (Stang et al., 2012; Reich et al., 2013). An example of coded diseases is the ICD-10, which for instance can be used as a tool to standardise the broad spectrum of malignant diseases.

In some surveillance studies, it is preferable to use broader definitions with a higher sensitivity to identify all potential cases and then apply a narrower and more precise definition with a high positive predictive value to reduce the number of false positives and resulting in more accurate cases. In contrast, in formal epidemiological studies, a specific event definition is used and validated to determine its precision; however, the 'validation' does not test alternative definitions, so it is not possible to determine sensitivity or specificity.

Surrogate endpoints should be avoided unless they have been validated. Some criteria to assess the validity of a surrogate outcome include:

- The surrogate has been shown to be in the causal pathway of the disease. This can be supported by the following evidence: correlation of biomarker response to pathology and improved performance relative to other biomarkers; biological understanding and relevance to toxicity (mechanism of response); consistent response across mechanistically different compounds and similar response across sex, strain and species; the presence of dose-response and temporal relationship to the magnitude of response; specificity of response to toxicity; that is, the biomarker should not reflect the response to toxicities in other tissues, or to physiological effects without toxicity in the target organ.
- At least one well conducted trial using both the surrogate and true outcome (Grimes and Schulz, 2005; la Cour et al., 2010). Several statistical methods are used to assess these criteria and if they are fulfilled the validity of the surrogate is increased. However, many times some uncertainty remains, making it difficult to apply surrogates in epidemiological studies (la Cour et al., 2010).

The data on health outcomes over the whole EU is potentially very extensive. If it can be managed effectively, it will open the prospect of greater statistical power for epidemiological studies assessing deleterious effects using very large sample sizes. Necessary prerequisites for these studies which may

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定することを可能にする(Rappaport、2012 年)。これらの主要な化学物質を特定し、症例と対照の独立したサンプル で疾患との関連性を検証した後、これらの化学物質は、大規模な集団からの血液を対象とした分析において、ばく露ま たは疾患進行のバイオマーカーとして使用することができる。

エクスポソームの概念に関連して、オミクス技術は複雑な化学物質の混合物への累積ばく露に対する生物学的反応 の特性やシグネチャーを測定する可能性を持っている。重要な進歩は、特定の生物学的試料中の個々のばく露を個 別に評価することなく、エクスポソームを特徴づけることができるユニークな生物学的マトリックスを特定することであろう。 オミクスデータの非標的特性は、ばく露に対する生物学的反応をより全体的な方法で捉え、ばく露に関連した健康影 響を裏付けるメカニズム論的な情報を提供することになる。重要なことは、オミクスツールは、多様なばく露がどのように して共通の経路で作用し、同じ健康影響を引き起こす仕組みを明らかにすることができるということである。

改良されたばく露評価は関連性を検出する力を高めるが、どのような個々の研究においても、各被験者のばく露評 価を実施するために使用する供給源と被験者の総数のバランスを最適化することにより、研究の全体的な力を最大化 することが必要である。

# 4.5. 健康影響

農薬については、これらの化学物質が単一の疾患領域に関連して特定の効果を示していないため、健康影響は広範囲にわたる。それぞれの健康影響について、文献には複数の定義が存在していて、程度の差こそあれ異なるデータベース間での再現性は不明であり、一般化できないという制限がある。健康影響の適切な定義は、観察による疫学研究の妥当性と再現性にとって非常に重要であり、これらの定義の一貫性と明確さは研究間で考慮する必要がある。前向きな観察研究では、明確な健康影響の定義、包含基準と除外基準、標準化されたデータ収集があるが、後ろ向き研究では通常、主にコード化されたデータに基づいた健康影響の同定に頼っており、疾患の分類とコード化は時間の経過とともに変化する可能性がある。主要な健康影響を定義するために使用された実際のコードの詳細な記述と検証作業の結果は、今後の研究活動にとって貴重なものである(Stang ら、2012 年; Reich ら、2013 年)。コード化された疾患の例としては、例えば ICD-10 があり、これは広範囲の悪性疾患を標準化するためのツールとして使用することができる。

いくつかのサーベイランス研究では、すべての潜在的な症例を特定するために感度の高いより広い定義を使用し、 その後、偽陽性の数を減らし、結果としてより正確な症例を得るために、高い陽性予測値のより狭く、より正確な定義を 適用することが望ましいとされている。対照的に、正式な疫学研究では、特定のイベントの定義が使用され、その精度 を決定するために検証される。しかしながら、「検証」では新たな定義をテストしないので、感度や特異度を測定できな いでだろう。

代替エンドポイントは、有効性が確認されていない限り避けるべきである。代替健康影響の妥当性を評価する基準に は、以下のようなものがある。

- ・代替指標が疾患の原因経路内にあることが示されていること。これは以下のエビデンスによって裏付けられる:バイオマーカーの反応が病理学と相関しており、他のバイオマーカーと比較して性能が向上していること;生物学的な理解と毒性との関連性(反応のメカニズム);メカニズム的に異なる化合物に対する一貫した反応と性、系統、種の違いによる類似した反応;用量反応の存在と反応の大きさと時間的関係;毒性に対する反応の特異性;すなわち、バイオマーカーは他の組織の毒性に対する反応や、標的臓器の毒性を伴わない生理学的効果を伴わない生理的効果を反映してはならない。
- ・代替健康影響と真の健康影響の両方を使用した少なくとも1 つのよく実施された試験があること(Grimes 及び Schulz、2005年; la Courら、2010年)。これらの基準を評価するために、いくつかの統計的手法が使用されて、 それらが満たされていれば、代替指標の妥当性が高まる。しかし、多くの場合、不確実性が残っているため、疫学 研究に代替指標を適用することは困難である(la Courら、2010年)。

EU 全体の健康影響に関するデータは非常に広範囲に及ぶ可能性がある。これを効果的に管理することができれば、非常に大規模なサンプルサイズを用いて悪影響を評価する疫学研究において、より大きな統計力を発揮できる可能性がある。これらの研究に必要な前提条件は、新たな軽微な影響、慢性的な影響、または層別化した場合の小集団

detect new subtle effects, chronic effects or effects on subpopulations when stratified are beyond the remit of risk assessment. They include trans-national approaches to health informatics where harmonised diagnostics, data storage and informatics coupled with legally approved access to anonymised personal data for societal benefit are established. Health records should include adequate toxidrome classification. The latter may in turn require improvements in medical and paramedical training to ensure the quality of the input data.

Another opportunity for biological monitoring to be employed is where the investigation involves the so-called biomarkers of effect. That is a quantifiable biochemical, physiological, or other change that, depending on the magnitude, is associated with an established or possible health impairment or disease. Biomarkers of effect should reflect early biochemical modifications that precede functional or structural damage. Thus, knowledge of the mechanism ultimately leading to toxicity is necessary to develop specific and useful biomarkers, and vice versa, an effect biomarker may help to explain a mechanistic pathway of the development of a disease. Such biomarkers should identify early and reversible events in biological systems that may be predictive of later responses, so that they are considered to be preclinical in nature. Advances in experimental -omics technologies will show promise and provide sound information for risk assessment strategies, i.e. on mode of action, response biomarkers, estimation of internal dose and dose–response relationships (DeBord et al., 2015). These technologies must be validated to assess their relevance and reliability. Once validated, they can be made available for regulatory purposes.

## 5. Contribution of vigilance data to pesticides risk assessment

In addition to the formal epidemiological studies discussed in Sections 2–4, other human health data can be generated from ad hoc reports or as a planned process, i.e. through monitoring systems that have been implemented at the national level by public health authorities or authorisation holders. Consistent with Sections 2–4, this section first reviews how such a monitoring system should operate, what the current situation is regarding the monitoring of pesticides and what recommendations for improvement can be made.

#### 5.1. General framework of case incident studies

A continuous process of collection, reporting and evaluation of adverse incidents has the potential to improve the protection of health and safety of users and others by reducing the likelihood of the occurrence of the same adverse incident in different places at later times, and also to alleviate consequences of such incidents. This obviously also requires timely dissemination of the information collected on such incidents. Such a process is referred to as vigilance.<sup>10</sup>

For example in the EU, the safety monitoring of medicines is known as pharmacovigilance; the pharmacovigilance system operates between the regulatory authorities in Member States, the European Commission and the European Medicines Agency (EMA). In some Member States, regional centres are in place under the coordination of the national Competent Authorities. Manufacturers and health care professionals report incidents to the Competent Authority at the national level, which ensures that any information regarding adverse reactions is recorded and evaluated centrally and also notifies other authorities for subsequent actions. The records are then centralised by the EMA which supports the coordination of the European pharmacovigilance system and provides advice on the safe and effective use of medicines.

#### 5.2. Key limitations of current framework of case incident reporting

Several EU regulations require the notification and/or collection and/or reporting of adverse events caused by pesticides in humans (occurring after acute or chronic exposure in the occupational setting, accidental or deliberate poisoning, etc.). These include:

 Article 56 of EC Regulation 1107/2009 requires that 'The <u>holder of an authorisation</u> for a plant protection product shall immediately notify the Member States [...] In particular, potentially

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への影響を検出する可能性があるが、リスク評価の範囲を超えている。これらの研究には、調和のとれた診断、データ 保存及び社会的利益のための匿名化された個人データへの法的に承認されたアクセスと相まって、健康情報学への 国境を越えたアプローチが含まれている。健康記録には、適切な中毒症候群の分類が含まれていなければならない。 後者は、入力データの品質を保証するために、医療と医療補助のトレーニングの改善を必要とするかもしれない。

生物学的モニタリングが採用されるもう一つの機会は、調査がいわゆる影響のバイオマーカーを含む場合である。こ れは、定量化可能な生化学的、生理学的、またはその他の変化であり、その大きさに応じて、確立された、または可能 性のある健康障害や病気に関連している。影響のバイオマーカーは、機能的または構造的損傷に先行する初期の生 化学的変化を反映している必要がある。このように、最終的に毒性につながるメカニズムの知識は、特定の有用なバイ オマーカーを開発するために必要であり、その逆もまた然りで、影響のバイオマーカーは、疾患の発生のメカニズムの 経路を説明するのに役立つかもしれない。このようなバイオマーカーは、生物学的システムにおける初期の可逆的な事 象を特定するものであり、後の反応を予測するものでなければならず、その性質上、前臨床的なものと考えられる。実 験的ーオミクス技術の進歩は有望であり、リスク評価戦略、すなわち作用機序、反応バイオマーカー、内部ばく露量の 推定、用量ー反応関係に関する確かな情報を提供するだろう(DeBord 6、2015 年)。これらの技術は、その妥当性と 信頼性を評価するために検証されなければならない。妥当性が確認されれば、それらの技術は規制目的で利用できる ようになる。

# 5. 農薬リスク評価への警戒データの貢献

第2-4節で議論した正式な疫学調査に加えて、その他のヒトの健康データは、その場限りの報告書から、あるいは計 画的なプロセスとして、すなわち公衆衛生当局や認可者によって国家レベルで実施されているモニタリングシステムを 通じて、生成することができる。第2-4節に沿って、本節ではまず、このようなモニタリングシステムがどのように運用され るべきか、農薬のモニタリングに関する現状はどうなっているのか、そして改善のためにどのような勧告ができるのかを レビューする。

# 5.1. ケースインシデント研究の一般的な枠組み

有害事象の収集、報告、評価を継続的に行うことは、同じ有害事象が後から別の場所で発生する可能性を減らすこ とで、利用者やその他の人々の健康と安全の保護を向上させ、また、そのような事象の結果を緩和する可能性がある。 そのためには、当然ながら、収集した情報をタイムリーに発信する必要がある。このようなプロセスを警戒(vigilance)と 呼んでいる<sup>10</sup>。

例えば、EU では、医薬品の安全性監視は医薬品安全性監視 (pharmacovigilance)して知られており、医薬品安 全性監視システムは、加盟国の規制当局、欧州委員会、欧州医薬品庁 (EMA)の間で運営されている。一部の加盟国 では、国内の管轄当局の調整の下に地域センターが設置されている。製造業者や医療従事者は、国レベルの管轄当 局に事件を報告する。これにより、有害事象に関するあらゆる情報が記録され、一元的に評価され、その後の対応につ いて他の当局に通知することができる。記録は EMA によって一元化され、欧州の医薬品安全性監視システムの調整 をサポートし、医薬品の安全で効果的な使用に関するアドバイスを提供する。

# 5.2. ケースインシデント報告の現在の枠組みの主な限界

いくつかの EU の規制では、ヒトに農薬が原因で発生した有害事象(職業環境での急性または慢性ばく露後に発生 したもの、偶発的または故意の中毒など)の通知及び/または収集及び/または報告を義務付けている。これらには 以下のものが含まれる。

・ EC 規則 1107/2009 の第 56 条は、「植物保護製剤(農薬)の認可を受けた者は、直ちに加盟国に通知しなけれ

<sup>&</sup>lt;sup>10</sup> The concept of <u>survey</u> refers to a single effort to measure and record something, and <u>surveillance</u> refers to repeated standardized surveys to detect trends in populations in order to demonstrate the absence of disease or to identify its presence or distribution to allow for timely dissemination of information. <u>Monitoring</u> implies the intermittent analysis of routine measurements and observations to detect changes in the environment or health status of a population, but without eliciting a response. <u>Vigilance</u> is distinct from surveillance and mere monitoring as it implies a process of paying close and continuous attention, and in this context addresses specifically post marketing events related to the use of a chemical.

<sup>10</sup> 調査という概念は、何かを測定し記録するための単一の努力を意味し、サーベイランスとは、疾病の不在を証明したり、疾病の存在や分布を特定して情報を適時に発信できるようにするために、集団の傾向を検出するために、標準化された調査を繰り返すことを意味する。モニタリングとは、集団の環境や健康状態の変化を検出するために、日常的な測定や観察を断続的に分析することを意味するが、反応を引き出すことはない、監視は、綿密かつ継続的に注意を払うプロセスを意味するため、監視や単なるモニタリングとは異なり、この背景では特に化学物質の使用に関連した販売後の事象を扱う。

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harmful effects of that plant protection product, or of residues of an active substance, its metabolites, a safener, synergist or co-formulant contained in it on human health [...] shall be notified. To this end the authorisation holder shall record and report all suspected adverse reactions in humans, in animals and the environment related to the use of the plant protection product. The obligation to notify shall include relevant information on decisions or assessments by international organisations or by public bodies which authorise plant protection products or active substances in third countries'.

Article 7 of EC Directive 128/2009 establishing a framework for Community action to achieve the sustainable use of pesticides requires that: '2. <u>Member States</u> shall put in place systems for gathering information on pesticide acute poisoning incidents, as well as chronic poisoning developments where available, among groups that may be exposed regularly to pesticides such as operators, agricultural workers or persons living close to pesticide application areas. 3. To enhance the comparability of information, <u>the Commission</u>, in cooperation with the Member States, shall develop by 14 December 2012 a strategic guidance document on monitoring and surveying of impacts of pesticide use on human health and the environment'. However, at the time of publishing this scientific opinion, this document has still not been released.

There are three additional regulations that apply, although indirectly, to pesticides and reporting:

- EC Regulation 1185/2009 concerning statistics on pesticides requires that Member States shall collect data on pesticide sales and uses according to a harmonised format. The statistics on the placing on the market shall be transmitted yearly to the Commission and the statistics on agricultural use shall be transmitted every 5 year.
- Article 50 of Regulation (EC) 178/2002, laying down the general principles and requirements of food law, set up an improved and broadened rapid alert system covering food and feed (RASFF). The system is managed by the <u>Commission</u> and includes as members of the network Member States, the Commission and the Authority. It reports on non-authorised occurrences of pesticides residues and food poisoning cases.
- Article 45 (4) of EC Regulation 1272/2008 (CLP Regulation): <u>importers and downstream users</u> placing hazardous chemical mixtures on the market of an EU Member State will have to submit a notification to the Appointed Body/Poison Centre of that Member State. The notification needs to contain certain information on the chemical mixture, such as the chemical composition and toxicological information, as well as the product category to which the mixture belongs. The inclusion of information on the product category in a notification allows Appointed Bodies/Poison Centres to carry out comparable statistical analysis (e.g. to define risk management measures), to fulfil reporting obligations and to exchange information among MS. The product category is therefore not used for the actual emergency health response as such, but allows the identification of exposure or poisoning trends and of possible measures to prevent future poisoning cases. When formally adopted, the new Regulation will apply as of 1 January 2020.

While there are substantial legislative provisions, to this date a single unified EU 'phytopharmacovigilance'<sup>11</sup> system akin to the pharmacovigilance system does not exist for PPP. Rather, a number of alerting systems have been developed within the EU to alert, notify, report and share information on chemical hazards that may pose a risk to public health in Member States. These systems cover different sectors including medicines, food stuffs, consumer products, industrial accidents, notifications under International Health Regulations (IHR) and events detected by EU Poisons Centres and Public Health Authorities. Each of these systems notify and distribute timely warnings to competent authorities, public organisations, governments, regulatory authorities and public health officials to enable them to take effective action to minimise and manage the risk to public health (Orford et al., 2014).

In the EU, information on acute pesticide exposure/incident originates mainly from data collected and reported by Poison Control Centres (PCC's). PCC's collect both cases of acute and chronic exposure/poisoning they are aware of, in the general population and in occupational settings. Cases are usually well-documented and information includes circumstances of exposure/incident, description of the suspected causal agent, level and duration of exposure, the clinical course and treatment and an assessment of the causal relationship. In severe cases, the toxin and/or the metabolites are usually

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#### Epidemiological studies and pesticides

ばならない」と規定している。この目的のために、認可保有者は、植物保護製剤(農薬)の使用に関連して、ヒト、動 物及び環境におけるすべての疑われる有害な反応を記録し、報告しなければならない。通知義務には、国際機関 や第三国の植物保護製剤(農薬)や有効成分を認可する公的機関による決定や評価に関する関連情報も含まれ ていなければならない。

・農薬の持続可能な使用を達成するための共同体行動の枠組みを確立した EC 指令 128/2009 の第7条は、次の ように要求している。加盟国は、作業者、農業労働者、農薬散布地域の近くに住む人など、定期的に農薬にばく露 される可能性のある集団の間で、農薬による急性中毒事故や慢性中毒の発生状況に関する情報を収集するシス テムを設置しなければならない。3.3.情報の類似性を高めるために、欧州委員会は加盟国と協力して、2012年12 月 14 日までに「農薬使用が比トの健康と環境に与える影響のモニタリングと調査に関する戦略的ガイダンス文書」 を作成する。しかし、この意見書を発表した時点では、この文書はまだ公表されていない。

間接的ではあるが、農薬と報告に適用される追加の規制が3つある。

- ・ 農薬の統計に関する EC 規則 1185/2009 は、加盟国が調和のとれたフォーマットに従って農薬の販売と使用に 関するデータを収集することを要求している。上市に関する統計は毎年欧州委員会に、農業利用に関する統計は 5年ごとに送信されなければならない。
- ・食品法の一般原則と要件を定めた規則(EC)178/2002の第50条では、食品と飼料を対象とした改良・拡大された迅速警報システム(RASFF)が設定されている。このシステムは欧州委員会によって管理されており、ネットワーク加盟国、欧州委員会、当局が加盟している。それは残留農薬の認可されていない事例や食中毒の事例を報告する。
- ・ EC 規則 1272/2008 (CLP 規則)第 45 条(4): EU 加盟国の市場に危険な化学物質の混合物を市場に出す輸入 業者と顧客ユーザーは、その加盟国の任命機関/毒物センターに通知書を提出しなければならない。通知書に は、化学成分や毒物学的情報、混合物が属する製品カテゴリーなど、混合物に関する特定の情報を記載する必 要がある。通知書に製品分類に関する情報を含めることで、指定団体/毒物取締センターは、同等の統計解析 (例えば、リスク管理措置の実施)を行い、報告義務を果たし、MS 間で情報交換を行うことができる。したがって、 製品カテゴリーは実際の緊急時の医療対応には使用されないが、ばく露や中毒の傾向を特定し、将来の中毒事 例を防ぐための対策をとることができる。正式に採択された場合、新規則は 2020 年 1 月 1 日から適用される。

実質的な立法規定がある一方で、今日までのところ、医薬品安全性監視システムに類似した単一の EU「植物薬理 監視」<sup>11</sup>システムは PPP には存在しない。むしろ、加盟国の公衆衛生にリスクをもたらす可能性のある化学物質のハザ ードについて警告、通知、報告、情報共有を行うための多くの警告システムが EU 内で開発されている。これらのシステ ムは、医薬品、食品、消費者製品、労働災害、国際保健規則(IHR)に基づく通知、EU 毒物センターや公衆衛生当局 によって検知した事象など、さまざまな分野をカバーしている。これらのシステムのそれぞれは、管轄官庁、公的機関、 政府、規制当局、公衆衛生当局にタイムリーな警告を通知し、配布して、公衆衛生へのリスクを最小限に抑え、管理す るための効果的な行動をとることを可能にしている(Orford ら、2014 年)。

EUでは、急性農薬ばく露・事故に関する情報は、主に毒物管理センター(PCC)によって収集・報告されたデータに 基づいている。PCCは、一般集団や職業環境において、自分たちが知っている急性と慢性のばく露/中毒の両方の 事例を収集している。通常、症例は十分に文書化されており、情報にはばく露・事故の状況、原因物質と疑われるもの の説明、ばく露のレベルと期間、臨床経過と治療、因果関係の評価が含まれている。重症の場合は、通常、血液や尿 中の毒素や代謝物の測定が行われる。しかし、センターに報告された症例の追跡調査は、長期化する可能性のある影 響を特定するために、さらに注意を払う必要がある。

毒物センターのデータを使用するには、2 つの重要な障害がある:各国の毒物センターからの報告書は常に公表さ

<sup>&</sup>lt;sup>11</sup> 'phytovigilance' would refer to a vigilance system for plants; as pesticides are intended to be 'medicines' for crops, the term 'phytopharmacovigilance' is considered to be the more appropriate one here. Furthermore, it is a broad term used in France covering soil, water, air, environment, animal data, etc.

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<sup>11「</sup>フィトビジランス(phytovigilance)」は植物に対する警戒システムを意味し、農薬は作物の「薬」であることを意図しているため、ここでは「フィトファーマコビジランス(phytopharmacovigilance)」という用語がより適切であると考えられている。さらに、フランスでは、土壌、水、大気、環境、動物のデータなどをカバーする広い用語として使われている。

measured in blood or urine. However, follow-up of cases reported to the centres merits further attention to identify potential long-term protracted effects.

There are two key obstacles to using Poison Centres data: official reports from national Poisons Centres are not always publicly available and when they are, there is a large heterogeneity in the format of data collections and coding, and assessment of the causal relationship. Indeed, each Member State has developed its own tools for collection activities resulting in difficulties for comparing and exchanging exposure data. In 2012, the European Commission funded a collaborative research and development project to support the European response to emerging chemical events: the Alerting and Reporting System for Chemical Health Threats, Phase III (ASHTIII) project. Among the various tools and methodologies that were considered, methods to exchange and compare exposure data. However, results of a consultation with the PCC community showed that further coordination of data coding and collection activities is supported. It was concluded that more support and coordination is required at the EU and Member States level so that exposures data can be compared between Member States (Orford et al., 2015).

In addition to data collected by PCC's, several Member States have set up programmes dedicated to occupational health surveillance.<sup>12</sup> The purpose of these programmes is to identify the kinds of jobs, types of circumstances and pesticides that cause health problems in workers in order to learn more about occupational pesticide illnesses and injuries and how to prevent them. They are based on voluntary event notification by physicians (sometimes self-reporting by users) of any case of suspected work-related pesticide injury or illness or poisoning. In addition to medical data, information gathered includes data regarding type of crop, mode of application, temperature, wind speed, wearing of personal protection equipment, etc. Once collected, these data are examined and a report is released periodically; they provide a useful support to evaluate the safety of the products under re-registration. These data also highlight emerging problems and allow definition of evidence-based preventive measures for policy-makers. At EU level, the European Agency for Safety and Health at Work (EU-OSHA)<sup>13</sup> has very little in the way of monitoring of occupational pesticide-related illnesses data. In the USA, a programme specifically dedicated to pesticides funded and administered by the National Institute for Occupational Safety and Health (NIOSH) is in operation in a number of States.<sup>14</sup>

In summary, currently human data may be collected in the form of case reports or case series, poison centres information, coroner's court findings, occupational health surveillance programmes or post-marketing surveillance programmes. However, not all this information is present in the medical data submitted by applicants mainly because the different sources of information are diverse and heterogeneous by nature, which makes some of them sometimes not accessible.

- Data collected through occupational health surveillance of the plant production workers or if they do so, the medical data are quite limited being typically basic clinical blood measurements, physical examinations, potentially with simple indications of how and where exposed took place, and there usually is no long-term follow up. Furthermore, worker exposures in modern plants (especially in the EU) are commonly very low, and often their potential exposure is to a variety of pesticides (unless it is a facility dedicated to a specific chemical).
- Moreover, the reporting of data from occupational exposure to the active substances during
  manufacture is often combined with results from observations arising from contact with the
  formulated plant protection product as the latter information results from case reports on
  poisoning incidents and epidemiological studies of those exposed as a result of PPP use.
  Indeed, the presence of co-formulants in a plant protection product can modify the acute
  toxicological profile. Thus, to facilitate proper assessment, when reporting findings collected in
  humans it should be clearly specified whether it refers to the active substance per se or a PPP.

With regard to the requirements of specific data on diagnoses of poisoning by the active substance or formulated plant protection products and proposed treatments, which are also part of chapter 5.9 of the EC Regulation 283/2013, information is often missing or limited to those cases where the toxic mode of action is known to occur in humans and a specific antidote has been identified.

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れているわけではなく、公表されている場合でも、データ収集の形式やコーディング、因果関係の評価には大きな不均 ー性がある。実際、各加盟国は独自の収集活動のためのツールを開発しており、ばく露データの比較や交換には困難 が伴う。2012 年、欧州委員会は、新たな化学物質事象への欧州の対応を支援するための共同研究開発プロジェクト 「化学物質健康脅威のための警告・報告システムフェーズ III(ASHTIII)プロジェクト」に資金を提供した。検討された 様々なツールや方法論の中で、欧州の PCC からのばく露データを交換・比較する方法が開発された。実現可能な研 究として、ワークパッケージ 5 には、加盟国が農薬ばく露データを比較できるようにするための、調和のとれた強固なコ ード化システムの開発が含まれていた。しかし、PCC コミュニティとの協議の結果、データのコーディングと収集活動の さらなる調整が必要であることが示された。その結果、ばく露データを加盟国間で比較できるようにするためには、EUと 加盟国レベルでの更なる支援と調整が必要であると結論づけられた(Orford ら、2015 年)。

PCC が収集したデータに加えて、いくつかの加盟国は、労働衛生監視に特化したプログラムを立ち上げた<sup>12</sup>。これら は、業務上の農薬による傷害や病気、中毒が疑われる症例について、医師による自発的なイベント通知(使用者による 自己申告の場合もある)に基づいている。医療データに加えて、収集された情報には、作物の種類、散布方法、温度、 風速、個人用保護具の着用状況などに関するデータが含まれる。一度収集されたこれらのデータは調査され、定期的 に報告書が発行され、再登録中の製品の安全性を評価するための有用な情報となる。これらのデータは調査され、定期的 に報告書が発行され、再登録中の製品の安全性を評価するための有用な情報となる。これらのデータはまた、新たな 問題を浮き彫りにし、政策立案者のためのエビデンスに基づく予防措置を策定することを可能にする。EU レベルでは、 欧州労働安全衛生庁(EU-OSHA)<sup>13</sup>は、職業上の農薬関連疾病データのモニタリング方法をほとんど持っていない。 米国では、国立労働安全衛生研究所(NIOSH)が資金を提供し、農薬に特化したプログラムがいくつかの州で実施さ れている<sup>14</sup>。

要約すると、現在、ヒトのデータは、症例報告書や症例集積、毒物センターの情報、検視官の裁判結果、労働衛生 監視プログラムや市販後の監視プログラムの形で収集されている。しかし、申請者が提出した医療データには、このよう な情報がすべて含まれているわけではない。これは、さまざまな情報源が多様で異質な性質を持っているため、アクセ スできないものもあるためである。

- ・工場生産労働者の労働衛生監視を通じて収集されたデータ、あるいはそれが行われたとしても、医療データは非常に限られており、一般的には基本的な臨床血液測定、身体検査、潜在的にはどこでどのようにばく露されたかという単純な指標であり、通常は長期的なフォローアップは行われていない。さらに、最新の工場(特に EU)での労働者のばく露は一般的に非常に低く、多くの場合、潜在的なばく露は(特定の化学物質に特化した施設でない限り)様々な農薬へのばく露である。
- ・さらに、製造中の有効成分への職業上ばく露からのデータの報告は、しばしば調合された植物保護製剤(農薬)との接触から生じる観察結果と組み合わされる。実際、植物保護製剤(農薬)中の共配合剤の存在は、急性毒性学的プロフィルを変更することができる。したがって、適切な評価を容易にするために、ヒトで収集した結果を報告する際には、それ自体が有効成分なのか PPP なのかを明確に特定しなければならない。

EC 規則 283/2013 の第 5.9 章の一部でもある有効成分や配合された植物保護製剤(農薬)や提案された治療法に よる中毒の診断に関する特定のデータの要求に関しては、情報が欠落していたり、毒性作用のモードがヒトで起こること が知られていて特定の解毒剤が特定されている場合に限定されていたりすることがよくある。

<sup>&</sup>lt;sup>12</sup> For example: Phyt'attitude in France is a vigilance programme developed by the Mutualité Sociale Agricole: http://www.msa. fr/lfr/sst/phyt-attitude

<sup>&</sup>lt;sup>13</sup> https://osha.europa.eu/en/about-eu-osha

<sup>&</sup>lt;sup>14</sup> SENSOR programme: https://www.cdc.gov/niosh/topics/pesticides/overview.html

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<sup>&</sup>lt;sup>12</sup> 例えばフランスの Phyt'attitude は、Sociale Agricole, Mutualite, Sociale Agricole によって開発された警戒プログラムである: http://www.msa。

<sup>13</sup> https://osha.europa.eu/en/about-eu-osha

 $<sup>^{14}\ {\</sup>rm SENSOR}\ {\mathcal T}{\it \Box}{\it D}{\it \neg}{\it \bot}{\it :} {\rm https://www.cdc.gov/niosh/topics/pesticides/overview.html}$ 

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# 5.3. Proposals for improvement of current framework of case incident reporting

In order to avoid duplication and waste of effort, a logical next step would be to now develop, with all concerned public and private sector actors, an EU 'phytopharmacovigilance' system for chemicals similar to the ones that have been put in place for medicines. This network could be based on committed and specifically trained occupational health physicians and general practitioners in rural areas, and resources should be allocated by Member States to establish and to successfully maintain the system. Indeed such a network would be useful in detecting acute effects; it would also act as a sentinel surveillance network for specific health effects (such as asthma, sensitisation, etc.) or for the detection of emerging work-related disease. In fact, while much experience has already been gained on how to gradually build such a system, it is nevertheless envisioned that this will take a number of vears to be put in place. Several difficulties will arise because of the nature of the data collected (the sources of information are potentially diverse), the quality and completeness of the collected information for every case (especially the circumstances), the grading of severity and accountability of the observed effects (the link between the observed effect and the product). Rules should be defined so that they are identical from one 'evaluator' to another. The network should be stable over time (e.g. continuity in national organisations involved, consistent methodology employed, etc.), to ensure that the phytopharmacovigilance system fully complies with the objectives, i.e. monitoring changes over time. The use of phytopharmacovigilance data is unlikely to be limited to risk assessment purposes and may have an impact on risk management decisions (e.g. revisions in the terms and conditions of product authorisations or ultimately product withdrawal); this should be clear to all stakeholders from the outset.

Such a system may not merit being established solely for chemicals that are (predominantly) used as pesticides. However, given the legislative provisions already in place for pesticides, its development may need to be prioritised for pesticides.

In conclusion, the European Commission together with the Member States should initiate the development of an EU-wide vigilance framework for pesticides. These should include:

- harmonisation of human incident data collection activities at the EU level:
- coordination of the compilation of EU-wide databases;
- improving the collaboration between Poison Centres and regulatory authorities at national level in order to collect all the PPP poisonings produced in each Member State;
- guidance document on monitoring the impact of pesticide use on human health with harmonisation of data assessment for causal relationships;
- regular EU-wide reports.

#### 6. Proposed use of epidemiological studies and vigilance data in support of the risk assessment of pesticides

This section briefly reviews the risk assessment process (Section 6.1) based on experimental studies and discusses what information epidemiological studies could add to that process. Next, the assessment of the reliability of epidemiological studies is addressed in Section 6.2. In Section 6.3, the relevance of one or more studies found to be reliable is assessed.

#### 6.1. The risk assessment process

Risk assessment is the process of evaluating risks to humans and the environment from chemicals or other contaminants and agents that can adversely affect health. For regulatory purposes, the process used to inform risk managers consists of four steps (EFSA, 2012a). On the one hand, information is gathered on the nature of toxic effects (hazard identification) and the possible doseresponse relationships between the pesticide and the toxic effects (hazard characterisation). On the other hand, information is sought about the potential exposure of humans (consumers, applicators, workers, bystanders and residents) and of the environment (exposure assessment). These two elements are weighed in the risk characterisation to estimate that populations be potentially exposed to quantities exceeding the reference dose values, that is, to estimate the extra risk of impaired health in the exposed populations. Classically, this is used to inform risk managers for regulatory purposes.

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a) Step 1. Hazard identification.

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# 5.3. ケースインシデント報告の現行枠組みの改善提案

重複と努力の無駄を避けるために、論理的な次のステップは、すべての関係する公的部門と民間部門の関係者と一 緒に、医薬品のために実施されているものと同様の化学物質のための EU の「植物薬理監視」システムを開発すること であろう。このネットワークは、献身的で特別な訓練を受けた地方の産業保健医や開業医を基盤とすることができ、シス テムを確立し、成功裏に維持するために加盟国が供給源を配分すべきである。実際、このようなネットワークは急性の 影響を検出するのに有用であろう。また、特定の健康影響(喘息、感作など)や新たな職業関連疾患の検出のためのセ ンチネルサーベイランスネットワークとしても機能するであろう。実際、このようなシステムを段階的に構築する方法につ いては、すでに多くの経験が得られているが、それにもかかわらず、これが実施されるまでには何年もかかることが想定 されている。収集されるデータの特性(情報源は多様である可能性がある)、収集された情報の質と完全性(特に状況)、 観察された効果の重症度と説明責任(観察された効果と製品との間のリンク)など、いくつかの困難が生じる。ルールは、 ある「評価者」から別の「評価者」まで同一であるように定義されなければならない。植物薬理監視システムが目的に完 全に適合していることを保証するために、ネットワークは長期的に安定していなければならない(例えば、関与する国の 組織の継続性、採用された一貫した方法論など)。植物薬剤モニタリングデータの使用は、リスク評価の目的に限定さ れることはなく、リスク管理上の政策決定(例えば、製品認可の条件の改定や最終的には製品の取り下げなど)に影響 を及ぼす可能性があるが、これは最初からすべての利害関係者に明確でなければならない。

このようなシステムは、(主に)農薬として使用されている化学物質のみを対象としたものでは意味がないかもしれない。しかし、すでに農薬に関する法律の規定があることを考えると、このシステムの開発は農薬に優先して行われる必要があるかもしれない。

結論として、欧州委員会は加盟国とともに、EU 全体の農薬の警戒枠組みの開発に着手すべきである。これには以下が含まれるべきである。

- ・ EU レベルでのヒトでの健康影響データ収集活動の調和
- ・ EU 全体のデータベースの編集の調整
- 各加盟国で発生したすべての PPP 中毒を収集するために、各国レベルでのポイズンセンターと規制当局との連携を改善すること
- ・ 因果関係のデータ評価の整合化を伴う農薬使用がとトの健康に及ぼす影響のモニタリングに関するガイダンス文書
- ・ EU 全体を対象とした定期的な報告書

# 6. 農薬のリスク評価を支援するための疫学研究と監視データの利用の提案

本節では、実験的研究に基づくリスク評価プロセス(第 6.1 節)を概説し、そのプロセスに疫学的研究がどのような情報を付加しうるかを論じる。 次に、第 6.2 節では、疫学研究の信頼性の評価について述べる。 6.3 節では、信頼性があると認められた 1 つ以上の研究の関連性を評価する。

# 6.1. リスク評価プロセス

リスクアセスメントとは、健康に悪影響を及ぼす可能性のある化学物質やその他の汚染物質、薬剤によるヒトや環境 へのリスクを評価するプロセスである。規制目的のために、リスク管理者に情報を提供するために使用されるプロセスは、 4 つのステップで構成されている(EFSA、2012 年 a)。一方では、毒性影響の特性(ハザード同定)と、農薬と毒性影 響の間に考えられる用量反応関係(ハザード特性評価)に関する情報が収集される。一方で、ヒト(消費者、散布者、労 働者、居合わせただけの者、住民)と環境へばく露可能性についての情報が求められる(ばく露評価)。これら2つの要 素は、集団が基準ばく露量を超える量にばく露される可能性があることを推定するために、リスク特性評価の中で考慮 される。通常、これは規制目的のためのリスク管理者への情報提供に用いられる。

#### a) ステップ 1. ハザードの同定

疫学的研究と監視データは、農薬ばく露と健康影響とが関連する可能性を示すことができるため、ハザードの特定 に関連している。この背景では、疫学的データは、実験モデルでは検出されなかった影響を「ホライズン・スキャニング」

Epidemiological studies and vigilance data are relevant for hazard identification as they can point to potential link between pesticide exposure and health. In this context, epidemiological data can provide invaluable information in 'scanning the horizon' for effects not picked up in experimental models. Importantly, these studies also provide information about potentially enhanced risks for vulnerable population subgroups, sensitive parts of the lifespan, and gender selective effects.

b) Step 2, Hazard characterisation (dose-response assessment). As previously discussed a classic dose-response framework is not normally considered when using epidemiological data as the exposure dose is rarely assigned. The challenge presented when high guality epidemiological studies are available is to see whether these can best be integrated into the scheme as numerical input. A doseresponse framework is rarely considered when using epidemiological data for risk assessment of pesticides. However, previous scientific opinions of the EFSA CONTAM Panel have used epidemiology as basis for setting reference values, particularly in the case of cadmium, lead, arsenic and mercury, which are the most well-known and data rich (EFSA, 2009a,b, 2010b, 2012b). Even when they may not form the basis of a dose-response assessment, vigilance and epidemiological data may provide supportive evidence to validate or invalidate a dose-response study carried out in laboratory animals. Characterisation of the relationships between varying doses of a chemical and incidences of adverse effects in exposed populations requires characterisation of exposure or dose, assessment of response and selection of a dose-response model to fit the observed data in order to find a no-effect level. This raises two questions; can a dose-response be derived from epidemiological data to identify a no-effect level. If not, can epidemiological information otherwise contribute to the hazard characterisation?

Understanding dose-response relationships could also be relevant where adverse health outcomes are demonstrated to be associated with uses with higher exposures than EU good plant protection practice would give rise to, but where no association is observed from uses with lower exposures. It is clear that in this context the statistical summary of an epidemiological study defining RR or OR is potentially useful quantitative information to feed into the hazard characterisation process, when the study design meets the necessary standards.

c) Step 3. Exposure assessment. Data concerning the assessment of exposure are often hard to estimate in complex situations where a variety of uncontrolled 'real-world' factors confound the analysis. As discussed previously, contemporary biological monitoring is rarely carried out in the general human population for practical reasons including high cost, test availability and logistics. However, it is anticipated that in the near future biomonitoring studies and data on quantitative exposure to pesticides will increase.

Step 4. Risk characterisation. In this final step, data on exposure are compared with health-based reference values to estimate the extra risk of impaired health in the exposed populations. Human data can indeed help verify the validity of estimations made based on extrapolation from the full toxicological database regarding target organs, dose-response relationships and the reversibility of toxic effects, and to provide reassurance on the extrapolation process without direct effects on the definition of reference values (London et al., 2010).

Epidemiological data might also be considered in the context of uncertainty factors (UFs). An UF of 10 is generally used on animal data to account for interspecies variability of effects and this is combined with a further factor of 10 to account for variation in susceptibility of different parts of the human population. However, there are cases where only human data are considered (when this is more critical than animals data) and a single factor of 10 for intraspecies variability will apply. It is noted that at this moment Regulation (EC) No 1107/2009 Article 4(6) stipulates that: 'In relation to human health, no data collected on humans shall be used to lower the safety margins resulting from tests on animals'. The implication of this is that for risk assessment epidemiological data may only be used to increase the level of precaution used in the risk assessment, and not to decrease UFs even where relevant human data are available.

#### Assessment of the reliability of individual epidemiological studies 6.2.

Factors to be considered in determining how epidemiology should be considered for a WoE assessment are described below and have been extensively outlined by available risk of bias tools for observational epidemiological studies.<sup>15</sup> The following examples represent factors to look for not an exhaustive list:

• Study design and conduct. Was the study design appropriate to account for the expected distributions of the exposure and outcome, and population at risk? Was the study conducted primarily in a hypothesis generating or a hypothesis-testing mode?

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する上で、非常に貴重な情報を提供することができる。重要なことは、これらの研究はまた、脆弱な集団のサブグルー プ、牛涯における感受性の高い時期、性別による選択的影響など、リスクが高まる可能性についての情報を提供するこ とである。

b) ステップ 2. ハザードの特性評価(用量反応評価)

前述したように、通常、疫学的データを使用する場合には、ばく露量が割り当てられることはほとんどないため、古典 的な用量反応の枠組みは考慮されない。質の高い疫学研究が利用可能な場合の課題は、それらを数値入力としてス キームに統合するのが最善かどうかを見極めることである。農薬のリスク評価に疫学的データを使用する場合、用量反 応フレームワークが考慮されることはほとんどない。しかし、EFSA CONTAM パネルのこれまでの科学的見解では、基 準ばく露量を設定するための基礎として疫学を使用してきた、特にカドミウム、鉛、ヒ素、水銀の場合は、最もよく知られ ていてデータが豊富である(EFSA、2009年a,b、2010年b、2012年b)。これらが用量反応評価の基礎とならない場 合でも、監視と疫学的データは、実験動物を用いた用量反応研究の妥当性を検証したり、無効にしたりするための裏 付けとなるエビデンスを提供することがある。化学物質の様々な用量とばく露された集団における有害な影響の発生率 との間の関係を特性評価するためには、ばく露または用量の特性評価、反応の評価、無影響量を特定するために観察 されたデータが適合する用量反応モデルの選択が必要である。2 つの課題が提起される。すなわち、無影響量を特定 するために、疫学的データから用量反応を導き出すことができるのか、ということである。もしそうでない場合、疫学的情 報はハザードの特性評価に貢献できるのか、ということである。

用量反応関係を理解することは、EU の優れた植物保護対策が予想されるよりも高いばく露量の使用による有害な 健康影響が関連していることが証明されるが、低いばく露量の使用では関連性が観察されない。この背景では、RR ま たは OR を明らかにした疫学研究の統計的要約は、研究デザインが必要な基準を満たしている場合には、ハザード特 性評価プロセスに投入するための有用な定量的情報となる可能性があることは明らかである。

#### c) ステップ 3. ばく露評価

ばく露の評価に関するデータは、制御されていない様々な「実社会」の要因が解析を混乱させる複雑な状況では、 推定が困難なことが多い。前述したように、現代の生物学的モニタリングは、コストの高さ、実施可能性、ロジスティック スなどの実際的な理由から、一般のビト集団ではほとんど実施されていない。しかし、近い将来、農薬への定量的ばく 露に関する生物学的モニタリング研究やデータが増加することが予想されている。

ステップ 4. リスクの特性評価。この最後のステップでは、ばく露に関するデータを健康ベースの基準値と比較し、ば く露された集団における健康障害のリスクを推定する。ヒトのデータは、標的臓器、用量反応関係、毒性影響の可逆性 に関する完全な毒性学的データベースからの外挿に基づいて行われた推定の妥当性を検証するのに役立ち、基準値 の定義に直接影響を与えずに外挿のプロセスを再確認するのに役立つ(London ら、2010 年)。

疫学的データは、不確実性因子(UF)との関連で考慮されることもある。一般的に動物データでは、影響の種間変 動を考慮するために 10 の UF が使用され、これにさらに 10 の係数を加えてヒト集団の異なる部分の感受性の変動を 考慮する。しかし、ヒトのデータのみを考慮する場合(動物のデータよりも重要な場合)もあり、種族間のばらつきを考慮 した 10 の係数が適用される。現時点では、規則(EC)No 1107/2009 の第4条(6)が次のように規定していることに留 意する。「ヒトの健康に関連して、ヒトから収集したデータがない場合、動物試験に由来する安全マージンを低下させて はならない」と規定している。このことの意味するところは、リスク評価において疫学的データはリスク評価で使用される 警戒レベルを高めるためにのみ使用され、関連するヒトのデータが入手可能であっても UF を低下させるために使用さ れてはならないということである。

# 6.2. 個々の疫学研究の信頼性の評価

WOE 評価のために疫学をどのように考慮すべきかを決定する際に考慮すべき因子は以下に記載されており、観察 疫学的研究のためのバイアスのリスクツールで広く概説されている15。以下の例は、網羅的なリストではないが注目すべ

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<sup>&</sup>lt;sup>15</sup> Assessing Risk of Bias and Confounding in Observational Studies of Interventions or Exposures: Further Development of the RTI Item Bank (https://www.ncbi.nlm.nih.gov/books/NBK154464/) and Cochrane handbook. 37

<sup>15</sup> 介入またはばく露の観察研究におけるバイアスと交絡因子のリスクの評価。RTI アイテムバンクのさらなる発展 (https://www.ncbi.nlm.nih.gov/books/NBK154464/)とコクランハンドブック

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- Population. Did the study sample the individuals of interest from a well-defined population? Did the study have adequate statistical power and precision to detect meaningful differences for outcomes between exposed and unexposed groups?
- Exposure assessment. Were the methods used for assessing exposure valid, reliable and adequate? Was a wide range of exposures examined? Was exposure assessed at quantitative level or in a categorical or dichotomous (e.g. ever vs never) manner? Was exposure assessed prospectively or retrospectively?
- Outcome assessment. Were the methods used for assessing outcomes valid, reliable and adequate? Was a standardised procedure used for collecting data on health outcomes? Were health outcomes ascertained independently from exposure status to avoid information bias?
- Confounder control: were potential confounding factors appropriately identified and considered? How were they controlled for? Were the methods used to document these factors valid, reliable and adequate?
- Statistical analysis. Did the study estimate quantitatively the independent effect of an exposure on a health outcome of interest? Were confounding factors appropriately controlled in the analyses of the data?
- Is the *reporting* of the study adequate and following the principles of transparency and the guidelines of the STROBE statement (or similar tools)?

Study evaluation should provide an indication on the nature of the potential limitations each specific study may have and an assessment of overall confidence in the epidemiological database.

Furthermore, the nature and the specificity of the outcome with regards to other known risk factors can influence the evaluation of human data for risk assessment purposes, particularly in case of complex health endpoints such as chronic effects with long induction and latency periods.

Table 2 shows the main parameters to be evaluated in single epidemiological studies and the associated weight (low, medium and high) for each parameter. Specific scientific considerations should be applied on a case-by-case basis, but it would be unrealistic to implement these criteria in a rigid and unambiguous manner.

	Table 2:	Study guality	considerations	for weighting	epidemiological	observational studies <sup>(a),(b)</sup>
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Parameter	High	Moderate	Low
Study design and conduct	Prospective studies. Prespecified hypothesis (compound and outcome specific)	Case-control studies. Prospective studies not adequately covering exposure or outcome assessment	Cross-sectional, ecological studies Case–control studies not adequately covering exposure or outcome assessment
Population	Random sampling. Sample size large enough to warrant sufficient power Population characteristics well defined (including vulnerable subgroups)	Questionable study power, not justified in detail Non-representative sample of the target population Population characteristics not sufficiently defined	No detailed information on how the study population was selected Population characteristics poorly defined
Exposure assessment	Accurate and precise quantitative exposure assessment (human biomonitoring or external exposure) using validated methods Validated questionnaire and/or interview for chemical-specific exposure answered by subjects	Non-valid surrogate or biomarker in a specified matrix and external exposure Questionnaire and/or interview for chemical-specific exposure answered by subjects or proxy	and/or interview; information collected for groups of chemicals No chemical-specific exposure information collected;
Outcome Assessment	Valid and reliable outcome assessment. Standardised and validated in study population Medical record or diagnosis confirmed	individuals Standardised outcome, not validated in population, or screening tool; or, medical record non-confirmed	general evaluated Non-standardised and non-validated health outcome Inappropriate or self-reported outcomes.

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き因子を示している。

- 研究デザインと実施。研究デザインは、ばく露と健康影響及びリスクのある集団の予想される分布を考慮して適切 なものであったか?その研究は主に仮説生成モードまたは仮説検証モードで実施されたか?
- 集団。研究は、十分に定義された集団から目的の個人をサンプリングしたか?研究は、ばく露群と非ばく露群の健康影響について有意な差を検出するのに十分な統計力と精度を有していたか。
- ・ばく露の評価。ばく露の評価に使用された方法は有効で、信頼性があり、適切であったか?広範囲のばく露が調査されたか?ばく露は定量的レベルで評価されたのか、それともカテゴリカルまたは二分法(例:経験対未経験)で 評価されたのか?ばく露は前向きに評価されたか、あるいは後ろ向きに評価されたか?
- ・健康影響の評価。健康影響の評価に使用された方法は有効で、信頼性が高く、適切であったか?健康影響に関するデータ収集には標準化された手順が用いられていたか。情報の偏りを避けるために、健康影響はばく露状態とは独立して把握されていたか?
- ・交絡因子の管理:潜在的な交絡因子が適切に特定され、考慮されていたか?それらはどのように管理されていたか?これらの因子を記録するために使用された方法は有効で、信頼性があり、適切であったか?
- 統計解析。研究は、対象となる健康影響に対するばく露の独立した影響を定量的に推定したか?データの解析において交絡因子が適切に管理されていたか。
- ・研究の報告は適切であり、透明性の原則とSTROBE 声明(または同様のツール)のガイドラインに従っているか。

研究の評価は、それぞれの研究が持つ可能性のある潜在的な限界の特性と、疫学的データベースの全体的な整合 性の評価を示すものでなければならない。

さらに、他の既知のリスク因子に関する健康影響の特性と特異性は、リスク評価目的のためのヒトデータの評価に影響を与える可能性があり、特に誘発期間や潜伏期間の長い慢性的な影響のような複雑な健康エンドポイントの場合には、その評価に影響を与える可能性がある。

表 2 は、単一の疫学研究で評価すべき主なパラメータと、各パラメータの関連する程度(低、中、高)を示している。 特定の科学的考察はケースバイケースで適用されるべきであるが、これらの基準を厳格かつ明確な方法で実施することは非現実的である。

# 表 2:疫学的観察研究の重み付けのための研究の質に関する考察(ω)、(ω)

パラメータ	吉	中	低
試験のデザ	前向き研究 特定の仮説(化合物と	症例対照研究。ばく露または	横断、生態学的研究
インと実施	健康影響の特定)	健康影響評価を十分にカバー	症例対照研究では、ばく露や健康
		していない前向き研究	影響評価が十分にカバーされて
			いない
集団	ランダムサンプリング。十分な検出	疑わしい研究検出力、詳細に	研究集団の選定方法についての
	力を保証するのに十分な大きさの	正当化されていない	詳細な情報がない
	サンプルサイズ		
	母集団の特性が十分に把握されて	標的集団の代表的なサンプル	母集団の特徴が十分に解明さ
	いる(脆弱なサブグループを含む)	ではない	ていない
		母集団の特性が十分に解明さ	
		れていない	
ばく露評価	検証された方法を用いた正確かつ	特定のマトリックス中の非有	乏しいサロゲート
	精密な定量的ばく露評価(ヒトのバ	効なサロゲートまたはバイオ	質の低いアンケート及び/ま
	イオモニタリングまたは外部ばく	マーカーと外部ばく露	はインタビュー;化学物質のグ
	露)	被験者または代理人が回答し	ープについて収集された情報
	被験者が回答した化学物質ばく露	た化学物質ばく露に関するア	化学物質に特化したばく露情
	に関する有効なアンケート及び/ま	ンケート及び/またはインタ	は収集されていない;農薬の使)
	たはインタビュー	ビュー	の有無の一般的な評価
健康影響評	有効で信頼性の高い健康影響評価	標準化された健康影響、母集	標準化されていない、検証され
価	研究集団において標準化され、妥当	団で有効性が確認されていな	いない健康影響
	性が確認されていること	い、またはスクリーニングツ	不適切な健康影響、または自己
	カルテまたは診断結果が記載され	ール、またはカルテが不明確	告された健康影響

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Parameter	High	Moderate	Low
Confounder control	Adequate control for important confounders relevant to scientific question, and standard confounders Careful consideration is given to clearly indicated confounders	Confounders are partially controlled for Moderately control of confounders and standard variables Not all variables relevant for scientific question are considered	No control of potential confounders and effect modifiers in the design and analysis phases of the study
Statistical Analysis	Appropriate to study design, supported by adequate sample size, maximising use of data, reported well (not selective) Statistical methods to control for confounding are used and adjusted and unadjusted estimates are presented. Subgroups and interaction analysis are conducted	Acceptable methods, analytic choices that lose information, not reported clearly Post hoc analysis conducted but clearly indicated	Only descriptive statistics or questionable bivariate analysis is made Comparisons not performed or described clearly Deficiencies in analysis (e.g. multiple testing)
Reporting	Key elements of the Material and Methods, and results are reported with sufficient detail Numbers of individuals at each stage of study is reported A plausible mechanism for the association under investigation is provided	Some elements of the Material and Methods or results are not reported with sufficient detail Interpretation of results moderately addressed	Deficiencies in reporting (interpretation of effect estimates, confounder control) Selective reporting Paucity of information on relevant factors that may affect the exposure-health relationship. Misplaced focus of the inferential objectives Not justified conclusions

(a): Overall study quality ranking based on comprehensive assessment across the parameters.

(b): Adapted from US-EPA (2016), based in turn on Muñoz-Quezada et al. (2013) and LaKind et al. (2014).

If the above assessment is part of the evidence synthesis exercise, where epidemiological research is being assessed and quantitatively summarised, it permits more accurate estimation of absolute risk related to pesticide exposure and further quantitative risk assessment.

In the particular case of pesticide epidemiology data, three basic categories are proposed as a first tier to organise human data with respect to risk of bias and reliability<sup>16</sup>: (a) low risk of bias and high reliability (all or most of the above quality factors have been addressed with minor methodological limitations); (b) medium risk of bias and medium reliability (many of the above quality factors have been addressed with moderate methodological limitations); (c) high risk of bias and low reliability, because of serious methodological limitations or flaws that reduce the validity of results or make them largely uninterpretable for a potential causal association. The latter studies are considered unacceptable for risk assessment mainly because of poor exposure assessment, misclassification of exposure and/or health outcome, or lack of statistical adjustment for relevant confounders. Risk assessment should not be based on results of epidemiological studies that do not meet well-defined data guality standards. Furthermore, results of exploratory research will need to be confirmed in future research before they can be used for risk assessment.

### 6.3. Assessment of strength of evidence of epidemiological studies

This section briefly discusses some important issues specifically related to combining and summarising results from different epidemiological studies on the association between pesticides and human health.

The approach for weighting epidemiological studies is mainly based on the modified Bradford Hill criteria, which are a group of conditions that provide evidence bearing on a potentially causal relationship between an incidence and a possible consequence (strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy) (Table 3). Clearly, the Epidemiological studies and pesticides

	ていること	な場合	
交絡因子コ ントロール	科学的な課題に関連する重要な交 絡因子と標準的な交絡因子の適切 なコントロール 明らかに示された交絡因子を慎重 に考慮	交絡因子は部分的にコントロ ール 交絡因子と標準変数を中程度 にコントロール 科学的な課題に関連するすべ ての変数が考慮されているわ けではない	研究のデザイン及び解析段階で 潜在的な交絡因子及び効果修飾 因子をコントロールしていない
統計解析	研究デザインが適切であること、適 切なサンプルサイズに支えられて いること、データを最大限に利用し ていること、よく報告されているこ と(選択的ではない) 交絡因子をコントロールするため の統計的手法が用いられており、調 整済み及び未調整の推定値が提示 されている	受け入れ可能な方法、情報を 失う分析的な選択、明確に報 告されていない 事後分析を実施したが、明確 に示された	記述的統計、または二変量分析の 疑わしいものだけが作られてい る 比較が行われていない、または明 確に記載されていない 分析の不備(多変量解析など)
報告	材料と方法の主要な要素と結果は、 十分に詳細に報告されている 研究の各段階における参加者数が 報告されている 調査中の関連性のについて信憑性 のあるメカニズムが示されている	材料と方法のいくつかの要素 や結果は、十分な詳細が報告 されていない 結果の解釈は中程度に対応	報告の不備(効果推定値の解釈、 交絡因子コントロール) 選択的報告 ばく露と健康の関係に影響を及 ぼす可能性のある関連因子に関 する情報の不足 推論目的の焦点がずれている 正当化された結論ではない

(a):パラメータ全体の総合的な評価に基づく総合的な研究品質ランキング。

(b): Muñoz-Ouezada ら (2013) と LaKind ら (2014) を順に引用した US-EPA (2016) からの引用。

上記の評価が、疫学研究が評価され定量的にまとめられているエビデンス総合演習の一部である場合、農薬ばく露 に関連する絶対的リスクをより正確に推定し、さらに定量的なリスク評価を行うことが可能となる。

農薬疫学データの場合には、バイアスのリスクと信頼性に関してヒトデータを整理するための第一段階として、3 つの 基本的なカテゴリーが提案されている16。(a)バイアスのリスクが低く、信頼性が高い(上記の品質要因のすべて、また は大部分が軽微な方法論的限界で対処されている):(b)バイアスのリスクが中程度で信頼性が中程度(上記の品質要 因の多くが中程度の方法論的限界で対処されている);(c)バイアスのリスクが高く、信頼性が低い(結果の妥当性を低 下させる、または潜在的な因果関係をほとんど解釈できない、といった重大な方法論的限界や欠陥があるため)。後者 の研究は、主にばく露評価の不備、ばく露及び/または健康影響の誤分類、または関連する交絡因子の統計的調整 の欠如により、リスク評価には受け入れられないと考えられている。リスク評価は、十分に定義されたデータ品質基準を 満たしていない疫学研究の結果に基づくべきではない。さらに、予備的研究の結果は、リスク評価に使用する前に、将 来の研究で確認する必要がある。

## 6.3. 疫学研究のエビデンスの強さの評価

このセクションでは、農薬ととト健康影響との関連性に関する様々な疫学研究から得られた結果を組み合わせて要約 することに関連したいくつかの重要な問題について簡潔に論じている。

疫学研究の重み付けのアプローチは、主に修正された Bradford Hill 基準に基づいている。これは、事象と起こりえ る結果(強さ、一貫性、特異性、時間性、生物学的勾配、妥当性、統一性、実験と類推)との間の潜在的な因果関係を 示すエビデンスを提供する条件のグループである(表 3)。明らかに、これらの基準を満たせば満たすほど、意味のある

<sup>&</sup>lt;sup>16</sup> These categories are in accordance with those currently used by EFSA for the peer review of pesticide active substances: acceptable, supplementary and unacceptable. 39

<sup>16</sup> これらのカテゴリーは、現在 EFSA が農薬有効成分のピアレビューに使用している、許容可能、補助的、非許容のカテゴリーに準拠している。 EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna 39

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more of these criteria that are met the stronger the basis for invoking the association as evidence for a meaningful association. However, Bradford Hill was unwilling to define what causality was and never saw the criteria as sufficient or even absolutely necessary but simply of importance to consider in a common-sense evaluation.

Table 3:	Considerations for WoE analysis based on the modified Bradford Hill criteria for evidence	
	integration	

Category	Considerations
Strength of Association	The assessment of the strength of association (not only the magnitude of association but also statistical significance) requires examination of underlying methods, comparison to the WoE in the literature and consideration of other contextual factors including the other criteria discussed herein
Consistency of Association	Associations should be consistent across multiple independent studies, particularly those conducted with different designs and in different populations under different circumstances. This criterion also applies to findings consistent across all lines of evidence (epidemiology, animal testing, <i>in vitro</i> systems, etc.) in light of modern data integration
Specificity	The original criteria of evidence linking a specific outcome to an exposure can provide a strong argument for causation has evolved and may have new and interesting implications within the context of data integration. Data integration may elucidate some mechanistic specificity among the varied outcomes associated with complex exposures. The lack of specificity can help to narrow down specific agents associated with disease
Temporality	Evidence of a temporal sequence between exposure to an agent and appearance of the effect within an appropriate time frame constitutes one of the best arguments in favour of causality. Thus, study designs that ensure a temporal progression between the two measures are more persuasive in causal inference
Biological Gradient (Dose-response)	Increased effects associated with greater exposures, or duration of exposures, strongly suggest a causal relationship. However, its absence does not preclude a causal association
Biological Plausibility	Data explained and supported by biologically plausible mechanisms based on experimental evidence strengthen the likelihood that an association is causal. However, lack of mechanistic data should not be taken as evidence against causality
Coherence	The interpretation of evidence should make sense and not to conflict with what is known about the biology of the outcome in question under the exposure-to-disease paradigm. If it does, the species closest to humans should be considered to have more relevance to humans
Experimental Evidence	Results from randomised experiments provide stronger evidence for a causal association than results based on other study designs. Alternatively, an association from a non- experimental study may be considered as causal if a randomised prevention derived from the association confirms the finding
Sequence of Key events	Provide a clear description of each of the key events (i.e. measurable parameters from a combination of <i>in vitro</i> , <i>in vivo</i> or human data sources) that underlie the established MoA/AOP for a particular health outcome. A fully elucidated MoA/AOP is a not requirement for using epidemiology studies in human health risk assessment

Adapted from Höfler (2005), Fedak et al. (2015) and US-EPA (2016).

For predictive causality, care must be taken to avoid the logical fallacy *post hoc ergo propter hoc* that states 'Since event Y followed event X, event Y must have been caused by event X'. Höfler (2005) quotes a more accurate 'counterfactual' definition as follows 'but for E, D will not occur or would not have occurred, but given E it will/would have occurred'. Yet, more detailed descriptions using symbolic logic are also available (Maldonado and Greenland, 2002). Rothman and Greenland (2008) stated that 'the only *sine qua non* for a counterfactual effect is the condition that the cause must precede the effect. If the event proposed as a result or "effect" precedes its cause, there may be an association between the events but certainly no causal relationship'.

#### 6.3.1. Synthesis of epidemiological evidence

Systematic reviews and meta-analysis of observational studies can provide information that strengthens the understanding of the potential hazards of pesticides, exposure-response characterisation, exposure scenarios and methods for assessing exposure, and ultimately risk characterisation (van den Brandt, 2002). Systematic reviews entail a detailed and comprehensive plan

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関連性の証拠としてその関連性を提起する根拠が強くなる。しかし、Bradford Hill は、因果関係とは何かを明確にすることを目的とせず、基準を十分に、あるいは絶対的に必要なものとしてかんがえず、単に常識的な評価の中で考慮することが重要であると考えている。

#### 表 3:エビデンス統合のための修正された Bradford Hill 基準に基づく WOE 解析の考察

カテゴリー	考察事項
関連性の強さ	関連性の強さ(関連性の大きさだけでなく、統計的有意性も)の評価には、基礎となる方法の 検討、文献の WOE との比較及びここで議論されている他の基準を含む他の背景的要因の考慮 が必要である。
関連性の一貫性	関連性は、複数の独立した研究、特に異なる計画で、異なる状況下で異なる集団で実施された 研究において一貫性があるべきである。この基準は、現代のデータ統合に照らして、すべての エビデンス系統(疫学、動物実験、in vitro システムなど)にまたがって一貫性のある結果に も適用される。
特異性	特定の結果をばく露に結びつけるエビデンスの独自の基準は、因果関係についての強力な諱 論を提供できるようになり、データ統合の背景の中で新たな興味深い意味合いを持つように なったかもしれない。データの統合は、複雑なばく露に関連した様々な結果の中から、いくつ かのメカニズム論的な特定性を明らかにするかもしれない。特異性の欠如は、疾患に関連する 特異的な薬剤を絞り込むのに役立つかもしれない。
時間性	薬剤へのばく露と適切な時間枠内での影響の出現との間の時間的順序のエビデンスは、因果関係を支持する最良の論拠の一つを構成する。このように、2つの尺度間の時間的進行を確実にする研究デザインは、因果関係の推論においてより説得力がある。
生物学的勾配(用量反 応)	より大きなばく露またはばく露の持続時間に関連した影響の増加は、因果関係を強く示唆している。しかし、その不在は因果関係を排除するものではない。
生物学的妥当性	実験的エビデンスに基づいた生物学的に信憑性のあるメカニズムによって説明され、支持されたデータは、関連性が因果関係にある可能性を強化する。しかし、メカニズム論的データの 欠如は因果関係に反するエビデンスとして捉えられるべきではない。
統一性	エビデンスの解釈は理にかなったものでなければならず、ばく露-疾病パラダイムの下で問題となっている健康影響の生物学について知られていることと矛盾するものであってはならない。もしそうであれば、ヒトに最も近い種の方がヒトとの関連性が高いと考えるべきである。
実験的エビデンス	無作為化実験の結果は、他の研究デザインに基づく結果よりも因果関係の強いエビデンスを 提供する。あるいは、非実験的研究からの関連は、関連から導き出された無作為化予防が結論 を導く場合には、因果関係があると考えられる。
重要事象の結果	特定の健康影響について確立された MOA/AOP の基礎となる重要事象(すなわち、in vitro、 in vivo、またはヒトのデータ源を組み合わせた測定可能なパラメータ)をそれぞれ明確に説明 する。完全に解明された MOA/AOP は、ヒト健康リスク評価に疫学研究を使用するための要 件ではない。

Höfler (2005)、Fedak ら(2015)及び US-EPA(2016)からの引用。

予測的因果関係については、「事象 Y が事象 X の後に続いたので、事象 Y は事象 X によって引き起こされたに違 いない」という論理的誤謬を避けるために注意を払わなければならない。Höfler(2005 年)は、より正確な「反事実」の 定義を次のように引用している:「しかし、E があれば、D は発生しないか、発生しなかっただろうが、E があれば発生す るだろう/しただろう」。しかし、記号論理を用いたより詳細な記述もある(Maldonado 及び Greenland、2002 年)。 Rothman 及び Greenland (2008 年)は、「反事実効果の唯一の必須条件は、原因が効果に先行しなければならない という状態である」と述べている。結果または「影響」として提案された事象がその原因に先行している場合、事象間の 関連性はあるかもしれないが、因果関係は確かにない」と述べている。

# 6.3.1. 疫学的証拠の統合

観察による研究のシステマティックレビューとメタアナリシスは、農薬の潜在的なハザード、ばく露反応の特徴、ばく露 シナリオとばく露評価の方法、そして最終的にはリスクの特性評価の理解を強化する情報を提供することができる(van den Brandt、2002 年)。システマティックレビューは、特定のトピックに関するすべての関連研究を特定し、評価し、統 合することでバイアスを低減することを目的とした、詳細で包括的な計画と事前に定められた検索戦略を伴う。システマ ティックレビューの主なステップは以下の通りである:研究課題の策定、包含基準と除外基準の定義、異なるデータベ www.efsa.europa.eu/efsajourna EFSA Journal 2017;15(10):5007

and search strategy defined a priori aimed at reducing bias by identifying, appraising and synthesising all relevant studies on a particular topic. The major steps of a systematic review are as follows: formulation of the research question; definition of inclusion and exclusion criteria; search strategy for studies across different databases; selection of studies according to predefined strategy; data extraction and creation of evidence tables; assessment of methodological quality of the selected studies; including the risk of bias; synthesis of data (a meta-analysis can be performed if studies allow); and interpretation of results and drawing of conclusions (EFSA, 2010a). Evidence synthesis is, however, challenging in the field of pesticide epidemiology as standardisation and harmonisation is difficult. Nonetheless, evidence synthesis should play a pivotal role in assessing the robustness and relevance of epidemiological studies.

Statistical tools have been developed that can help assess this evidence. When multiple studies on nearly identical sets of exposures and outcomes are available, these can provide important scientific evidence. Where exposure and outcomes are quantified and harmonised across studies, data from individual epidemiological studies with similar designs can be combined to gain enough power to obtain more precise risk estimates and to facilitate assessment of heterogeneity. Appropriate systematic reviews and quantitative synthesis of the evidence needs to be performed regularly (e.g. see World Cancer Research Fund approach to continuous update of meta-analysis for cancer risk factor<sup>17</sup>). Studies should be evaluated according to previously published criteria for observational research and carefully examine possible selection bias, measurement error, sampling error, heterogeneity, study design, and reporting and presentation of results.

Meta-analysis is the term generally used to indicate the collection of statistical methods for combining and contrasting the results reported by different studies (Greenland and O'Rourke, 2008). Meta-analysis techniques could be used to examine the presence of diverse biases in the field such as small study effects and excess significance bias. Meta-analyses, however, do not overcome the underlying biases that may be associated with each study design (i.e. confounding, recall bias or other sources of bias are not eliminated). The extent to which a systematic review or meta-analysis can draw conclusions about the effects of a pesticide depends strongly on whether the data and results from the included studies are valid, that is, on the quality of the studies considered. In particular, consistent findings among original studies resulting from a consistent bias will produce a biased conclusion in the systematic review. Likewise, a meta-analysis of invalid studies may produce a misleading result, vielding a narrow confidence interval around the wrong effect estimate.

In addition to summarising the basic study characteristics of the literature reviewed, a typical metaanalysis should include the following components: (a) the average effect size and effect size distribution for each outcome of interest and an examination of the heterogeneity in the effect size distributions; (b) subgroup analysis in which the variability present in the effect size distribution is systematically analysed to identify study characteristics that are associated with larger or smaller effect sizes; (c) publication bias analysis and other sensitivity analyses to assess the validity of conclusions drawn (Wilson and Tanner-Smith, 2014).

In a meta-analysis, it is important to specify a model that adequately describes the effect size distribution of the underlying population of studies. Meta-analysis using meaningful effect size distributions will help to integrate quantitative risk into risk assessment models. The conventional normal fixed- and random-effects models assume a normal effect size population distribution, conditionally on parameters and covariates. Such models may be adequate for estimating the overall effect size, but surely not for prediction if the effect size distribution exhibits a non-normal shape (Karabatsos et al., 2015).

#### 6.3.2. Meta-analysis as a tool to explore heterogeneity across studies

When evaluating the findings of different studies, many aspects should be carefully evaluated. Researchers conducting meta-analyses may tend to limit the scope of their investigation to the determination of the size of association averaged over the considered studies. The motivation often is that aggregating the results yields greater statistical power and precision for the effect of interest. Because individual estimates of effect vary by chance, some variation is expected. However, estimates must be summarised only when meaningful. An important aspect that is often overlooked is heterogeneity of the strength of associations across subgroups of individuals. Heterogeneity between

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ースにまたがる研究の検索戦略、事前に定めた戦略に従った研究の選択、データ抽出とエビデンス表の作成、選択し た研究の方法論的品質の評価、バイアスのリスクを含めた評価、データの統合(研究が許せばメタアナリシスを行うこと もできる)、結果の解釈と結論の導き出し(EFSA、2010 年 a)。しかし、農薬疫学の分野では、標準化と調和が困難で あるため、エビデンスの統合は困難である。それにもかかわらず、疫学研究の強固性と妥当性を評価する上で、エビデ ンス統合は極めて重要な役割を果たすべきである。

このエビデンスの評価に役立つ統計ツールが開発されている。ほぼ同一のばく露と転帰に関する複数の研究が利 用可能な場合、これらの研究は重要な科学的証拠を提供することができる。ばく露と転帰が研究間で定量化され、調 和されている場合には、類似した計画の個々の疫学研究からのデータを組み合わせることで、より正確なリスク推定値 を得るのに十分な検出力を得ることができ、不均一性の評価を容易にすることができる。適切なシステマティックレビュ ー及びエビデンスの定量的な統合を定期的に行う必要がある(例えば、世界がん研究基金のがんリスク因子のメタアナ リシスの継続的な更新のためのアプローチ17)。研究は、以前に発表された観察による研究の基準に従って評価され、 可能性のある選択バイアス、測定誤差、サンプリング誤差、異質性、研究デザイン及び結果の報告と提示について慎 重に検討されるべきである。

メタアナリシスとは、一般的に、異なる研究で報告された結果を組み合わせて比較するための統計的手法の集積を 示すために使用される用語である(Greenland 及び O'Rourke、2008 年)。メタアナリシスの技術は、小さな研究効果 や過剰な有意性バイアスなど、研究分野における多様なバイアスの存在を調べるために使用されることがある。しかし、 メタアナリシスは、各研究デザインに関連している可能性のある根本的なバイアスを克服するものではない(すなわち、 交絡、想起バイアス、または他のバイアスの原因が排除されない)。システマティックレビューやメタアナリシスが農薬の 影響について結論を導き出すことができる範囲は、含まれた研究から得られたデータや結果が有効かどうか、つまり検 討された研究の質に大きく依存する。特に、一貫したバイアスの結果として、オリジナルの研究間で一貫した結論が得 られれば、システマティックレビューでは偏った結論が得られることになる。同様に、無効な研究のメタアナリシスでは、 誤った影響推定値に狭い信頼性間隔が生じるなど、誤解を招く結果になる可能性がある。

レビューされた文献の基本的な研究の特徴を要約することに加えて、典型的なメタアナリシスには以下の要素が含ま れるべきである。(a) 対象となる各健康影響の平均影響量と影響量分布及び影響量分布の不均一性の検討(b) 影響 量分布に存在する変動性を系統的に解析し、効果量の大小に関連する研究の特徴を特定するサブグループ解析(c) 引き出された結論の妥当性を評価するための出版バイアス解析及びその他の感度分析(Wilson 及び Tanner Smith, 2014年)。

メタアナリシスでは、基礎となる研究集団の影響量分布を適切に記述するモデルを指定することが重要である。意味 のある影響量分布を用いたメタアナリシスは、定量的リスクをリスク評価モデルに統合するのに役立つ。従来の正規の 固定影響モデル及びランダム影響モデルは、パラメータと共変量に条件付きで正規の影響量母集団分布を仮定して いる。このようなモデルは、全体的な影響量を推定するのには適切かもしれないが、影響量分布が非正規の形状を示 す場合には予測には確実に適していない(Karabatsosら、2015年)。

# 6.3.2. 研究間の異質性を探索するツールとしてのメタアナリシス

異なる研究の結果を評価する際には、多くの側面を慎重に評価すべきである。メタアナリシスを行う研究者は、調査 の範囲を、考慮した研究を平均した関連性の大きさの結果に限定する傾向があります。その動機は、多くの場合、考慮 した研究を平均した関連性の強さの決定に限定する傾向がある。影響の個々の推定値は偶然性によって変化するた め、ある程度のばらつきは予想される。しかし、推定値は意味のある場合にのみ要約されなければならない。見落とさ れがちな重要な側面として、サブグループを超えた個人間の関連の強さの不均一性がある。研究間の不均一性は評 価され、存在する場合には定量化される必要がある(Higgins、2008 年)。メタアナリシスでは、異なる研究からの結果 の間の不均一性は、同質性と同じくらい有益であるかもしれない。観察された結果の矛盾の根底にある理由を探ること

<sup>&</sup>lt;sup>17</sup> World Cancer Research Fund International, Continuous Update Project (CUP) http://www.wcrf.org/int/research-we-fund/ continuous-update-project-cup 41

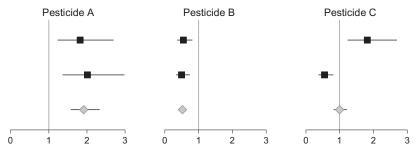
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<sup>17</sup> 世界がん研究基金インターナショナル。継続的更新プロジェクト(CUP) http://www.wcrf.org/int/research-we-fund/ continuous-updateproject-cup

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studies needs to be assessed and quantified when present (Higgins, 2008). In meta-analysis, heterogeneity among results from different studies may indeed be as informative as homogeneity. Exploring the reasons underlying any observed inconsistencies of findings is generally conducive of great understanding.

Figure 1 shows three forest plots from a fictitious example in which each of three pesticides (A, B and C) is evaluated in meta-analysis of two studies. It is assumed that both studies for each pesticide are of the highest quality and scientific rigor. No biases are suspected.



**Figure 1:** Forest plots from a fictitious example in which each of three pesticides (A, B and C) is evaluated in a meta-analysis of two studies. The x-axis in each plot represents the estimated risk ratio of the disease of interest comparing exposed and unexposed individuals. The squares denote the estimated risk ratio in each study and the grey diamonds the summarised risk ratio. The horizontal lines indicate 95% confidence intervals

The following text contains short comments on the interpretation of the results in Figure 1, one pesticide at a time.

- Exposure to pesticide A seems to double the risk of the disease. The results are consistent
  between the two studies and the confidence intervals do not contain the null value, one. These
  results, however, do not imply that (a) the risk ratio would be about 2 in any other study that
  was conducted on the same exposure and disease; or that (b) the risk ratio is two in any
  group of individuals (e.g. males or females, young or old).
- Exposure to pesticide B seems to halve the risk of the disease. The results are consistent between the two studies and the confidence intervals do not contain the null value, one. These results, however, do not imply that (a) the risk ratio would be about a half in any other study that was conducted on the same exposure and disease; or that (b) the risk ratio is about a half in any group of individuals (e.g. males or females, young or old).
- Exposure to pesticide C seems to double the risk of the disease in one study and to halve the
  risk in the other. The results are inconsistent between the two studies and the confidence
  intervals do not contain the null value, one. These results, however, do not imply that (a) the
  risk ratio would be about one in any other study that was conducted on the same exposure
  and disease; or that (b) the risk ratio is about one in any group of individuals (e.g. males or
  females, young or old).

#### What evidence can the results shown in Figure 1 provide?

The risk ratio reported by any study can be generalised to other populations only if all the relevant factors have been controlled for (Bottai, 2014; Santacatterina and Bottai, 2015). In this context, relevant factors are variables that are stochastically dependent with the health outcome of interest. For example, cardiovascular diseases are more prevalent among older subjects than among younger individuals. Age is therefore a relevant factor for cardiovascular diseases. The evidence provided by the results shown in Figure 1 are potentially valid only if this step was taken in each of the studies considered. If that was the case for the studies, then, there is evidence that exposure to pesticide A doubles the risk in the specific group of individuals considered by each of the two studies. If the risk ratios are summary measures over the respective study populations, then none of the findings should be generalised. However, if the risk ratios for pesticide A were not adjusted for any factor, and the underlying populations were very different

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## は、一般的に大きな理解につながる。

図1は、3つの農薬(A、B、C)のそれぞれが2つの研究のメタアナリシスで評価されている仮想例の3つのフォレストプロットを示している。各農薬の両方の研究が、最高の品質と科学的な厳密さを持っていると仮定している。バイアスが疑われない。

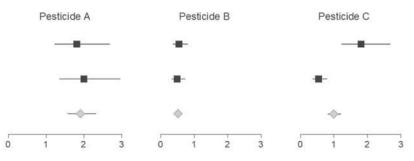


図 1:3 つの農薬(A, B, C)のそれぞれが 2 つの研究のメタアナリシスで評価されている仮想例のフォレストプロット。 各プロットの x 軸は、ばく露した個体とばく露していない個体を比較した、対象疾患の推定リスク比を表している。 四角は各研究における推定リスク比を示し、灰色のダイヤモンドは要約されたリスク比を示している。横線は 95%信頼区間を示す。

以下の文章には、図1の結果の解釈について、農薬を1つずつ、短くコメントしている。

- ・農薬Aへのばく露は、病気のリスクを2倍にするようである。結果は2つの研究の間で一致しており、信頼区間には帰無値である1が含まれていない。しかし、これらの結果は、(a)同じばく露と疾患について実施された他の研究では、リスク比が約2であること、または(b)個人のどのグループ(例えば、男性か女性か、若年者か高齢者)でもリスク比が2であることを暗示するものではない。
- ・農薬 B へのばく露は、病気のリスクを半減させるようである。結果は2つの研究の間で一致しており、信頼区間には帰無値である1は含まれていない。しかし、これらの結果は、(a)同じばく露と疾患について実施された他の研究では、(a)リスク比が約半分になること、または(b)個人のどのグループ(例えば、男性か女性か、若年者か高齢者か)でもリスク比が約半分になることを暗示するものではない。
- ・農薬 C へのばく露は、一方の研究では病気のリスクが2倍になり、他方の研究ではリスクが2分の1になるようである。結果は2つの研究の間で矛盾しており、また、信頼区間には帰無値である1が含まれていない。しかし、これらの結果は、(a)同じばく露と疾患について実施された他の研究では、リスク比が約1であること、または(b)個人のどのグループ(例えば、男性か女性か、若年者か高齢者か)でもリスク比が約1であることを暗示するものではない。

図1に示された結果は、どのようなエビデンスを提供できるか?

どのような研究で報告されたリスク比も、すべての関連因子がコントロールされている場合にのみ、他の集団に一般 化することができる(Bottai, 2014 年; Santacatterina 及び Bottai, 2015 年)。この背景では、関連因子とは、対象と なる健康影響に確率的に依存する変数のことである。例えば、心血管疾患は、若年者よりも高齢者の方が多い。したが って、年齢は心血管疾患の関連因子である。図1に示された結果から得られるエビデンスは、検討した各研究でこのス テップを踏んだ場合にのみ有効となる可能性がある。もしそうであれば、2 つの研究のそれぞれで考慮された個人の特 定のグループでは、農薬A へのばく露がリスクを2倍にするというエビデンスがある。リスク比がそれぞれの研究集団の 要約測定値であるならば、どの結論も一般化されるべきではない。しかし、農薬Aのリスク比がいかなる因子でも調整さ れておらず、基礎となる母集団が2 つの研究で大きく異なっていた場合、関連因子が存在せず、農薬A はどのサブグ across the two studies, then there would still be evidence that there may be no relevant factors and pesticide A doubles the risk in any subgroup of individuals. Pesticide B appears to halve the risk, and the estimated confidence intervals are narrower for pesticide B than for pesticide A. Generalisability of the findings, however, holds for pesticide B under the conditions stated above for pesticide A. As for pesticide C, the forest plot provides evidence that exposure to this pesticide raises the risk of the disease in the group of individuals in one of the studies and decreases it in the group considered in the other study. Again, if the risk ratios are summary measures over the respective study populations, then none of the findings should be generalised. Investigating the reasons behind the inconsistency between the two studies on pesticide C can provide as much scientific insight as investigating the reasons behind the similarity between the studies on pesticide A or pesticide B.

In general, the overall summary measures provided by forest plots, such as the silver diamonds in each of the three panels of Figure 1, are of little scientific interest. When evaluating the findings of different studies, many aspects should be carefully evaluated. An important aspect that is often overlooked is heterogeneity of the strength of associations across subgroups of individuals. When information about subgroup analysis is provided in the publications that describe a study, this should be carefully evaluated. Sensitivity analyses should complement the results provided by different studies. These should aim to evaluate heterogeneity and the possible impact of uncontrolled for relevant factors along with information and sampling error. A synoptic diagram is displayed in Figure 2.

#### Bias

• Information error, such as measurement error, effect size magnification

#### **Relevant Factors**

- Which were considered and which were not considered
- How were they distributed in each study
- What population is the resulting inference on

## Sampling Error

• Standard errors, not p-values, of the estimates of the parameters of interest

#### Sensitivity Analyses

• Range of the parameters of interest that are consistent with observed data

#### Figure 2: Items to consider when evaluating and comparing multiple studies

### 6.3.3. Usefulness of meta-analysis for hazard identification

Human data can be used for many stages of risk assessment. Single epidemiological studies, if further studies on the same pesticide are not available, should not be used as a sole source for hazard identification, unless they are high quality studies (according to criteria shown in Table 2). Evidence synthesis techniques which bring together many studies, such as systematic reviews and meta-analysis (where appropriate) should be utilised instead. Although many meta-analyses have been carried out for the quantitative synthesis of data related to chronic diseases, their application for risk assessment modelling is still limited.

Importantly, evidence synthesis will provide a methodological assessment and a risk of bias assessment of the current evidence highlighting areas of uncertainties and identifying associations with robust and credible evidence.

Figure 3 shows a simple methodology proposed for the application of epidemiological studies into risk assessment. The first consideration is the need of combining different epidemiological studies

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ループの個人においてもリスクを2倍にするというエビデンスが残っている。農薬Bはリスクを半減させるようであり、推定された信頼区間は農薬Aよりも農薬Bの方が狭い。しかしながら、農薬Aについての上記の条件のもとでは結果を一般化できる可能性は農薬Bについても維持されている。農薬Cに関しては、フォレストプロットから、この農薬へのばく露が、一方の研究では個人のグループで病気のリスクを上げ、他方の研究ではリスクを下げるというエビデンスが得られた。繰り返しになるが、リスク比がそれぞれの研究集団の要約測定値であるならば、どの結果も一般化されるべきではない。農薬Cに関する2つの研究間の矛盾の背後にある理由を調査することは、農薬Aまたは農薬Bに関する研究間の類似性の背後にある理由を調査するのと同じくらい多くの科学的予測を提供することができる。

ー般的に、図1の3つのパネルのそれぞれにある銀色のダイヤモンドのようなフォレストプロットによって提供される 全体的な要約評価尺度は、ほとんど科学的な関係を持たない。異なる研究の結果を評価する際には、多くの側面を慎 重に評価しなければならない。見落とされがちな重要な側面は、サブグループを超えた個人間の関連の強さの不均一 性である。研究を記述した出版物でサブグループ解析に関する情報が提供されている場合、これは慎重に評価される べきである。感度分析は、異なる研究で得られた結果を補完すべきである。これらの解析は、異質性と、情報とサンプリ ング誤差とともに、関連する因子を制御していない場合の影響を評価することを目的とすべきである。図2に総観図を 示す。

バイアス	
測定誤差などの情報誤差、効果の大きさの倍率	
関連する要因	
どれが検討され、どれが検討されなかったか 各研究ではどのように分布していたか についての推論の結果がどの母集団であるか	
サンプリング誤差	
関心のあるパラメータの推定値のp値ではなく標準誤差	
感度分析	
観測されたデータと一致する関心のあるパラメータの範囲	

#### 図 2:複数の研究を評価・比較する際に考慮すべき項目

# 6.3.3. ハザード同定のためのメタアナリシスの有用性

ヒトのデータはリスク評価の多くの段階で利用できる。単一の疫学研究(同じ農薬に関する追加研究が入手できない 場合)は、質の高い研究(表 2 に示す基準による)でない限り、単独のハザード同定のための情報源として使用すべき ではない。代わりに、システマティックレビューやメタアナリシス(必要に応じて)など、多くの研究をまとめたエビデンス統 合技術を利用すべきである。慢性疾患に関連するデータの定量的な統合のために多くのメタアナリシスが実施されて いるが、リスク評価モデリングへの応用はまだ限られている。

重要なことは、エビデンス統合は、現在のエビデンスの方法論的評価とバイアスのリスク評価を提供し、不確実性の 領域を強調し、強固で信頼性の高いエビデンスとの関連を特定することである。

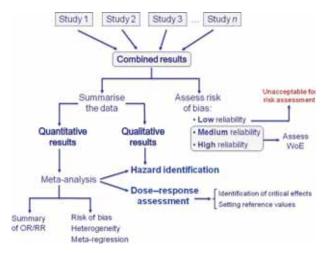
図 3 は、疫学研究をリスク評価に適用するために提案された簡単な方法論を示している。最初の考慮事項は、同じ 健康影響を扱う異なる疫学研究を組み合わせる必要性である。これは、EFSA のシステマティックレビューのためのガ イダンス(EFSA、2010 年 a)で提案されている基準に従って行うことができる。次に、研究デザインと実施、母集団、ば く露評価、健康影響評価、交絡因子の管理、統計解析、結果の報告など、WOE 評価のための 6.2 節に記載されてい

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addressing the same outcome. This can be made following criteria proposed by EFSA guidance for systematic reviews (EFSA, 2010a). Then, the risk of bias is assessed based on the factors described in Section 6.2 for a WoE assessment, namely: study design and conduct, population, exposure assessment, outcome assessment, confounder control, statistical analysis and reporting of results. Those studies categorised as of low reliability will be considered unacceptable for risk assessment. The remaining studies will be weighted and used for hazard identification.

If quantitative data are available, a meta-analysis can be conducted to create summary data and to improve the statistical power and precision of risk estimates (OR, RR) by combining the results of all individual studies available or meeting the selection criteria. As meta-analyses determine the size of association averaged over the considered studies, they provide a stronger basis for hazard identification. Moreover, under certain circumstances, there is the possibility to move towards risk characterisation metrics because these measured differences in health outcomes (OR, RR) can be converted to dose-response relationships (Nachman et al., 2011). Although quite unusual in practice, this would allow for the identification of critical effects in humans and/or setting reference values without the need of using animal extrapolation.

Since heterogeneity is common in meta-analyses, there is a need to assess which studies could be combined quantitatively. Heterogeneity can be genuine, representing diverse effects in different subgroups, or might represent the presence of bias. If heterogeneity is high (I<sup>2</sup> greater than 50%), individual studies should not be combined to obtain a summary measure because of the high risk of aggregating bias from different sources. Sources of heterogeneity should be explored through sensitivity analysis and/or meta-regression. Furthermore, the presence of diverse biases in the meta-analysis should be examined, such as small study effects, publication bias and excess significance bias. It is important to find models that adequately describe the effect size distribution of the underlying studied populations.



#### Figure 3: Methodology for utilisation of epidemiological studies for risk assessment

6.3.4. Pooling data from similar epidemiological studies for potential dose-response modelling

As in other fields of research, findings from a single epidemiological study merit verification through replication. When the number of replications is abundant, it may be worthwhile to assess the entire set of replicate epidemiological studies through a meta-analysis and ascertain whether, for key outcomes, findings are consistent across studies. Such an approach will provide more robust conclusions about the existence of cause-effect relationships.

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る要素に基づいてバイアスのリスクを評価する。信頼性が低いと分類された研究は、リスク評価のために受け入れられ ないと考えられる。残りの研究は重み付けを行い、ハザードの特定に使用する。

定量的データが利用可能な場合、メタアナリシスを実施して要約データを作成し、利用可能な、あるいは選択基準を 満たすすべての個々の研究の結果を組み合わせることで、統計的な検出力とリスク推定値(OR, RR)の精度を向上さ せることができる。メタアナリシスは、関連の大きさが検討した研究の平均値に決定するので、ハザード同定のためのよ り強力な基盤を提供する。さらに、特定の状況下では、健康影響におけるこれらの測定された差(OR, RR)を用量反応 関係に変換できるため、リスク特性の測定基準に移行する可能性がある(Nachman 6、2011 年)。実際には非常に珍 しいことではあるが、これにより、動物からの外挿法を使用することなく、ヒトにおける重大影響の同定や基準値の設定 が可能になる。

メタアナリシスでは不均一性が一般的であるため、どの研究を定量的に組み合わせることができるかを評価する必要 がある。異質性は、異なるサブグループにおける多様な影響を表す真正なものである場合もあれば、バイアスの存在を 表す場合もある。異質性が高い場合(I<sup>2</sup>が 50%を超える場合)は、異なる情報源からのバイアスを集約するリスクが高い ため、要約尺度を得るために個々の研究を組み合わせるべきではない。感度分析及び/またはメタ回帰によって異質 性の原因を探るべきである。さらに、メタアナリシスにおける多様なバイアスの存在、例えば、小規模な研究効果、出版 バイアス、過剰な有意性バイアスなどを調べるべきである。基礎となる研究集団の影響量分布を適切に記述するモデ ルをみつけることが重要である。

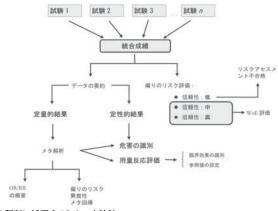


図3:疫学研究をリスク評価に活用するための方法論

# 6.3.4. 潜在的な用量反応モデル化のための類似の疫学研究からのデータの蓄積

他の研究と同様に、単一の疫学研究から得られた結果は、複製によって検証する価値がある。複製の数が豊富な場 合には、メタアナリシスによって複製された疫学研究の全セットを評価し、主要な健康影響事象について、研究間で結 果が一貫しているかどうかを確認することについては価値があるかもしれない。このようなアプローチにより、因果関係 の存在についてより強固な結論が得られるであろう。

ハザードが同定されると、リスク評価の次のステップは、異なるばく露レベルでの有害な影響のリスクを推定するための用量反応評価を実施することであり、特定の集団に対して健康への有害な影響が認められない濃度以下のばく露レベルを推定する。しかし、このステップでは、個人レベルでの完全に定量的な(または少なくとも半定量的な)ばく露データが必要である。定量的統合から得られた要約推定値は、研究間の相対比較が可能となるようなばく露の連続変数の変化(またはばく露のある一定割合の変化)に対する OR を示すものであれば、リスク評価にとってより有益であり、研www.efsa.europa.eu/efsajourna

Once a hazard has been identified, the next step in risk assessment is to conduct a dose-response assessment to estimate the risk of the adverse effect at different levels of exposure and/or the concentration level below which no appreciable adverse health effect can be assumed for a given population. However, this step requires fully quantitative (or at least semi-quantitative) exposure data at an individual level. Summary estimates resulting from quantitative synthesis would be more informative for risk assessment if they present an OR for a given change in the continuous variable of exposure (or per a given percentile change in exposure) as this allows for relative comparisons across studies and could be of help to derive health-based reference values. Only within such a framework can data from human studies with similar designs be merged to gain enough power to model proper dose-response curves (Greenland and Longnecker, 1992; Orsini et al., 2012).

Conversely, meta-analytical approaches may be of limited value if a combined OR is calculated based on meta-analyses interpreting exposure as a 'yes' or a 'no' (ever vs never) because exposures are not necessarily to active ingredients in the same proportion in all studies included. Even though in these cases, meta-analyses may consistently find an increased risk associated with pesticide exposure, for risk assessment the exposure needs to characterise the effect of specific pesticide classes or even better individual pesticides as their potency may differ within the same class (Hernández et al., 2016).

This approach would allow points of departure to be identified (e.g. benchmark doses (BMD)) and would be relevant for the integration of epidemiological studies into quantitative risk assessment. Although BMD modelling is currently used for analysing dose–response data from experimental studies, it is possible to apply the same approach to data from observational epidemiological studies (Budtz-Jørgenson et al., 2004). The EFSA Scientific Committee confirmed that the BMD approach is a scientifically more advanced method compared to the no observed-adverse-effect level (NOAEL) approach for deriving a Reference Point, since it makes extended use of the dose–response data from experimental and epidemiological studies to better characterise and quantify potential risks. This approach, in principle, can be applicable to human data (EFSA Scientific Committee, 2017b), although the corresponding guidelines are yet to be developed.

Dose-response data from observational epidemiological studies may differ from typical animal toxicity data in several respects and these differences are relevant to BMD calculations. Exposure data often do not fall into a small number of well-defined dosage groups. Unlike most experimental studies, observational studies may not include a fully unexposed control group, because all individuals may be exposed to some extent to a chemical contaminant. In this case, the BMD approach still applies since fitting a dose-response curve does not necessarily require observations at zero exposure. However, the response at zero exposure would then need to be estimated by low-dose extrapolation. Hence, the BMD derived from epidemiological data can be strongly model-dependent (Budtz-Jørgensen et al., 2001).

Epidemiology data need to be of sufficient quality to allow the application of the BMD approach, especially in terms of assigning an effect to a specific pesticide and its exposure. Clear rules and guidance, and definition of model parameters need to be considered for such a BMD approach, which might differ from BMD approaches from controlled experimental environments. Although the BMD modelling approach has been applied to epidemiological data on heavy metals and alcohol (Lachenmeier et al., 2011), currently, few individual studies on pesticides are suitable for use in dose–response modelling, much less in combination with other studies. However, future studies should be conducted and similarly reported so that they could be pooled together for a more robust assessment.

#### 7. Integrating the diverse streams of evidence: human (epidemiology and vigilance data) and experimental information

This section first considers in Section 7.1 the different nature of the main streams of evidence, i.e. originating either from experimental studies or from epidemiological studies. The approach used is that recommended by the EFSA Scientific Committee Guidance on WoE (EFSA Scientific Committee, 2017b), which distinguishes three successive phases to assess and integrate these different streams of information: reliability, relevance and consistency. The first step, consists in the assessment of the reliability of individual studies be they epidemiological (addressed in Section 6) or experimental (beyond the scope of this Scientific Opinion). Then, the relevance (strength of evidence) of one or more studies found to be reliable is assessed using principles of epidemiology (addressed in Section 6) and toxicology. Next, Section 7.2 considers how to bring together different streams of relevant information from epidemiological and experimental studies, which is considered in a WoE approach, to assess consistency and biological plausibility for humans.

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究間の相対比較が可能となり、健康に基づく基準値を導き出すのに役立つ。このような枠組みの中でのみ、類似した 計画のヒト研究からのデータを統合して、適切な用量反応曲線をモデル化するのに十分な力を得ることができる (Greenland 及び Longnecker、1992年; Orsini ら、2012年)。

逆に、メタアナリシス的アプローチは、すべての研究で同じ割合で含まれる有効成分へのばく露が必ずしも必要では ないため、ばく露を「はい」または「いいえ」(これまでにあり、または決してない)と解釈するメタアナリシスに基づいて複 合 OR が計算された場合には、限られた価値しかないかもしれない。これらのケースでは、メタアナリシスは一貫して農 薬ばく露に関連したリスクの増加を示しているが、リスク評価のためには、ばく露は特定の農薬クラスの影響を特性評価 する必要があり、同じクラス内でも効力が異なる可能性があるため、個々の農薬の影響を特性評価する必要がある (Hernándezら、2016 年)。

このアプローチは、Point of Departure を特定することを可能にし(例えば、ベンチマーク用量(BMD))、疫学研究 を定量的リスク評価に統合することに関連するであろう。現在、BMD モデリングは実験研究からの用量反応データの 解析に使用されているが、観察による疫学研究からのデータにも同様のアプローチを適用することが可能である (Budtz-Jørgenson 5、2004 年)。EFSA 科学委員会は、BMD アプローチは、実験研究や疫学研究からの用量反応 データを利用して潜在的なリスクをよりよく特徴付け、定量化するために、Reference Pointを得るための NOAEL(no observed-adverse-effect level)アプローチと比較して、より科学的に進んだ方法であると結論づけている。このアプロ ーチは、原則としてヒトのデータにも適用可能である(EFSA Scientific Committee、2017 年 b)が、対応するガイドラ インはまだ作成されていない。

観察による疫学研究からの用量反応データは、いくつかの点で典型的な動物試験の毒性データとは異なる可能性 があり、これらの違いは BMD の計算に関連する。ばく露データは、多くの場合、少数の十分に定義された用量群に当 てはまらないことが多い。ほとんどの実験研究とは異なり、観察による研究には完全に未ばく露の対照群が含まれてい ない場合がある。この場合、用量反応曲線を作成することは必ずしもばく露量ゼロでの観察を必要としないため、BMD アプローチが適用される。しかし、ばく露量ゼロでの反応は低用量外挿法で推定する必要がある。したがって、疫学デ ータから得られる BMD はモデル依存性が強い(Budtz-Jørgensen 6、2001年)。

疫学データは、BMD アプローチを適用するためには、特に特定の農薬とそのばく露に影響を与えるという点で、十 分な品質のものでなければならない。このような BMD アプローチについては、明確なルールとガイダンス、モデルパラ メータの定義を考慮する必要があり、制御された実験環境からの BMD アプローチとは異なる可能性がある。BMD モ デリングアプローチは重金属やアルコールに関する疫学データに適用されているが(Lachenmeier ら、2011 年)、現 在のところ、農薬に関する個別の研究は、用量反応モデリングに使用するのに適しているものはほとんどなく、他の研 究と組み合わせて使用することはあまりない。しかし、今後も研究は実施され、同様の報告がなされ、それらの研究を蓄 積して、より強固な評価を行うことができるようにすべきである。

# 7. 多様なエビデンスの統合:ヒト(疫学データと警戒データ)と実験の情報

本節では、まず第 7.1 節で、実験研究や疫学研究に由来する主なエビデンスの特性の違いについて考察する。使用したアプローチは、EFSA Scientific Committee Guidance on WOE(EFSA Scientific Committee、2017 年 b) で推奨されているもので、これらの異なる情報を評価し統合するために、信頼性、関連性、一貫性の 3 つの段階を区別している。最初の段階では、疫学的研究(セクション 6 で述べている)や実験的研究(この意見書の範囲を超えている) である個々の研究の信頼性の評価で構成されている。次に、信頼性があると判断された 1 件以上の研究の関連性(エビデンスの確実性)を疫学(第 6 節に記載)と毒物学の原則を用いて評価する。次に、第 7.2 節では、WOE アプローチで検討された疫学的及び実験的研究からの様々な関連情報を、ヒトに対する一貫性と生物学的妥当性を評価する ために、どのようにしてまとめるかを検討する。

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# 7.1. Sources and nature of the different streams of evidence Comparison of experimental and epidemiological approaches

In the regulatory risk assessment of pesticides, the information on the toxic effects is based on the results of a full set of experiments as required by Regulation (EC) 283/2013 and 284/2013, and conducted according to OECD guidelines. They are carried out in vivo or in vitro, so there will always be some high-quality experimental data available for pesticides as required to be provided by applicants under Regulation (EC) 1107/2009. A number of categories are established for rating the reliability of each stream of evidence according to the EFSA peer review of active substances: acceptable, supplementary and unacceptable. The data quality and reliability of in vivo or in vitro toxicity studies should be assessed using evaluation methods that better provide more structured support for determining a study's adequacy for hazard and risk assessments. Criteria have been proposed for conducting and reporting experimental studies to enable their use in health risk assessment for pesticides (Kaltenhäuser et al., 2017).

Animal (in vivo) studies on pesticide active substances conducted according to standardised test guidelines and good laboratory practices (GLP, e.g. OECD test guidelines) are usually attributed higher reliability than other research studies. Notwithstanding, since there is no evidence that studies conducted under such framework have a lower risk of bias (Vandenberg et al., 2016), evidence from all relevant studies, both GLP and non-GLP, should also be considered and weighted. Thus, data from peer-reviewed scientific literature should be taken into account for regulatory risk assessment of pesticide active substances, provide they are of sufficient quality after being assessed for methodological reliability. Their contribution to the overall WoE is influenced by factors including test organism, study design and statistical methods, as well as test item identification, documentation and reporting of results (Kaltenhäuser et al., 2017).

The internal validity of in vitro toxicity studies should be evaluated as well to provide a better support for determining a study's adequacy for hazard and risk assessments. In silico modelling can be used to derive structure-activity relationships (SAR) and to complement current toxicity tests for the identification and characterisation of the mode or mechanisms of action of the active substance in humans. These alternative toxicity testing (and non-testing) approaches could be helpful in the absence of animal data, e.g. to screen for potential neurodevelopmental or endocrine disruption effects of pesticides, and to increase confidence in animal testing. Considering the demand for minimising the number of animal studies for regulatory purposes, non-animal testing information can provide relevant stand-alone evidence that can be used in the WoE assessment.

A number of toxicological issues are amenable for systematic review, from the impact of chemicals on human health to risks associated with a specific exposure, the toxicity of chemical mixtures, the relevance of biomarkers of toxic response or the assessment of new toxicological test methods (Hoffmann et al., 2017). For instance, in a previous Scientific Opinion EFSA used a systematic review for the determination of toxicological mechanisms in the frame of AOP approach (Choi et al., 2016; EFSA Scientific Committee, 2017c).

Besides toxicity data on the active substance, such data may also be required on metabolites or residues if human exposure occur through the diet or drinking water. Results from these studies are then considered in relation to expected human exposures estimated through food consumption and other sources of exposure. The strength of this approach is that in vivo studies account for potential toxic metabolites, though not always animal metabolic pathways parallels the ones of humans.

Experimental studies in laboratory animals are controlled studies where confounding is eliminated by design, which is not always the case with epidemiological studies. Animals used in regulatory studies are, however, typically inbred, genetically homogeneous and due to the controlled environment they lack the full range of quantitative and qualitative chemical susceptibility profiles. Nevertheless, animal surrogates of human diseases are being challenged by their scientific validity and translatability to humans, and the lack of correlation often found between animal data and human outcomes can be attributed to the substantial interspecies differences in disease pathways and disease-induced changes in gene expression profiles (Esch et al., 2015). Thereby, many experimental models do not capture complex multifactorial diseases making animal-to-human extrapolation subject to considerable uncertainty. Current risk assessment is therefore by its nature predictive and may be insufficient because it is chemical-specific and humans are exposed to a large number of chemicals from environmental, dietary and occupational sources or because of different toxicokinetic differences. In recognition of the uncertain nature of animal-to-human extrapolation, the regulatory risk assessment advice does not just consider the relevant point(s) of departure (NOAEL, LOAEL or BMDL) that have

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Epidemiological studies and pesticides

# 7.1. 異なるエビデンスの起源と特性 実験的アプローチと疫学的アプローチの比較

農薬の規制リスク評価では、毒性影響に関する情報は、規則(EC)283/2013 及び 284/2013 で要求され、OECD ガイドラインに従って実施された一連の実験結果に基づいている。これらの実験は in vivo または in vitro で実施され ているため、規則(EC)1107/2009 に基づき申請者が提供することが要求されているように、農薬については常に質の 高い実験データが存在している。EFSA の有効成分のピアレビューによると、各エビデンスの信頼性を評価するために、 許容範囲、補足範囲、不許容範囲といういくつかのカテゴリーが設定されている。in vivo または in vitro 毒性試験の データの質及び信頼性は、ハザード及びリスク評価のための試験の妥当性を判断するためのより構造的な裏付けをよ り良く提供する評価方法を用いて評価されるべきである。農薬の健康リスク評価に使用できるように、実験的研究の実 施と報告のための基準が提案されている(Kaltenhäuserら、2017年)。

標準化された試験ガイドラインや優良試験所規範(GLP、例えば OECD 試験ガイドライン)に従って実施された農薬 有効成分の動物(in vivo)試験は、通常、他の研究よりも信頼性が高いとされている。しかし、このような枠組みの下で 実施された研究の方がバイアスのリスクが低いというエビデンスはないため(Vandenbergら、2016年)、GLPと非GLP の両方を問わず、関連するすべての研究から得られたエビデンスも考慮し、重み付けを行うべきである。このように、査 読された科学文献からのデータは、方法論的信頼性を評価した後に十分な品質のものであれば、農薬有効成分の規 制リスク評価のために考慮されるべきである。WOE 全体への貢献は、試験系、試験計画、統計的方法、試験項目の特 定、結果の文書化、報告などの要因によって左右される(Kaltenhäuserら、2017年)。

ハザード及びリスク評価のための試験の妥当性を判断するためのより良い補助とするために、in vitro 毒性試験の 内部妥当性も評価されるべきである。in silico モデルは、構造活性相関(SAR)を導出し、ヒトにおける有効成分の作 用様式や作用機序の同定や特性評価のための現行の毒性試験を補完するために使用することができる。これらの代 替毒性試験(及び非試験)アプローチは、動物データがない場合、例えば農薬の潜在的な神経発達や内分泌かく乱 作用をスクリーニングし、動物試験の信頼性を高めるのに役立つ可能性がある。規制目的のために動物試験の数を最 小限にすることが求められていることを考慮すると、動物試験以外の情報はWOE評価に使用できる適切な独立したエ ビデンスを提供することができる。

化学物質のヒト健康への影響から特定の化学物質へのばく露に伴うリスク、化学物質の混合物の毒性、毒性反応の バイオマーカーの関連性、あるいは新しい毒性試験法の評価まで、多くの毒性学的問題がシステマティックレビューの 対象となっている(Hoffmann ら、2017年)。例えば、以前の Scientific Opinion では、EFSA は AOP アプローチの 枠内で毒性学的メカニズムの決定にシステマティックレビューを使用した(Choi ら、2016 年: EFSA Scientific Committee、2017年c)。

有効成分の毒性データの他に、食事や飲料水を通じてヒトにばく露された場合には、代謝物や残留物についてもデ ータが必要となる場合がある。これらの研究から得られた結果は、食品消費やその他のばく露源から推定される予想さ れるとトばく露量との関連で検討される。このアプローチの長所は、動物の代謝経路が必ずしもとトの代謝経路と類似し ているとは限らないにもかかわらず、in vivo 試験では潜在的な毒性代謝物を考慮していることである。

実験動物を用いた実験研究は、交絡因子が排除されるように計画された研究であるが、疫学研究では必ずしもそうと は限らない。しかし、規制研究に使用される動物は、一般的には近親交配されて遺伝的に同一であり、制御された環 境のために、定量的及び定性的な化学物質感受性のすべての特性を欠いている。それにもかかわらず、ヒト疾患の動 物代替は、その科学的妥当性ととトへの外挿性が問われており、動物データととトの結果との間にしばしば見られる相 関性の欠如は、疾患経路や遺伝子発現プロフィルにおける疾患誘発性の変化における実質的な種間差に起因してい ると考えられている(Esch ら、2015年)。そのため、多くの実験モデルは複雑な多因子疾患を捉えていないため、動物 からヒトへの外挿はかなりの不確実性を伴うものとなっている。したがって、現在のリスク評価はその特性上、予測的なも のであり、化学物質に特化したものであり、ヒトは環境、食事、職業上の源からの多数の化学物質にばく露されているた め、あるいは異なる毒物動態の違いのために、十分ではないかもしれない。動物からとトへの外挿の不確実性を考慮し て、規制当局のリスク評価のアドバイスは、単に安全性が確認されている関連の Point of Departure (NOAEL、 EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

been identified as safe but lowers these values using uncertainty factors (UFs) to propose safe reference dose values, either for acute or chronic toxicity.

Given the limitations of studies in laboratory animals, epidemiological studies in the 'real world' are needed, even if they have limitations of their own. Epidemiological studies incorporate the true (or estimated) range of population exposures, which usually are intermittent and at inconsistent doses instead of occurring at a consistent rate and dose magnitude (Nachman et al., 2011). Since epidemiological studies are based on real-world exposures, they provide insight into actual human exposures that can then be linked to diseases, avoiding the uncertainty associated with extrapolation across species. Hence, it can be said that they address the requirements of Regulation 1107/2009 Article 4, which stipulates that the risk assessment should be based on good plant protection practice and realistic use conditions. Thus, epidemiological studies assist problem formulation and hazard/risk characterisation whilst avoiding the need for high dose extrapolation (US-EPA, 2010).

Epidemiological studies therefore provide the opportunity to (a) identify links with specific human health outcomes that are difficult to detect in animal models; (b) affirmation of the human relevance of effects identified in animal models; (c) ability to evaluate health effects for which animal models are unavailable or limited (Raffaele et al., 2011). Epidemiological evidence will be considered over experimental animal evidence only when sufficiently robust pesticide epidemiological studies are available. However, in epidemiological studies, there are always a variety of factors that may affect the health outcome and confound the results. For example, when epidemiological data suggest that exposures to pesticide formulations are harmful they usually cannot identify what component may be responsible due to the complexity of accurately assessing human exposures to pesticides. While some co-formulants are not intrinsically toxic, they can be toxicologically relevant if they change the toxicokinetics of the active substance. In addition, confounding by unmeasured factor(s) associated with the exposure can never be fully excluded; however, a hypothetical confounder (yet unrecognised) may not be an actual confounder and has to be strongly associated with disease and exposure in order to have a meaningful effect on the risk (or effect size) estimate, which is not always the case.

Many diseases are known to be associated with multiple risk factors; however, a hazard-by-hazard approach is usually considered for evaluating the consequences of individual pesticide hazards on vulnerable systems (Figure 4A). Specifically, single-risk analysis allows a determination of the individual risk arising from one particular hazard and process occurring under specific conditions, while it does not provide an integrated assessment of multiple risks triggered by different environmental stressors (either natural or anthropogenic) (Figure 4B). Risk assessment would benefit by developing procedures for evaluating evidence for co-occurrence of multiple adverse outcomes (Nachman et al., 2011), which is more in line with what happens in human setting. For these reasons, if appropriately conducted, epidemiological studies can be highly relevant for the risk assessment process.

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LOAEL、または BMDL)を考慮するのではなく、不確実性因子(UF)を用いてこれらの値を下げ、急性毒性または慢性毒性の安全な参照用量値を提案するものである。

実験動物を用いた研究の限界を考えると、たとえそれ自体に限界があるとしても、「実社会」での疫学研究が必要で ある。疫学的研究では、集団のばく露の真の範囲(または推定された範囲)を組み入れているが、これは通常、一貫し た速度と用量の大きさで発生するのではなく、断続的で一貫性のない用量で発生する(Nachman 6、2011 年)。疫学 的研究は実社会のばく露に基づいているため、実際のといのばく露についての予測を提供し、それを疾病に結びつけ ることができ、種を超えた外挿に関連する不確実性を回避することができる。したがって、リスク評価は、優れた植物保 護対策(Good Plant Protection Practice)と現実的な使用条件に基づいて行われるべきであると規定した規制 1107/2009 の第4条の要件に対応していると言える。このように、疫学的研究は、高用量外挿の必要性を回避しつつ、 問題の定式化とハザード/リスクの特性評価を支援する(US-EPA、2010 年)。

したがって、疫学的研究は、(a)動物モデルでは検出が困難な特定のといの健康影響との関連性を特定する。(b)動 物モデルで特定された影響のとトへの関連性を明らかにする(c)動物モデルが利用できない、または限定された、健康 影響を評価する能力を提供する(Raffaele ら、2011 年)。疫学的証拠は、十分に強固な農薬の疫学的研究が利用可 能な場合にのみ、動物実験によるエビデンスよりも考慮される。しかし、疫学研究では、健康影響に影響を与え、結果 を混乱させる様々な要因が常に存在する。例えば、疫学的データが農薬製剤へのばく露が有害であることを示唆して いても、通常、農薬へのとトばく露を正確に評価することの複雑さから、どの成分が原因であるかを特定することはでき ない。一部の製剤補助剤は本質的には毒性がないが、有効成分の毒物動態を変化させる場合には、毒性学的に関連 性がある可能性がある。さらに、ばく露に関連した測定されていない因子による交絡を完全に排除することはできない が、仮説的な交絡因子(まだ認識されていない)は実際の交絡因子ではない可能性があり、リスク(または効果量)の推 定値に意味のある影響を与えるためには、疾患やばく露と強く関連していなければならないが、必ずしもそうとは限らな い。

多くの病気は複数のリスク因子と関連していることが知られているが、脆弱なシステムに対する個々の農薬のハザードの影響を評価するためには、通常、ハザードごとのアプローチが考慮される(図 4A)。特に、単一リスク解析では、特定の条件下で発生する特定のハザードやプロセスに起因する個々のリスクを決定することができるが、異なる環境ストレス要因(自然現象または人為的要因のいずれか)によって引き起こされる複数のリスクを統合的に評価することはできない(図 4B)。リスク評価は、複数の有害な健康影響の同時発生のエビデンスを評価するための手順を開発することが有用である(Nachman 6、2011 年)が、これはよりとトの環境で起こることと一致している。これらの理由から、適切に実施されれば、疫学研究はリスク評価プロセスとの関連性が高い。

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Classical single hazard approach: Α driven by regulatory frameworks

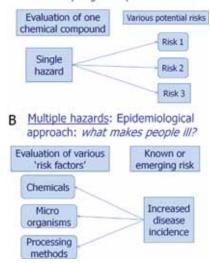


Figure 4: Role of epidemiological studies when compared to classical toxicological studies

In parallel with epidemiological data, vigilance data can provide an additional stream of evidence, especially for acute toxicity. Cases are usually well-documented and information can be used at different steps of the risk assessment; these include: level and duration of exposure, clinical course and assessment of the causal relationship. In severe cases, the toxin and/or the metabolites are usually measured in blood or urine which allows for comparison with animal data and in some cases for setting toxicological values.

In summary, experimental studies or epidemiological studies and vigilance data represent two different approaches to collect and assess evidence i.e. one emanating from controlled exposures (usually to a single substance) using experimental study design and a relatively homogeneous surrogate population, the other reflecting the changes observed in a heterogeneous target population from mixed (and varying) exposure conditions using non-experimental study design (ECETOC, 2009). Epidemiology and toxicology each bring important and different contributions to the identification of human hazards. This makes both streams of evidence complementary, and their combination represents a powerful approach. Animal studies should always inform the interpretation of epidemiological studies and vice versa; hence, they should not be studied and interpreted independently.

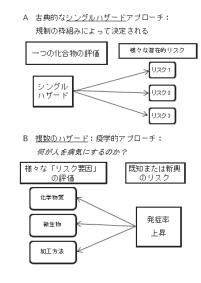
# 7.2. Principles for weighting of human observational and laboratory animal experimental data

Following the identification of reliable human (epidemiological or vigilance) studies and the assessment of the relevance of the pooled human studies, the separate lines of evidence that were found to be relevant need to be integrated with other lines of evidence that were equally found to be relevant.

The first consideration is thus how well the health outcome under consideration is covered by toxicological and epidemiological studies. When both animal and human studies are considered to be available for a given outcome/endpoint, this means that individual studies will first have been assessed for reliability and strength of evidence (Sections 6.2 and 6.3, respectively, for epidemiological studies)

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#### 図 4:古典的な毒性学的研究と比較した場合の疫学的研究の役割

疫学的データと並行して、特に急性毒性については、警戒データが追加のエビデンスの流れを提供することができ る。通常、症例は十分に文書化されており、リスク評価のさまざまな段階で情報を利用することができる。これらの情報 には、ばく露のレベルと期間、臨床経過、因果関係の評価などが含まれる。重度のケースでは、毒素及び/または代 謝物が通常、血液または尿中で測定され、動物データとの比較が可能であり、場合によっては毒性値を設定することが できる。

要約すると、実験的研究、疫学的研究、及び警戒データは、エビデンスを収集し評価するための 2 つの異なるアプ ローチを表している。すなわち、実験的な研究計画と比較的均質な代理母集団を用いた(通常は単一物質に対する) 管理されたばく露から得られるものと、非実験的な研究デザインを用いた混合ばく露条件(及び変化する)から不均質 な対象集団で観察される変化を反映したものである(ECETOC、2009年)。疫学と毒物学は、それぞれがヒトへのハザ ードの特定に重要かつ異なる貢献をしている。このことは、両方のエビデンスの流れを補完的なものにしており、それら を組み合わせることで強力なアプローチが可能になる。動物実験は常に疫学的研究の解釈に情報を与えるものである べきであり、その逆もまた然りであるため、これらを独立して研究・解釈すべきではない。

# 7.2. ヒトの観察データと実験動物の実験データの重み付けの原則

信頼性の高いとト(疫学的または警戒研究)研究の特定し、プールされたとト研究の関連性の評価した後、関連性が あると判断された別個のエビデンスを、同様に関連性があると判断された他のエビデンスと統合する必要がある。

したがって、最初の検討事項は、検討対象の健康影響がどれだけ毒性学的及び疫学的研究でカバーされているか ということである。特定の健康影響/エンドポイントについて動物試験とヒト試験の両方が利用可能であると考えられる 場合、これは、様々なエビデンス源の重み付けに先立って、個々の試験が信頼性とエビデンスの強固さを評価されるこ とになる(疫学的研究については、それぞれ 6.2 項と 6.3 項)。異なるデータセットは補完的で確実なものであるが、個 別には不十分な場合があり、ヒトの健康リスクを適切に特定するための課題となる可能性がある。良好な観察データが EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna

prior to the weighting of the various sources of evidence. Although the different sets of data can be complementary and confirmatory, individually they may be insufficient and pose challenges for characterising properly human health risks. Where good observational data are lacking, experimental data have to be used. Conversely, when no experimental data is available, or the existing experimental data were found not to be relevant to humans, the risk assessment may have to rely on the available and adequate observational studies.

A framework is proposed for a systematic integration of data from multiple lines of evidence (in particular, human and experimental studies) for risk assessment (Figure 5). Such integration is based on a WoE analysis accounting for relevance, consistency and biological plausibility using modified Bradford Hill criteria (Table 3). For a comparative interpretation of human and animal data, this framework should rely on the following principles (adapted from ECETOC, 2009; Lavelle et al., 2012):

- Although the totality of evidence should be assessed, only the studies that are found to be reliable (those categorised as acceptable or supplementary evidence) are considered further. If the data from the human or the experimental studies is considered to be of low reliability (categorised as unacceptable), no risk assessment can be conducted.
- A WoE approach should be followed where several lines of evidence are found to be relevant. For pesticide active substances, experimental studies following OECD test guidelines are deemed high reliability unless there is evidence to the contrary. The strength of evidence from animal studies can be upgraded if there is high confidence in alternative pesticide toxicity testing or non-testing methods (e.g. *in vitro* and *in silico* studies, respectively). As for epidemiological evidence, the conduct of meta-analysis provides a more precise estimate of the magnitude of the effect than individual studies and also allows for examining variability across studies (see Section 6.3).
- Next, the studies that are found to be more relevant for the stage being assessed are to be given more weight, regardless of whether the data comes from human or animal studies. Where human data are of highest relevance, and supported by a mechanistic scientific foundation, they should take precedence for each stage of the risk assessment. When human and experimental data are of equal or similar relevance, it is important to assess their concordance (consistency across the lines of evidence) in order to determine whether and which data set may be given precedence.
- In case of concordance between human and experimental data, the risk assessment should use all the data as both yield similar results in either hazard identification (e.g. both indicate the same hazard) or hazard characterisation (e.g. both suggest similar safe dose levels). Thus, both can reinforce each other and similar mechanisms may be assumed in both cases.
- In case of non-concordance, the framework needs to account for this uncertainty. For hazard identification, the data suggesting the presence of a hazard should generally take precedence. For dose-response, the data resulting in the lower acceptable level should take precedence. In every situation of discordance, the reasons for this difference should be considered. If the reason is related to the underlying biological mechanisms, or toxicokinetic differences between humans and animal models, then confidence in the risk assessment will increase. Conversely, if the reason cannot be understood or explained, then the risk assessment may be less certain. In such cases, efforts should be made to develop a better understanding of the biological basis for the contradiction.

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#### Epidemiological studies and pesticides

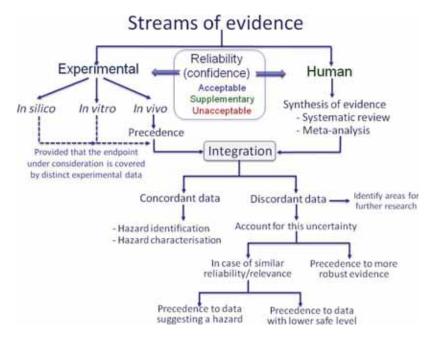
不足している場合は、実験データを使用しなければならない。逆に、実験データが利用できない場合や、既存の実験 データがとトに関連していないことが判明した場合には、リスク評価は利用可能で適切な観察による研究に頼らなけれ ばならないかもしれない。

リスク評価のために、複数のエビデンス(特にヒト研究と実験研究)から得られたデータを体系的に統合するためのフレームワークが提案されている(図 5)。このような統合は、修正された Bradford Hill 基準(表 3)を用いて、関連性、一 貫性、生物学的妥当性を考慮した WOE 解析に基づいている。ヒトと動物のデータを比較解釈するためには、この枠組 みは以下の原則に依存すべきである(ECETOC、2009 年; Lavelle ら、2012 年)。

- エビデンスの全体を評価すべきであるが、信頼性があると判断された研究(許容可能なエビデンスまたは補足的エ ビデンスに分類された研究)のみがさらに検討される。とト研究または実験研究から得られたデータの信頼性が低い(許容できないと分類される)と考えられる場合は、リスク評価を行うことはできない。
- ・複数のエビデンスが関連していることが判明した場合には、WOE アプローチに従うべきである。農薬有効成分については、OECDの試験ガイドラインに従った実験研究は、それに反するエビデンスがない限り、信頼性が高いとみなされる。動物実験からのエビデンスの強固さは、代替的な農薬毒性試験または非試験方法(例えば、それぞれ in vitro 試験と in silico 試験)に高い信頼性がある場合に向上させることができる。疫学的証拠については、メタアナリシスを実施することで、個々の研究よりも効果の大きさをより正確に推定でき、また、研究間のばらつきを調べることができる(第 6.3 節参照)。
- 次に、評価されるステージに関連性が高いと判断された研究は、データがヒトまたは動物の研究からのものである かどうかに関わらず、より重要視されるべきである。ヒトのデータが最も関連性が高く、作用機序的な科学的根拠に 支えられている場合には、リスク評価の各段階で優先されるべきである。ヒトデータと実験データの関連性が同等ま たは類似している場合には、どのデータセットが優先されるかを判断するために、それらのデータの一致性(エビ デンス系統間の一貫性)を評価することが重要である。
  - ーヒトデータと実験データが一致している場合、リスク評価では、ハザードの特定(例:両方とも同じハザードを示 す)またはハザードの特性評価(例:両方とも同じような安全用量を示唆する)のいずれにおいても類似した結 果が得られるため、すべてのデータを使用すべきである。このように、両者は互いに補強し合うことができ、両 方のケースで同様のメカニズムを想定することができる。
  - -不一致の場合、フレームワークはこの不確実性を考慮する必要がある。ハザードの特定については、一般的 にハザードの存在を示唆するデータが優先されるべきである。用量反応については、低い許容量のデータが 優先されるべきである。不一致が生じた場合には、この相違の理由を検討すべきである。その理由が基礎と なる生物学的メカニズム、またはヒトと動物モデル間の毒物動態学的差異に関連している場合は、リスク評価 の信頼性が高まる。逆に、その理由が理解できない、あるいは説明できない場合は、リスク評価の信頼性が 低下するかもしれない。このような場合には、生物学的エビデンスにおける矛盾に対する考察を行うべきであ る。

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#### Figure 5: Methodology for the integration of human and animal data for risk assessment

Epidemiological studies provide complementary data to analyse risk and should be contextualised in conjunction with well-designed toxicological in vivo studies and mechanistic studies. The overall strength of the evidence achieved from integrating multiple lines of evidence will be at least as high as the highest evidence obtained for any single line. This integrated approach provides explicit guidance on how to weight and integrate toxicological and epidemiological evidence. This is a complex task that becomes even more difficult when epidemiological data deal with multifactorial, multihit, chronic diseases for which toxicological models, or disease-specific animal models, are limited.

#### Weighting all the different sources of evidence 7.3.

The WHO/IPCS defines the WoE approach as a process in which all of the evidence considered relevant for risk assessment is evaluated and weighted (WHO/IPCS, 2009). The WoE approach, taking the risk assessment of chemical substances as an example, requires the evaluation of distinct lines of evidence (in vivo, in vitro, in silico, population studies, modelled and measured exposure data, etc.). The challenge is to weight these types of evidence in a systematic, consistent and transparent way (SCENIHR, 2012). The weighting may be formally quantitative or rely on categorisation according to criterion referencing of risk.

An EFSA Working Group was established to provide transparent criteria for the use of the WoE approach for the evaluation of scientific data by EFSA's Panels and Scientific Committee (EFSA, 2015b). The aim of this Working Group was to provide support to stakeholders on how individual studies should be selected and weighted, how the findings integrated to reach the final conclusions and to identify uncertainties regarding the conclusions.

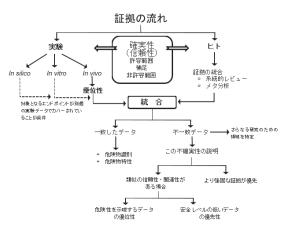
The WoE approach is not consistently considered in the risk assessment of pesticides in the peer review process of DAR or RAR. Expert judgement alone, without a structured WoE approach, has been more commonly used. A few examples can be found, such as the peer review of glyphosate (EFSA, 2015c), where the rapporteur Member State (RMS) considered all the data either from industry or

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Epidemiological studies and pesticides



#### 図 5:リスク評価のためのヒトと動物のデータ統合の方法論

疫学研究はリスクを解析するための補完的なデータを提供するものであり、十分に計画された毒物学的 in vivo 研 究やメカニズム研究と併せて文脈を考慮する必要がある。複数のエビデンスを統合して得られたエビデンスの総合的な 強度は、少なくとも単一のエビデンスで得られた最高のエビデンスと同程度になる。この統合的アプローチは、毒性学 的証拠と疫学的証拠をどのように重み付けして統合するかについての明確な指針を提供するものである。これは複雑 な作業であり、疫学的データが多因子性、多発性、慢性の疾患を対象としている場合には、毒物学的モデルや疾患特 異的動物モデルが限られているため、さらに困難な作業となる。

# 7.3. 異なる起源のエビデンスの重み付け

WHO/IPCSは、WOEアプローチをリスク評価に関連すると考えられるすべてのエビデンスが評価され、重み付けさ れるプロセスと定義している(WHO/IPCS, 2009 年)。WOE アプローチは、化学物質のリスク評価を例にとると、異な る一連のエビデンス(in vivo, in vitro, in silico, 母集団研究、モデル化されたばく露データや測定されたばく露デー タなど)の評価を必要とする。課題は、体系的で一貫性がある明確な方法で、これらのタイプのエビデンスを重み付け することである(SCENIHR、2012 年)。重み付けは、形式的には定量的なものであってもよいし、リスクの基準を参照 した分類に依存するものであってもよい。

EFSA のパネルと科学技術委員会による科学技術データの評価に WOE アプローチを使用するための明確な基準 を提供するために EFSA ワーキンググループが設立された(EFSA、2015 年 b)。このワーキンググループの目的は、 個々の研究がどのように選択され、どのように重み付けされるべきか、結論に到達するためにどのように知見を統合し、 結論に関する不確実性をどのように特定するかについて、利害関係者に支援を提供することであった。

WOE アプローチは、DAR や RAR のピアレビュープロセスにおける農薬のリスク評価において一貫して考慮されて いない。構造化された WOE アプローチを用いずに専門家の判断だけで行うことがより一般的になっている。いくつか の例を挙げると、グリホサートのピアレビュー(EFSA、2015年 c)では、報告者である加盟国(RMS)は、疫学的データ を含む産業界または公的文献からのすべてのデータを考慮し、確立された事後基準と、探索された毒性の各エンドポ イントについて「全体的な」NOAELを提案するために利用可能なすべてのデータを考慮した特定のWOEアプローチ を採用している。

US-EPA は最近、「健康リスク評価にヒトの疫学的データと事象データを組み込むためのフレームワーク」に従って、 EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna 50

from public literature, including epidemiological data, and took a specific WoE approach with established *ad hoc* criteria and considering all data available for proposing an 'overall' NOAEL for each endpoint of toxicity explored.

The US-EPA has recently applied specific criteria for the WoE approach to the peer review of the pesticide chlorpyrifos by following the 'Framework for incorporating human epidemiologic & incident data in health risk assessment'. In this specific case, a WoE analysis has been conducted to integrate quantitative and qualitative findings across many lines of evidence including experimental toxicology studies, epidemiological studies and physiologically based pharmacokinetic and pharmacodynamic (PBPK-PD) modelling. Chlorpyrifos was also used as an example for the EFSA Guidance on literature search under Regulation (EC) No 1107/2009. In addition, an EFSA conclusion (EFSA, 2014a) took into consideration the US-EPA review (2011) to revise its first conclusion produced in 2011.

In sum, a broader WoE approach can be applied to evaluate the available scientific data using modified Bradford Hill criteria as an organisational tool to increase the likelihood of an underlying causal relationship (Table 3). Although epidemiology increasingly contributes to establishing causation, an important step to this end is the establishment of biological plausibility (US-EPA, 2010; Adami et al., 2011; Buonsante et al., 2014).

#### 7.4. Biological mechanisms underlying the outcomes

A biological mechanism describes the major steps leading to a health effect following interaction of a pesticide with its biological targets. The mechanism of toxicity is described as the major steps leading to an adverse health effect. An understanding of all steps leading to an effect is not necessary, but identification of the key events following chemical interaction is required to describe a mechanism (of toxicity in the case of an adverse health effect). While many epidemiological studies have shown associations between pesticide exposures and chronic diseases, complementary experimental research is needed to provide mechanistic support and biological plausibility to the human epidemiological observations. Experimental exposures should be relevant to the human population provided that the biologic mechanisms in laboratory animals occur in humans.

Establishing biological plausibility as part of the interpretation of epidemiological studies is relevant and should take advantage of modern technologies and approaches (Section 7.6). In this context, the AOP framework can be used as a tool for systematically organising and integrating complex information from different sources to investigate the biological mechanisms underlying toxic outcomes and to inform the causal nature of links observed in both experimental and observational studies (Section 7.5).

The use of data to inform specific underlying biological mechanisms or pathways of the potential toxic action of pesticides is limited since only selected pesticide chemicals have been investigated for biological function in relation to a specific health outcome. It may be possible to formulate a mode of action (MoA) hypothesis, particularly where there is concordance between results of comparable animal studies or when different chemicals show the same pattern of toxicity. It is essential to identify the toxicant and the target organ as well as the dose–response curve of the considered effect and its temporal relationship. If the different key events leading to toxicity and a MoA hypothesis can be identified, it is sometimes possible to evaluate the plausibility of these events to humans (ECETOC, 2009).

Sulfoxaflor is an example where MoA has been extensively studied and has been also widely used as an example during the ECHA/EFSA MOA/HRF workshop held in November 2014. Sulfoxaflor induced hepatic carcinogenicity in both rats and mice. Studies to determine the MoA for these liver tumours were performed in an integrated and prospective manner as part of the standard battery of toxicology studies such that the MoA data were available prior to, or by the time of, the completion of the carcinogenicity studies. The MoA data evaluated in a WoE approach indicated that the identified rodent liver tumour MoA for sulfoxaflor would not occur in humans. For this reason, sulfoxaflor is considered not to be a potential human liver carcinogen.

Furthermore, sometimes MoA data may indicate a lack of possible effects. If there are biological data that indicate an adverse effect is not likely to occur in humans, this should inform the interpretation of epidemiological studies. Nevertheless, while primary target site selectivity between pests and humans plays an important role in pesticides safety, secondary targets in mammals must also be considered.

In the case of exposure to multiple pesticides, the decision to combine risks can be taken if the pesticides share a common mechanism of toxicity (act on the same nolecular target at the same target tissue, act by the same biochemical mechanism of action, and share a common toxic intermediate) which may cause the same critical effect or just based on the observation that they share the same target organ (EFSA 2013a,b). However, cumulative risk assessment is beyond the scope of this Opinion.

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農薬のクロルビリホスのピアレビューに WOE アプローチのための特定の基準を適用した。この特定のケースでは、実験的毒性研究、疫学研究、生理学的ベースの薬物動態・薬力学(PBPK-PD)モデリングを含む多くのエビデンスを横断して、定量的・定性的な知見を統合するために WOE 解析が実施された。クロルビリホスは、規則(EC)No 1107/2009に基づく文献検索に関する EFSA ガイダンスの例としても使用されている。さらに、EFSA の結論(EFSA、2014年a)は、2011年に出された最初の結論を修正するために US-EPA のレビュー(2011年)を考慮に入れている。

まとめると、根本的な因果関係の可能性を高めるための組織的なツールとして、修正された Bradford Hill 基準を使 用して、利用可能な科学的データを評価するために、より広範な WOE アプローチを適用することができる(表 3)。疫 学は因果関係の立証にますます貢献しているが、この目的のための重要なステップは、生物学的妥当性の立証である (US-EPA、2010年:Adami ら、2011:Buonsante ら、2014年)。

# 7.4. 健康影響の基礎となる生物学的メカニズム

生物学的メカニズムとは、農薬とその生物学的標的との相互作用に続く健康影響につながる主要なステップを記述 したものである。毒性のメカニズムは、健康への悪影響につながる主要なステップとして記述されている。影響につなが るすべてのステップを理解する必要はないが、化学的相互作用に続く重要なイベントを特定することは、メカニズム(健 康に悪影響を及ぼす場合の毒性)を記述するために必要である。多くの疫学研究で農薬ばく露と慢性疾患との関連性 が示されているが、ヒトの疫学的観察にメカニズム的な裏付けと生物学的な妥当性を与えるためには、補完的な実験研 究が必要である。実験でのばく露は、実験動物の生物学的メカニズムがヒトで発生することを条件に、ヒトの集団に関連 するものでなければならない。

疫学研究の解釈の一部として生物学的妥当性を確立することは重要であり、最新の技術とアプローチを活用すべき である(7.6 節)。この意味では、AOPの枠組みは、毒性結果の基礎となる生物学的メカニズムを調査し、実験研究と観 察による研究の両方で観察された関連性の因果関係を知るために、異なる情報源からの複雑な情報を体系的に整理 し、統合するためのツールとして利用できる(7.5 節)。

農薬の潜在的な毒性作用の基礎となる生物学的メカニズムや経路について特定の情報を提供するためのデータの 利用は、特定の健康影響に関連した生物学的機能について調査された農薬化学物質のみであるため、限られたもの である。特に、同等の動物実験の結果の間に一致点がある場合や、異なる化学物質が同じ毒性パターンを示す場合 には、作用機序(MOA)仮説を立てることが可能な場合がある。毒物と標的臓器を特定することは、考えられる影響の 用量反応曲線とその時間的関係と同様に不可欠である。毒性につながるさまざまな主要事象と MOA 仮説を特定でき れば、ヒトに対するこれらの事象の妥当性を評価することが可能になることもある(ECETOC、2009 年)。

農薬のスルホキサフロル (Sulfoxaflor)は、MOA が広範囲に研究されてきた例であり、2014 年 11 月に開催された ECHA/EFSA MOA/HRF ワークショップでも広く取り上げられている。スルホキサフロルはラットとマウスの両方で肝発 がん性を誘発した。これらの肝腫瘍に対する MOA を決定するための試験は、発がん性試験が終了する前または終了 するまでに MOA データが入手できるように、標準的な毒性試験のバッテリーの一部として、統合的かつ前向きな方法 で実施された。WOE アプローチで評価された MOA データは、スルホキサフロルの齧歯類での肝腫瘍の MOA がヒト では発生しないことを示している。この理由から、スルホキサフロルはヒトの潜在的な肝臓発がん性物質ではないと考え られている。

さらに、MOA データが影響の可能性がないことを示す場合もある。仮に、ヒトでは有害な影響が発生する可能性が ないことを示す生物学的データがある場合は、疫学研究の解釈に反映させる必要がある。それにもかかわらず、害虫と ヒトの間の一次標的部位選択性は農薬の安全性において重要な役割を果たしているが、哺乳類における二次標的も 考慮しなければならない。

複数の農薬にばく露した場合、リスクを組み合わせるかどうかの判断は、農薬が共通の毒性メカニズム(同じ標的組 織で同じ分子標的に作用し、同じ生化学的作用機序で作用し、共通の毒性中間体を共有する)を共有しており、同じ 重大影響を引き起こす可能性がある場合、あるいは単に同じ標的臓器を共有しているという観察に基づいて判断する ことができる(EFSA、2013 年 a,b)。しかし、累積リスク評価は本意見書の範囲を超えている。



#### 7.5. Adverse Outcome Pathways (AOPs)

The AOP methodology provides a framework to collect and evaluate relevant chemical, biological and toxicological information in such a way that is useful for risk assessment (OECD, 2013). An AOP may be defined as the sequence of key events following the interaction of a chemical with a biological target (molecular initiating event (MIE)) to the *in vivo* adverse outcome relevant to human health. All these key events are necessary elements of the MoA and should be empirically observable or constitute biologically based markers for such an event. An AOP is therefore a linear pathway from one MIE to one adverse outcome at a level of biological organisation relevant to risk assessment. The goal of an AOP is to provide a flexible framework to describe the cascade of key events that lead from a MIE to an adverse outcome in a causal linkage (EFSA PPR Panel, 2017). The 'key events' must be experimentally measurable and the final adverse effect is usually associated with an *in vivo* OECD Test Guideline. However, in some cases the adverse outcome may be at a level of biological organisation below that of the apical endpoint described in a test guideline (OECD, 2013).

A particular MIE may lead to several final adverse effects and, conversely, several MIEs may converge in the same final adverse effect. However, each AOP will have only one MIE and one final adverse effect, but may involve an unlimited number of intermediate steps (Vinken, 2013). It should be noted that key events at different levels of biological organisation provide a greater WoE than multiple events at the same level of organisation (OECD, 2013).

The essential biochemical steps involved in a toxic response are identified and retrieved from an indepth survey of relevant scientific literature or from experimental studies. Any type of information can be incorporated into an AOP, including structural data, 'omics-based' data and *in vitro*, *in vivo* or *in silico* data. However, *in vivo* data are preferred over *in vitro* data and endpoints of interest are preferred to surrogate endpoints (Vinken, 2013). The AOPs identified must not be incompatible with normal biological processes, since they need to be biologically plausible.

Qualitative AOPs (intended as an AOP including the assembly and evaluation of the supporting WoE following the OECD guidance for AOP development) should be the starting and standard approach in the process of integration of epidemiology studies into risk assessment by supporting (or identifying the lack of support for) the biological plausibility of the link between exposure to pesticides affecting the pathway and the adverse outcome. Accordingly, qualitative AOPs may be developed solely for the purpose of hazard identification, to support biological plausibility of epidemiological studies based on mechanistic knowledge (EFSA PPR Panel, 2017).

The AOP framework is a flexible and transparent tool for the review, organisation and interpretation of complex information gathered from different sources. This approach has the additional advantage of qualitatively characterising the uncertainty associated with any inference of causality and identifying whether additional mechanistic studies or epidemiological research would be more effective in reducing uncertainty. The AOP framework is therefore a useful tool for risk assessment to explore whether an adverse outcome is biologically plausible or not. For the purpose of analysing the biological plausibility, AOPs can serve as an important tool, particularly when the regulatory animal toxicological studies are useful tool for relevant biomarkers) observed in epidemiological studies is considered inadequate based on the AOP. By means of mechanistically describing apical endpoints, the AOP contributes to the hazard identification and characterisation steps in risk assessment. As the AOP framework is chemically agnostic, if complemented by the MoA and/or Integrated Approach on Testing and Assessment (IATA) framework, it will support the chemical specific risk assessment (EFSA PPR Panel, 2017).

AOP and MoA data can be used to assess the findings of epidemiological studies to weight their conclusions. Whether those findings are inconsistent with deep understanding of biological mechanisms, or simply empirical, they should be given less weight than other findings that are consistent with AOP or MoA frameworks once established. However, there are relatively few examples of well-documented AOPs and a full AOP/MoA framework is not a requirement for using epidemiological studies in risk assessment.

AOPs are thus a critical element to facilitate moving towards a mechanistic-based risk assessment instead of the current testing paradigm relying heavily on apical effects observed in animal studies. Shifting the risk assessment paradigm towards mechanistic understanding would reduce limitations of the animal data in predicting human health effects for a single pesticide, and also support the current efforts being made on cumulative risk assessment of pesticide exposure (EFSA PPR Panel, 2017).

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# 7.5. 有害転帰経路(AOPs)

AOP の解析法は、リスク評価に有用な方法で、関連する化学的、生物学的、毒物学的な情報を収集し、評価するた めの枠組みを提供している(OECD、2013年)。AOPは、化学物質と生物学的標的との相互作用(標的分子への作用 (Molecular initiating event:MIE))から、とトの健康に関連する in vivo での有害な事象に至るまでの一連の重要 事象として定義することができる。これらの重要事象はすべて MOA の必要な要素であり、経験的に観察可能であるか、 またはそのような事象の生物学的ベースのマーカーを構成するものでなければならない。したがって、AOP は、リスク 評価に関連する生物学的組織のレベルで、1 つの MIE から 1 つの有害な事象に至る直線的な経路である。AOP の 目的は、因果関係の連鎖において、1 つの MIE から 1 つの有害な事象へとつながる重要事象の段階を記述するため の、柔軟なフレームワークを提供することである(EFSA PPR パネル、2017年)。重要な事象は実験的に測定可能で なければならず、有害な事象は通常、in vivo での OECD 試験ガイドラインに関連している。しかし、場合によっては、 有害な事象が試験ガイドラインに記載されている先端エンドポイントよりも低いレベルの生物学的組織のレベルにあるこ ともある(OECD、2013年)。

特定の MIE はいくつかの毒性影響につながる可能性があり、逆に、複数の MIE は同じ毒性影響に収束する可能 性がある。しかし、各 AOP は 1 つの MIE と 1 つの毒性影響象のみであるが、無制限の数の中間ステップを伴うことも ある(Vinken、2013 年)。生物学的組織の異なるレベルでの重要事象は、同じレベルの組織での複数の事象よりも大 きな WOE をもたらすことに留意すべきである(OECD、2013 年)。

毒性反応に関与する重要な生化学的ステップは、関連する科学文献の詳細な調査や実験研究から同定され、検索 される。構造データ、「オミクスベース」データ、in vitro、in vivo、あるいは in silico のデータなど、あらゆるタイプの情 報を AOP に組み込むことができる。しかし、in vitro データよりも in vivo データの方が優先され、対象となるエンドポ イントは代替エンドポイントよりも優先される(Vinken、2013 年)。特定された AOP は、生物学的に妥当性をもつ必要 があるため、正常な生物学的プロセスと両立しないものであってはならない。

定性的 AOP (AOP 開発のための OECD ガイダンスに従った WOE の組み立てと評価を含む AOP として意図され ている) は、経路に悪影響を与える農薬へのばく露と有害な影響との間の関連性の生物学的妥当性を裏付ける(また は裏付けがないことを特定する)ことによって、疫学研究をリスク評価に統合するプロセスの出発点であり、標準的なア プローチであるべきである。したがって、定性的な AOP は、メカニズム的知見に基づく疫学研究の生物学的妥当性を 裏付けるために、ハザード同定の目的のためだけに開発される可能性がある(EFSA PPR パネル、2017 年)。

AOP フレームワークは、異なる情報源から収集された複雑な情報のレビュー、整理、解釈を行うための柔軟で透明 性の高いツールである。このアプローチには、因果関係の推論に関連する不確実性を定性的に特徴づけ、不確実性 を低減するためには、追加のメカニズム的研究や疫学研究がより効果的であるかどうかを特定するという付加的な利点 がある。したがって、AOP フレームワークは、有害な影響が生物学的にもっともらしいかどうかを探るためのリスク評価に 有用なツールである。生物学的に妥当性を解析する目的では、AOP は重要なツールとなり得る。特に、規制上の動物 を用いた毒性試験が陰性であっても、疫学研究で観察された先端エンドポイント(または関連するバイオマーカー)の 評価が AOP に基づいて不十分であると考えられる場合には、AOP は重要なツールとなり得る。先端エンドポイントをメ カニズム的に記述することにより、AOP はリスク評価におけるハザードの特定と特性評価のステップに貢献する。AOP フレームワークは化学的には不明確な点があるため、MOA 及び/または試験・評価に関する統合的アプローチ (IATA)フレームワークで補完されれば化学物質特有のリスク評価をサポートすることになる(EFSA PPR パネル、 2017 年)。

AOPとMOAのデータは、疫学研究の結果を評価し、その結論に重み付けをするために使用できる。それらの結論 が生物学的メカニズムの深い理解と矛盾するものであろうと、あるいは単に経験的なものであろうと、一度確立された AOPや MOAの枠組みと一致する他の結論よりも、それらの結論の重要性は低く設定されるべきである。しかし、十分 に文書化された AOP の例は比較的少なく、完全な AOP/MOA の枠組みはリスク評価に疫学的研究を用いるための 要件ではない。

したがって、AOP は、動物実験で観察された末端の影響(apical effects)に大きく依存する現在の試験パラダイム

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# 7.6. Novel tools for identifying biological pathways and mechanisms underlying toxicity

The elucidation of toxicity pathways brings the opportunity of identifying novel biomarkers of early biological perturbations in the toxicodynamic progression towards overt disease, particularly from advances in biomonitoring, in -omics technologies and systems biology (toxicology). The revolution of omics in epidemiology holds the promise of novel biomarkers of early effect and offers an opportunity to investigate mechanisms, biochemical pathways and causality of associations.

The growing recognition of the value of biomonitoring data in epidemiological investigations may help to reduce misclassification by providing objective measures of exposure and outcome. As long as biomarker data for exposure, outcome and susceptibility are increasingly generated, epidemiology will have a greater impact in the understanding of toxicodynamic progression as a function of pesticide exposure and eventually in risk assessment. A challenge for risk assessors will be to acknowledge where subtle and early changes along the toxicodynamic pathway are indicative of increased potential for downstream effects (Nachman et al., 2011). Omics data can be used for gaining insight to the MoA by identifying pathways affected by pesticides and as such can assist hazard identification, the first step in risk assessment.

Transcriptomic, metabolomic, epigenomic and proteomic profiles of biological samples provide a detailed picture, sometimes at individual molecule resolution, of the evolving state of cells under the influence of environmental chemicals, thus revealing early mechanistic links with potential health effects. Nowadays, the challenges and benefits that advances in -omics techniques can bring to regulatory toxicology are still being explored (Marx-Stoelting et al., 2015). Clear rules for assessing the specificity of these biomarkers are necessary.

Those -omic applications most relevant and advanced in the context of toxicology are analysis of MoA and the derivations of AOP, and biomarker identification, all of which potentially assist epidemiology too. For example, (a) transcriptomics: comparing gene expression (mRNA) profiles can be used for biomarker discovery, grouping expressed genes into functional groups (Gene Ontology categories) or for Gene Set Analysis. Such techniques may provide varying information regarding biological mechanisms. (b) Proteomics: studying the protein profile of samples, with sophisticated analysis of protein quantity and post-translational modifications which may be associated with changes in biological pathways following exposure and possible disease development, utilising informatics and protein databases for identification and quantification. (c) Metabolomics uses nuclear magnetic resonance spectroscopy or mass-spectrometry based techniques to produce data which are analysed via software, and databases, to identify markers (molecular signatures and pathways) that correlate with exposure or disease. (d) The use of the exposome (the totality of exposures received by an individual during life) might be better defined by using -omics technologies and biomarkers appropriate for human biomonitoring. Nevertheless, important limitations stemming from the lack of validation of these methodologies and their cost limit their use at large scale.

The application of -omics technologies to environmental health research requires special consideration to study design, validation, replications, temporal variance and meta-data analysis (Vlaanderen et al., 2010). For larger studies, intra-individual variability in the molecular profiles measured in biological samples should show less variability than the interindividual variation in profiles of gene expression, protein levels or metabolites, which are highly variable over time. It is important that these inter-individual variations should not be larger than variation related to exposure changes, but it is not certain if this will be true.

The biologically meaningful omics signatures identified by performing omics-exposure and omicshealth association studies provide useful data for advanced risk assessment. This approach supports moving away from apical toxicity endpoints towards earlier key events in the toxicity pathway resulting from chemical-induced perturbation of molecular/cellular responses (NRC, 2007).

#### 7.7. New data opportunities in epidemiology

The current technological landscape permits the digitisation and storage of unprecedented amount of data from many sources, including smart phones, text messages, credit card purchases, online activity, electronic medical records, global positioning system (GPS) and supermarket purchasing data. While some of these data sources may provide valuable information for risk assessment, many of them contain personal information that can outpace legal frameworks and arise questions about the ethics of its use for scientific or regulatory purposes. A specific example is constituted by data containing

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ではなく、メカニズムに基づくリスク評価への移行を促進するための重要な要素である。リスク評価のパラダイムをメカニ ズム的な理解へと移行させることで、単一の農薬のヒトへの健康影響を予測する上での動物データの限界を下げること になり、また、農薬ばく露の累積リスク評価に関する現在の取り組みを支援することにもなる(EFSA PPR パネル、2017 年)。

#### 7.6. 毒性の基礎となる生物学的経路とメカニズムを特定するための新しいツール

毒性経路の解明は、特にバイオモニタリング、オミクス技術、システムバイオロジー(毒性学)の進歩から、顕在化した 疾患への毒性力学的進行における初期の生物学的摂動の新規バイオマーカーを特定する機会をもたらしている。疫 学におけるオミクスの革命は、早期効果の新しいバイオマーカーの可能性を秘めており、関連のメカニズム、生化学的 経路、因果関係を調査する機会を提供している。

疫学調査におけるバイオモニタリングデータの価値が認識されつつあることは、ばく露と転帰の客観的な尺度を提供 することで、誤分類を減らすのに役立つかもしれない。ばく露、転帰、感受性に関するバイオマーカーデータが増加し ている限り、疫学は農薬ばく露の関数としての毒性力学的進行の理解と最終的にはリスク評価に大きな影響を与えるこ とになるであろう。リスク評価者にとっての課題は、毒性力学的経路に沿った軽微で初期の変化が、下流への影響の可 能性の増大を示唆していることを認識することである(Nachman ら、2011 年)。オミクスデータは、農薬の影響を受ける 経路を特定することで MOA への理解を深め、リスク評価の第一段階であるハザードの特定を支援することができる。

生物学的サンプルのトランスクリプトーム(Transcriptomic,)、メタボローム(metabolomic)、エピゲノム (epigenomic)、プロテオミクス(proteomic)のプロファイリングは、環境化学物質の影響下での細胞の進化状態の詳 細な画像を、時には個々の分子レベルで提供し潜在的な健康影響との早期のメカニズム的な関連性を明らかにするこ とができる。今日では、オミクス技術の進歩が規制毒性学にもたらす課題と長所については、まだ研究が進められてい る(Marx-Stoelting ら、2015 年)。これらのバイオマーカーの特異性を評価するための明確なルールが必要である。

毒物学の文脈において、最も有用であり進歩しているオミクス技術は、MOAの解析とAOPの誘導、バイオマーカー の同定であり、これらはすべて疫学にも役立つ可能性がある。例えば、(a)トランスクリプトミクス:遺伝子発現(mRNA) プロファイルの比較は、バイオマーカーの発見、発現遺伝子の機能グループ(Gene Ontology カテゴリー)へのグルー プ化、または遺伝子セット分析に使用することができる。これらの手法により、生物学的メカニズムに関する様々な情報 が得られる可能性がある。(b)プロテオミクス:試料のタンパク質プロファイリングを調べ、タンパク質の量や転写後の修 飾を高度に分析し、ばく露後の生物学的経路の変化や疾患の発症に関連している可能性がある。(c)メタボロミクスは、 核磁気共鳴分光法や質量分析法をベースにした技術を用いてデータを作成し、ソフトウェアやデータベースを介して 分析することで、ばく露や疾患と相関のあるマーカー(分子シグネチャーや経路)を特定するものである。(d)エクスポソ ーム(個人が生活の中で受けたばく露の総量)の利用は、ヒトのバイオモニタリングに適したオミクス技術とバイオマーカ ーを使用することで、より良い結果を得ることができるかもしれない。それにもかかわらず、これらの方法論の検証不足と そのコストの問題から、大規模な使用には限界がある。

オミクス技術を環境衛生研究に応用するには、研究デザイン、バリデーション、再現性、時間的分散、メタデータ分析 に特別な配慮が必要である(Vlaanderen ら、2010年)。大規模な研究では、生体サンプルで測定された分子プロファ イルの個人内変動は、時間の経過とともに大きく変動する遺伝子発現、タンパク質レベル、または代謝物のプロファイ ルの個人間変動よりも少ない変動を示す。これらの個人間変動がばく露変化に関連した変動よりも大きくならないことが 重要であるが、これが実現するかどうかは定かではない。

生物学的に意味のあるオミクスのシグネチャーは、オミクスーばく露及びオミクスー健康の関連性の研究を行うことに よって同定され、高度なリスク評価に有用なデータを提供する。このアプローチは、末端の毒性エンドポイントから、化 学物質によって誘発された分子/細胞応答の摂動に起因する毒性経路の初期の主要イベントへと移行することを支 持するものである(NRC、2007年)。

# 7.7. 疫学における新たなデータの機会

現在の技術的状況では、スマートフォン、テキストメッセージ、クレジットカードでの購入、オンラインでの行動、電子カ

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personal information related to health, which are considered sensitive or especially protected, such as electronic medical records, information from occupational or environmental questionnaires, geographic location, health or social security number, etc. These various forms of health information are being easily created, stored and accessed. Big data provide researchers with the ability to match or link records across a number of data sources. Linking of big data sources of health and heritable information offers great promise for understanding disease predictors (Salerno et al., 2017); however, there are challenges in using current methods to process, analyse and interpret the data systematically and efficiently or to find relevant signals in potential oceans of noise, as noted by the Board on Environmental Studies and Toxicology of the National Academies of Sciences, Engineering, and Medicine in its 2017 report.<sup>18</sup>

In addition, medico-administrative data, such as drug reimbursements drawn from National Health Insurance or hospital discharge databases, can be cross-linked with data on agricultural activities drawn from agricultural census or geographical mapping. It is acknowledged that in several instances this information can be obtained at group level only, and an important challenge will be to obtain data at individual level and/or on individual habits.

Biobanks also constitute new data sources from healthy or diseased populations. They consist of an organised collection of human biological specimens and associated information stored for diverse research purposes. These biosamples are available for application of novel technologies with potential for generating data valuable for exposure assessment or exposure reconstruction. If studies' design and conduct are harmonised, data and samples can be shared between biobanks to promote powerful pooled analyses and replications studies (Burton et al., 2010).

Large scale epidemiological studies with deep phenotyping provide also unprecedented opportunities to link well phenotyped study participants with the aforementioned data. For example, UK Biobank, has recruited over 500,000 individuals with questionnaire, medical history and physical measurements data as well as stored blood and urine samples with available genome wide association data for all 500,000 participants, and linkage to Hospital Episode Statistics, national registry data and primary care records. To gain information on air pollution and noise levels, the postcode of participants has been linked to air pollution or noise estimates. In addition, piloting of personal exposure monitoring will take place in order to collect individual level data on these exposures. These approaches could be extended to gain information on pesticide exposure, either through geographical linkage, linkage with purchasing and occupational registries, and personal exposure monitoring. Similar biobanks exist in many other EU countries (http://www.bbmri-eric.eu/BBMRI-ERIC has collected most EU studies).

#### 8. Overall recommendations

#### 8.1. Recommendations for single epidemiological studies:

The following recommendations for improving epidemiological studies are aimed to conform to the 'recognised standards' mentioned in Regulation (EU) No 1107/2009 to make them of particular value to risk assessment of pesticides ('where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards, epidemiological studies are of particular value and must be submitted'). Accordingly, these recommendations can indeed not be considered as a practical guidance for researchers on how to conduct such studies, but for those who are planning to conduct a study for further use in pesticide risk assessment.

#### a) Study design (including confounding)

- Since prospective epidemiological designs provide stronger evidence for causal inference, these studies are encouraged over the other designs for pesticide risk assessment.
- 2) Future epidemiological studies should be conducted using the appropriate sample size in order to properly answer the question under investigation. A power analysis should thus be performed at the study design stage.
- Future studies should take into consideration heterogeneity, subpopulations, exposure windows and susceptibility periods and conditions (pregnancy, development, diseases, etc.).

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#### Epidemiological studies and pesticides

ルテ、全地球測位システム(GPS)、スーパーマーケットの購買データなど、多くの情報源からの前例のない量のデータ のデジタル化と保存が可能になっている。これらのデータ源の中には、リスク評価のための貴重な情報を提供するもの もあるが、それらの多くには法的枠組みを超えて、科学的または規制上の目的のために使用することの倫理性につい て疑問が生じることもある。具体的な例としては、電子カルテ、職業や環境に関するアンケート、地理的な位置、健康や 社会保障番号など、機密性が高い、または特に保護されていると考えられる健康に関連する個人情報を含むデータが 挙げられる。これらの様々な形態の健康情報が容易に作成、保存、アクセスされている。ビッグデータは、研究者に多く のデータ源をまたいで記録を照合したり、リンクしたりする能力を提供する。健康情報と遺伝性情報のビッグデータ源の リンクは、病気の予測因子を理解するための大きな可能性を期待されている(Salerno 6、2017 年)。しかし、現在の方 法を用いてデータを体系的かつ効率的に処理、解析、解釈すること、あるいは大量データの中から関連するシグナル を特定することには課題がある(全米科学・工学・医学の環境研究・毒物学委員会が 2017 年の報告書で指摘している) <sup>18</sup>。

さらに、国民健康保険や退院データベースから抽出された薬剤費などの医療行政データは、農業人口調査や地理 的マッピングから抽出された農業活動に関するデータと相互にリンクさせることができる。このような情報が集団レベル でしか得られない場合もあることは認識されているが、個人レベル及び/または個人の習慣に関するデータを得ること が重要な課題である。

バイオバンクはまた、健康な集団や病気にかかった集団からの新たなデータ源を構成するものである。バイオバンク は、多様な研究目的のために保存されているとトの生物学的標本と関連情報の整理されたコレクションで構成されてい る。これらのバイオサンプルは、ばく露評価やばく露の再構成に有用なデータを生成する可能性のある新技術の応用 に利用可能である。研究の計画と実施が調和されていれば、データとサンプルはバイオバンク間で共有され、強力な 蓄積分析や反復研究を促進することができる(Burton 6、2010 年)。

深い表現型(deep phenotyping)を用いた大規模な疫学研究は、優れた表現型を持つ研究参加者と前述のデータ を結びつける前例のない機会を提供する。例えば、英国のバイオバンクでは、アンケート、病歴、身体測定データだけ でなく、50 万人以上の参加者全員のゲノムワイドな関連データを持つ血液と尿のサンプルを保存し、病院の事例統計、 国の登録データ、プライマリーケアの記録とリンクさせている。大気汚染や騒音レベルに関する情報を得るために、参 加者の郵便番号を大気汚染や騒音の推定値にリンクさせている。さらに、これらのばく露に関する個人レベルのデータ を収集するために、個人ばく露モニタリングの試験的実施が行われる予定である。これらのアプローチは、地理的リンク、 購買・職業登録とのリンク、個人のばく露モニタリングのいずれかを通じて、農薬ばく露に関する情報を得るために拡張 される可能性がある。同様のバイオバンクは、他の多くの EU 諸国にも存在する(http://www.bbmri-eric.eu/BBMRI-ERIC は、ほとんどの EU の研究を収集している)。

# 8. 全体的な推奨事項

# 8.1. 単一の疫学的研究に関する勧告

疫学的研究を改善するための以下の勧告は、規制(EU)No 1107/2009 で言及されている「認知された基準」に準拠し、農薬のリスク評価に特に価値のあるものとすることを目的としている(「利用可能で、ばく露レベルとばく露期間に関するデータを裏付けとし、認識された基準に従って実施された場合、疫学的研究は特に価値があり、提出しなければならない」としている)。したがって、これらの勧告は、このような研究をどのように実施するかについての研究者のための実践的な指針としてではなく、農薬リスク評価にさらに活用するための研究を計画している研究者のためのものと考えることができる。

#### a)研究デザイン(交絡を含む)

 前向き疫学的デザインは因果関係推論のためのより強力なエビデンスを提供するので、農薬リスク評価のための 他の計画よりもこれらの研究が奨励される。

<sup>&</sup>lt;sup>18</sup> National Academies of Sciences, Engineering, and Medicine; Division on Earth and Life Studies; Board on Environmental Studies and Toxicology; Committee on Incorporating 21st Century Science into Risk-Based Evaluations. Washington (DC): National Academies Press (US); 2017 Jan.

<sup>18</sup> 全米科学・工学・医学アカデミー、地球・生命研究部門、環境研究・毒物学委員会、リスクベースの評価に21世紀の科学を組み込む委員会。ワシントン(DC)。全米アカデミープレス(米国);2017年1月

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- 4) A wide range of potential confounding variables (including co-exposure to other chemicals, lifestyle, socioeconomic factors, etc.) should be measured or accounted for during the design stage (e.g. matching) of the study.
- 5) Consideration of host factors that may influence toxicity and act as effect modifiers. These will include genetic polymorphisms data (e.g. paraoxonase-1 genotype) or nutritional factors (e.g. iodine status) among others.
- 6) Collaboration between researchers is encouraged to build-up consortia that enhance the effectiveness of individual cohorts.

Collection and appropriately storage of relevant biological material should be undertaken for future exposure assessment, including the use of novel technologies.

# b) **Exposure** (measurement, data transformation for reporting and statistical analysis):

- 1) Collection of specific information on exposure should avoid as far as possible broad definitions of exposure, non-specific pesticide descriptions and broad exposures classifications such as 'never' vs. 'ever' categories. Nevertheless, these categories may be valuable under certain circumstances, e.g. to anticipate a class effect.
- 2) Studies which only look at broad classes of pesticides (generic groups of unrelated substances), or 'insecticides', 'herbicides', etc. or even just 'pesticides' in general are of much less use (if any) for risk assessment. Studies that investigate specific named pesticides and co-formulants are more useful for risk assessment.
- 3) Pesticides belonging to the same chemical class or eliciting the same mode of toxic action or toxicological effects might be grouped in the same category. Further refinement with information on frequency, duration and intensity of exposure might help in estimating exposure patterns.
- 4) In occupational epidemiology studies, operator and worker behaviour and proper use of PPE should be adequately reported as these exposure modifiers may significantly change exposures and thereby potential associations.
- 5) Improving the accuracy of exposure measurement is increasingly important, particularly for cohort studies. Long-term cohort studies which cover the etiologically relevant time period should improve the accuracy of measures of exposures by use of repeated biologic measures or repeated updates of self-reported exposures.
- 6) Indirect measures of environmental exposure for wider populations, including records on pesticide use, registry data, GIS, geographical mapping, etc., as well as data derived from large databases (including administrative databases) may be valuable for exploratory studies. If these data are not available, records/registries should be initiated. Likewise, estimation of dietary exposure to pesticide from food consumption databases and levels of pesticide residues from monitoring programmes can be used as well. As with direct exposure assessment, each method of indirect measurement should be reviewed for risk of bias and misclassification and weighted appropriately.
- 7) Whenever possible, exposure assessment should use direct measurements of exposure to named pesticides in order to establish different levels of exposure (e.g. personal exposure metering/biological monitoring), possibly in conjunction with other methods of exposure assessment which are more practicable or even necessary for large studies and historical exposures. New studies should explore novel ways of personal exposure monitoring. Results should be expressed using standardised units to normalise exposure across populations
- 8) The characterisation of exposure assessment over time can benefit by undertaken a more comprehensive exposure monitoring strategy coupled with information on exposure determinants over a longer time period collected from questionnaires or job-exposure matrices supported by biomonitoring data. Exposure assessment models can be comprehensively supported by HBM studies, which would allow identification of the critical exposure parameters. If such case, adjustments can then be made to the parameter assumptions within the models, leading to more realistic evaluations of exposure.
- 9) The use of the exposome concept and metabolomics in particular hold great promise for next-generation epidemiological studies both for better exposure measurement (biomarkers of exposure), for identification of vulnerable subpopulations and for biological interpretation of toxicity pathways (biomarkers of disease).

- 2) 今後の疫学研究は、調査対象の課題に適切に答えるために、適切な標本数を用いて実施されるべきである。その ためには、研究デザインの段階で検出力解析を行う必要がある。
- 3)今後の研究では、異質性、小集団、ばく露方法、感受性の時期や条件(妊娠、発育、疾患など)を考慮する必要 がある。
- 4)幅広い潜在的交絡変数(他の化学物質への共ばく露、ライフスタイル、社会経済的要因など)を研究の計画段階 (例:マッチング)で測定または考慮すべきである。
- 5) 毒性に影響を与え、効果を調節する宿主因子を考慮する。これらには、遺伝的多型データ(例:パラオキソナーゼ -1遺伝子型)や栄養因子(例:ヨウ素の状態)などが含まれる。
- 6)研究者間の共同研究は、個々のコホートの有効性を高めるコンソーシアムを構築するために奨励される。
- 将来のばく露評価のために、新規技術の利用を含め、関連する生物学的試料の収集と適切な保管を行うべきである。 b) ばく露(測定、報告のためのデータ変換、統計解析)
- 1) ばく露に関する特定の情報の収集は、可能な限り、ばく露の広範な定義、非特定の農薬の記述及び「一度もない」 対「今までにあり」のような広範なばく露の分類を避けるべきである。それにもかかわらず、これらのカテゴリーは、 特定の状況下では、例えばクラス効果を予測するために価値があるかもしれない。
- 2) 農薬の幅広いクラス(無関係な物質の一般的なグループ)、または「殺虫剤」、「除草剤」など、あるいは一般的な 「農薬」だけを対象とした研究は、リスク評価にはあまり役に立たない(あるとすれば)。特定の名前のついた農薬や 共調合製剤を調査した研究の方がリスク評価には有用である。
- 3)同じ化学クラスに属する農薬、または同じ毒性作用モードや毒性学的効果をもたらす農薬は、同じカテゴリーにグ ループ化されている可能性がある。ばく露の頻度、持続時間、ばく露の強度などの情報を追加することで、ばく露 パターンの推定に役立てることができるかもしれない。
- 4)職域疫学研究では、作業者や労働者の行動や PPE の適切な使用は、これらのばく露修飾がばく露を大幅に変 化させ、それによって潜在的な関連性を変化させる可能性があるため、適切に報告されなければならない。
- 5) ばく露測定の精度を向上させることは、特にコホート研究において、ますます重要になってきている。病因学的に 関連する期間をカバーする長期のコホート研究では、繰り返しの生物学的測定や自己報告されたばく露の繰り返 しの更新を使用することにより、ばく露の測定の精度を向上させるべきである。
- 6) 農薬の使用記録、登録データ、GIS、地理的マッピングなどを含むより広い集団の環境ばく露の間接的な尺度及 び大規模なデータベース(行政データベースを含む)から得られたデータは、探索的研究には貴重であるかもしれ ない。これらのデータが利用できない場合は、記録・登録を開始すべきである。同様に、食品消費データベースか らの農薬への食事ばく露の推定や、モニタリングプログラムからの残留農薬レベルの推定も利用できる。直接的な ばく露評価と同様に、間接測定の各方法は、偏りや誤分類のリスクを検討し、適切な重み付けを行うべきである。
- 7) 可能な限り、ばく露評価は、異なるばく露レベルを確立するために、指定された農薬へのばく露の直接測定を使 用すべきである(例えば、個人的なばく露測定/生物学的モニタリング)。新しい研究では、個人ばく露モニタリン グの新しい方法を探求すべきである。結果は、集団間のばく露を標準化するために、標準化された単位を用いて 表現されるべきである。
- 8)長期にわたるばく露評価の特性評価は、より包括的なばく露モニタリング戦略を実施し、アンケートやバイオモニタ リングデータに裏付けられた作業ばく露マトリックスから収集された長期にわたるばく露決定要因に関する情報と 相まって、利益を得ることができる。ばく露評価モデルは、重要なばく露パラメータを特定することを可能にする HBM の研究によって包括的にサポートされることができる。そのような場合には、モデル内のパラメータの仮定を 調整することで、より現実的なばく露の評価につなげることができる。
- 9) エクスポソームの概念とメタボロミクスの使用は、特に、より良いばく露測定(ばく露のバイオマーカー)、脆弱な小 集団の特定、毒性経路の生物学的解釈(疾患のバイオマーカー)のための次世代の疫学研究に大きな可能性を 秘めている。
- 10) 農薬混合物へのばく露(及び毒性)に関する知識の向上は、包括的なリスク評価に有益である。共通の標的に作 EFSA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna 55

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10) Improved knowledge on exposure (and toxicity) to pesticide mixtures will be beneficial for comprehensive risk assessment. Consideration of the joint action of combined exposures to multiple pesticides acting on common targets, or eliciting similar adverse effects, is relevant for cumulative risk assessment. This requires all the components of the mixture to be known as well as an understanding of the MoA, dose-response characteristics and potential interactions between components. Characterisation of the exposure is a key element for combined exposure to multiple pesticides where the pattern and magnitude of exposure changes over time.

#### c) Adverse Outcomes (measurement, data transformation for reporting and statistical analysis):

- Self-reported health outcomes should be avoided or confirmed by independent, blinded assessment of disease status by a medical expert assigned to the study.
- 2) Outcomes under study should be well defined and surrogate endpoints should be avoided unless they have been validated. Care must be taken when definitions of diseases and subclasses of diseases change over time (cancer, neurodegenerative disorders, etc.).
- 3) Use should be made of biological markers of early biological effect to improve the understanding of the pathogenesis of diseases. These quantitative biological parameters from mechanistic toxicology will enhance the usefulness of epidemiology because they improve the study sensitivity, reduce misclassification and enhance human relevance as compared to findings from studies in experimental animals. Since these refined endpoints are early events in the toxicodynamic pathway and often measured on a continuous scale, they might be preferable to more overt and traditional outcomes.
- 4) The use of biomarkers of effect may be helpful in assessing aggregate exposure to pesticides and informing cumulative risk assessment.
- Developing read across methods allowing health outcomes to be identified using epidemiological studies and to link acute and chronic incidents records with experimental findings.

#### d) Statistical (descriptive statistics, modelling of exposure-effect relationship):

- Statistical analysis should be based on *a priori* defined analytical (statistical) protocols, to avoid post hoc analyses for exploratory studies and report all the results, regardless of whether they are statistically significant or not.
- 2) Data should be reported in such a way that permit, where appropriate, mathematical modelling to estimate individual/population exposures and dose-response assessment irrespective of whether direct or indirect measures are used.
- Reports should include both unadjusted and adjusted proportions and rates of outcome of interest across studies that are based on underlying populations with different structure of relevant factors and exposures.
- 4) Possible relevant factors, and their role in the exposure-health outcome relationship, should be carefully identified, accurately measured and thoroughly assessed. Most often, relevant factors have been screened as potential confounders. When confounding effects were detected, these needed to be adjusted for using appropriate statistical methods that include sensitivity analysis.
- 5) Potentially useful analytical approaches, such as propensity score matching, mediation analyses, and causal inference are encouraged to be applied in pesticide epidemiology.
- 6) When the association between a given pesticide exposure and a disease is found to be statistically significant, particularly in (presumed) low powered studies, it would be general good practice to perform a power analysis/design calculation to determine the degree to which the statistically significant effect size estimate (e.g. OR or RR) may be artificially inflated or magnified.<sup>19</sup>

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用する複数の農薬への複合ばく露の共同作用を考慮すること、または類似の有害作用を誘発することは、累積リ スク評価に関連している。そのためには、混合物の全成分を把握し、MOA、用量反応特性、成分間の潜在的な 相互作用を理解しておく必要がある。ばく露の特性を把握することは、ばく露のパターンや大きさが時間の経過と ともに変化する複数の農薬への複合ばく露において重要な要素である。

c) 有害な健康影響(測定、報告のためのデータ変換、統計解析)。

- 1)自己申告による健康上の影響は、研究に指定された医療専門家による病状の独立した盲検評価により、回避する か、または結論を出すべきである。
- 2)研究の対象となる健康影響は十分に定義されているべきであり、妥当性が確認されている場合を除き、代替エンドポイントは避けるべきである。疾患や疾患のサブクラスの定義が時間の経過とともに変化する場合には注意が必要である(がん、神経変性疾患など)。
- 3)早期の生物学的効果を示す生物学的マーカーを利用して、疾患の病態の理解を深めるべきである。これらの定量 的な生物学的パラメータは、実験動物を用いた研究から得られた結果と比較して、研究の感度を向上させ、誤分 類を減らし、ヒトへの関連性を高めることができるため、疫学の有用性を高めることができる。これらの再定義された エンドポイントは、毒性力学的経路における初期のイベントであり、連続的なスケールで測定されることが多いため、 よりあからさまな従来の健康影響よりも好ましいかもしれない。
- 4) 効果のバイオマーカーの使用は、農薬への総体的ばく露を評価し、累積的なリスク評価に役立てることができるか もしれない。
- 5)健康影響を疫学研究を用いて特定し、急性・慢性の事故記録と実験結果を結びつけることを可能にするリードアク ロス手法(read across methods)を開発する。
- d)統計(記述的統計、ばく露と影響の関係のモデル化)。
- 1)統計解析は、事前に定められた解析(統計)プロトコールに基づき、探索的研究のための事後的な解析を避け、統計的に有意であるかどうかにかかわらず、すべての結果を報告すべきである。
- 2)データは、適切な場合には、直接または間接的な測定法が使用されているかどうかにかかわらず、個人/集団のばく露と用量反応評価を推定するための数学的モデル化を可能にするような方法で報告されなければならない。
- 3)報告書には、関連因子やばく露の構造が異なる基礎となる集団に基づいた研究において、未調整と調整の両方の割合と、対象となる結果の割合と率を含めるべきである。
- 4)考えられる関連因子及びばく露と健康転帰の関係におけるそれらの役割は、慎重に同定され、正確に測定され、 徹底的に評価されるべきである。ほとんどの場合、関連因子は潜在的な交絡因子としてスクリーニングされている。 交絡因子が検出された場合には、感度分析を含む適切な統計的手法を用いて、交絡因子を調整する必要がある。
- 5) プロペンシティスコアマッチング (propensity score matching)、メディエーション解析 (mediation analyses)、因 果推論などの潜在的に有用な解析手法を農薬疫学に適用することが奨励されている。
- 6)対象とした農薬ばく露と疾患との間の関連が統計的に有意であることが判明した場合、特に(推定される)検出力の低い研究では、統計的に有意な効果の大きさの推定値(例えば、オッズ比 OR または相対リスク RR)が人為的に増大するか、または拡大するかの程度を決定するために、検出力解析/設計計算を実行することが一般的に良い対応とされている<sup>19</sup>。

<sup>&</sup>lt;sup>19</sup> Additional information on power and sample size recommendations and related issues including effect size magnification and design calculations are provided in Annex D to this report. Specifically, a power calculation requires 3 values to be clearly reported by epidemiological studies: (i) the number of subjects in the non-exposed group (including individuals with and without the disease of interest); (iii) the number of subjects in the non-exposed group.

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<sup>&</sup>lt;sup>19</sup>検出力とサンプルサイズの推奨事項及び効果量の算出とデザインの計算を含む関連問題に関する追加情報は、本報告書の附属書 D に記載されている。特に、検出力の計算では、疫学研究で明確に報告されるべき3つの値が必要である。(i)非ばく露群の被験者数(対象となる疾患の有無を含む)、(iii)ばく露群の被験者数(対象となる疾患の有無を含む)、(iii)非ばく露群の福患者数。

#### e) Reporting of results:

- 1) These should follow practices of good reporting of epidemiological research outlined in the STROBE statement and in the EFSA guideline on statistical reporting (EFSA, 2014b) and include the further suggestions identified in this Opinion including effect size inflation estimates.
- 2) Although some epidemiological research will remain exploratory and post hoc in nature, this should be acknowledged and supported by appropriate statistical analysis.
- 3) Epidemiological studies are encouraged to provide access to raw data for further investigations and to deposit their full results and scripts or software packages used for analyses.
- 4) Report, or deposit using online sources, all results along with scripts and statistical tools used to allow the reproducibility of results to be tested.
- 5) Report all sources of funding and adequately report financial and other potential conflicts of interest.

As a general recommendation, the PPR Panel encourages development of guidance for epidemiological research in order to increase its value, transparency and accountability for risk assessment.<sup>20</sup> An increased quality of epidemiological studies, together with responsible research conduct and scientific integrity, will benefit the incorporation of these studies into risk assessment.

#### 8.2. Surveillance

- 1) Increase the reporting of acute and chronic incidents by setting up post-marketing surveillance programmes (occupational and general population) as required by article 7 of EU directive 2009/128; this should be fulfilled by developing surveillance networks with occupational health physicians and by boosting the collaboration between national authorities dealing with PPP and poison control information centres.
- 2) Develop a valid method for assessing the weight/strength of the causal relationship ('imputability') for acute and chronic incidents, and develop glossaries and a thesaurus to support harmonised reporting between EU member states.
- 3) Harmonised data from member states should be gathered at the EU level and examined periodically by the Commission/EFSA and a report should be released focussing on the most relevant findings.
- 4) Develop an EU-wide vigilance framework for pesticides.
- 5) There is scope for training improvements regarding pesticide toxidromes in toxicology courses for medical and paramedical staff responsible for diagnostic decisions, data entry and management.
- 8.3. Meta-analysis of multiple epidemiological studies
  - 1) Evidence from epidemiological studies might be pooled by taking into account a thorough evaluation of the methods and biases of individual studies, an assessment of the degree of heterogeneity among studies, development of explanations underlying any heterogeneity and a quantitative summary of the evidence (provided that it is consistent).
  - 2) For every evidence synthesis effort, studies should be reviewed using relevant risk of bias tools. Studies with different designs, or with different design features, may require (some) different questions for risk of bias assessments.
  - 3) Evidence syntheses should not be restricted to specific time frames; they should include the totality of evidence. These efforts are more relevant if focused on specific health outcome or disease categories.
  - 4) In evidence synthesis efforts, beyond the quantitative synthesis of the effect sizes, there should be consideration on the calculated predictive intervals, small study effects and asymmetry bias, conflicts of interest, confounding, excess significance bias,<sup>21</sup> and heterogeneity estimates.

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#### e)結果の報告

- 1)結果報告は、STROBE 声明及び統計報告に関する EFSA ガイドライン (EFSA, 2014 年 b) に概説されている疫 学研究の良好な報告の慣行に従うべきであり、効果量の推定値を含む本意見書で指摘されている更なる提案を 含むべきである。
- 2)いくつかの疫学研究は探索的で事後的な特性を持つものもあるがが、そのことを認識し、適切な統計解析によっ て裏付けられるべきである。
- 3) 疫学研究は、さらなる調査のために生データへのアクセスを提供し、その全結果と解析に使用したスクリプトやソフ トウェアパッケージを提供することが奨励されている。
- 4)結果の再現性を検証するために使用したスクリプトや統計ツールとともに、すべての結果を報告するか、またはオ ンライン源を利用して寄託する。
- 5) すべての資金源を報告し、財務上の問題やその他の潜在的な利害関係者を適切に報告する。

一般的な勧告として、PPR パネルは、リスク評価における疫学研究の価値、透明性、説明責任を高めるために、疫 学研究のためのガイダンスの開発を奨励している20。 疫学研究の質の向上は、責任ある研究の実施と科学的誠実さと ともに、リスク評価にこれらの研究を組み入れることに利益をもたらすであろう。

# 8.2. サーベイランス

- 1) EU 指令 2009/128 の第7 条で要求されている市販後サーベイランスプログラム(産業用及び一般集団)を設定 することにより、急性及び慢性の事故の報告を増加させる。これは、産業保健医とのサーベイランスネットワークを 構築し、PPPを扱う国の当局と毒物管理情報センターとの連携を強化することにより達成すべきである。
- 2) 急性・慢性事故の因果関係の程度/強度(「推定可能性」)を評価するための有効な方法を開発し、EU 加盟国 間の整合化された報告を支援するための用語集と類義語集を開発する。
- 3) EU 加盟国からの整合化されたデータを EU レベルで収集し、欧州委員会/EFSA が定期的に検討し、最も関 連性の高い結果に焦点を当てた報告書を発表すべきである。
- 4) 農薬に関する EU 全体の警戒体制を構築する。
- 5)診断決定、データ入力、管理を担当する医療・救急スタッフのための毒物学コースにおいて、農薬のトキシドロー ムに関する研修を改善する余地がある。

# 8.3. 複数の疫学研究のメタアナリシス

- 1) 個々の研究の方法とバイアスの徹底的な評価、研究間の異質性の程度の評価、異質性の根底にある説明の展開、 エビデンスの定量的な要約(一貫性があれば)を考慮に入れて、疫学研究からのエビデンスを蓄積することができ る。
- 2) すべてのエビデンス統合作業において、関連するバイアスのリスクツールを用いて研究をレビューすべきである。 計画が異なる研究や計画の特徴が異なる研究では、バイアスのリスク評価のために(いくつかの)異なる課題が必 要になるかもしれない。
- 3) エビデンス統合は、特定の期間に限定されるべきではなく、エビデンス全体を含めるべきである。これらの努力は、 特定の健康影響または疾患カテゴリーに焦点を当てれば、より適切である。
- 4) エビデンス統合の取り組みでは、効果量の定量的な統合に加えて、計算された予測間隔、小試験効果と非対称 性バイアス、対象となる対角線、交絡、過剰な有意性バイアス21及び不均一性の推定値についても考慮すべきで ある。

<sup>&</sup>lt;sup>20</sup> An example is the guideline developed by the Dutch Society for Epidemiology on responsible epidemiologic Research Practice (2017).

<sup>&</sup>lt;sup>21</sup> Excess significance bias refers to the situation in which there are too many studies with statistically significant results in the published literature on a particular outcome. This pattern suggests strong biases in the literature, with publication bias, selective outcome reporting, selective analyses reporting, or fabricated data being possible explanations (Ioannidis and Trikalinos, 2007).

<sup>20</sup> 例として、オランダ疫学協会が責任ある疫学的研究実践に関するガイドラインを作成した(2017年)

<sup>21</sup> 過剰シグニフィカンスバイアスとは、特定のアウトカムに関する公表されている文献の中に、統計的に有意な結果が得られた研究が多すぎる状況 を指す。このパターンは、出版バイアスを伴う文献の強いバイアスを示唆している。 選択的な結果報告、選択的な分析報告、または捏造されたデータが説明の対象となる可能性がある(Ioannidis and Trikalinos、2007年)。

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- 5) In the presence of heterogeneity, studies with highly selected populations, albeit unrepresentative of their respective populations, may prove valuable and deserve consideration as they may represent genuine and not statistical heterogeneity.
- 6) A more consistent reporting such as for age, race and gender across studies would enhance the meta-analyses.
- 7) Where quantitative data of individual pesticides are available from epidemiological studies, they can be combined or pooled for dose–response modelling, which could enable development of quantitative risk estimates and points of departure (BMDL, NOAEL).
- International consortium of cohort studies should be encouraged to support data pooling to study disease-exposure associations that individual cohorts do not have sufficient statistical power to study (e.g. AGRICOH).
- 8.4. Integration of epidemiological evidence with other sources of information
  - 1) All lines of evidence (epidemiology, animal, *in vitro* data) should be equally scrutinised for biases.
  - Validated and harmonised methods should be developed to combine observational studies, animal/basic science studies and other sources of evidence for risk assessment.
  - Experimental and human data should both contribute to hazard identification and to doseresponse assessment.
  - 4) A systematic integration of data from multiple lines of evidence should be based on a WoE analysis accounting for relevance, consistency and biological plausibility using modified Bradford Hill criteria. The principles underlying this framework are described in Section 7.2 and summarised in Figure 5.
  - 5) Epidemiological findings should be integrated with other sources of information (data from experimental toxicology, mechanism of action/AOP) by using a WoE approach. An integrated and harmonised approach should be developed by bringing together animal, mechanistic and human data in an overall WoE framework in a systematic and consistent manner.
  - 6) The AOP framework offers a structured platform for the integration of various kinds of research results.
  - 7) Animal, *in vitro* data and human data should be assessed as a whole for each endpoint. A conclusion can be drawn as to whether the results from the experiments are confirmed by human data for each endpoint and this could be included in the RARs.

# 9. Conclusions

This Scientific Opinion is intended to help the peer review process during the renewal of pesticides authorisation (and, where possible, during the approval process) under Regulation 1107/2009 which requires a search of the scientific peer-reviewed open literature, including existing epidemiological studies. These are more suitable for the renewal process of active substances, also in compliance with Regulation 1141/2010, which indicates that the dossiers submitted for renewal should include new data relevant to the active substance.

The four key elements of the terms of reference are repeated below and the parts of the text addressing the individual terms are identified in order. As they follow from the text passages grouped with each of the ToRs the recommendations relevant to each of the ToRs are also indicated as follows.

'The PPR Panel will discuss the associations between pesticide exposure and human health effects observed in the External scientific report (Ntzani et al., 2013) and how these findings could be interpreted in a regulatory pesticide risk assessment context. Hence, the PPR Panel will systematically assess the epidemiological studies collected in the report by addressing major data gaps and limitations of the studies and provide recommendations thereof'.

'The PPR Panel will specifically':

- Collect and review all sources of gaps and limitations, based on (but not necessarily limited to) those identified in the External Scientific report in regard to the quality and relevance of the available epidemiological studies. Responses in Section 3 pp. 20–24, Section 5.2 pp. 33–35: no Recommendations appropriate.
- Based on the gaps and limitations identified in point 1, propose potential refinements for future epidemiological studies to increase the quality, relevance and reliability of the findings

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- 5) 不均質性が存在する場合、高度に選択された母集団を用いた研究は、それぞれの母集団を代表するものではないが、統計的な不均質性ではなく真の不均質性を示すものである可能性があるため、価値があることが証明され、 検討に値する。
- 6)年齢、人種、性別など、研究間での一貫性のある報告があれば、メタアナリシスがより充実すると思われる。
- 7)個々の農薬の定量的データが疫学研究から得られる場合には、それらのデータを組み合わせたり、プールして用 量反応モデリングを行うことで、定量的なリスク推定値や Point of Departure (BMDL、NOAEL)の開発が可能 となる。
- 8)個々のコホートでは十分な統計的力がない疾病ばく露関連を研究するために、コホート研究の国際的なコンソーシアムがデータプールを支援するよう奨励されるべきである(例:AGRICOH)。

# 8.4. 疫学的証拠と他の情報源との統合

すべてのエビデンス(疫学、動物、試験管内データ)は、バイアスがなく、平等に精査されるべきである。
 リスク評価のために、観察による研究、動物/基礎科学研究、その他のエビデンス源を組み合わせるために、有効かつ調和のとれた方法を開発すべきである。

- 3)実験データとヒトデータの両方が、ハザードの特定と用量反応評価に寄与するべきである。
- 4)複数のエビデンスから得られたデータを体系的に統合することは、Bradford Hill 基準を修正したものを用いて、 関連性、一貫性、生物学的妥当性を考慮した WOE 解析に基づくべきである。この枠組みの基礎となる原則は 7.2 節に記載され、図5 に要約されている。
- 5) WOE アプローチを用いて、疫学的知見を他の情報源(実験毒性学からのデータ、作用機序/AOP)と統合すべ きである。統合された調和のとれたアプローチは、体系的かつ一貫した方法で WOE の全体的な枠組みの中で動 物、メカニズム、ヒトのデータをまとめることによって開発されなければならない。
- 6) AOP フレームワークは、様々な種類の研究成果を統合するための構造化されたプラットフォームを提供する。
- 7)動物データ、in vitro データ、ヒトデータは、それぞれのエンドポイントについて全体として評価されるべきである。 実験から得られた結果が各エンドポイントのヒトのデータと一致しているかどうかの結論を導き出すことができ、これ を RARs に含めることができる。

# 9. 結論

本意見書は、既存の疫学研究を含む科学的に査読された公表文献の検索を要求する規制 1107/2009 の下で、農 薬の認可更新時(可能であれば認可プロセス時)の査読プロセスを支援することを目的としている。これらは有効成分 の更新プロセスに適しており、更新のために提出される書類には有効成分に関連する新しいデータを含める必要があ ることを示す規則 1141/2010 にも準拠している。

以下では、参照条件の4つの重要な要素を繰り返し、個々の用語に対応する部分を順番に示している。それぞれの ToRs でグループ化された文章から導かれるように、それぞれのToR に関連する推奨事項も以下のように示す。

「PPR パネルは、外部科学報告書 (Ntzani ら、2013 年)で観察された農薬ばく露とたの健康影響との関連性と、こ れらの知見が規制上の農薬リスク評価の文脈でどのように解釈され得るかを検討する。したがって、PPR パネルは、報 告書で収集された疫学研究を体系的に評価し、研究の主要なデータギャップと限界に対処し、その提言を行う」。 PPR パネルは特に以下を行う。

- 1)利用可能な疫学研究の質と妥当性に関して外部科学報告書で明らかにされたものに基づいて(必ずしもこれに限 定されないが)ギャップと限界のすべての情報源を収集し、レビューする。セクション 3、20-24 頁、セクション 5.2 33-35頁:勧告は適切ではない。
- 2)ポイント1で明らかになったギャップと限界に基づき、調査結果の質、妥当性、信頼性を向上させ、それが農薬リスク評価にどのように影響を与えるかについて、将来の疫学的研究のための潜在的な改善点を提案する。これには、研究デザイン、ばく露評価、データの質とアクセス、健康影響の診断分類、統計解析が含まれる。(セクション 4の

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and how they may impact pesticide risk assessment. This may include study design, exposure assessment, data quality and access, diagnostic classification of health outcomes, and statistical analysis. Responses in Section 4 pp 24–33: recommendations in Sections 8.1, 8.2 and 8.3 pp. 54–58.

- 3) Identify areas in which information and/or criteria are insufficient or lacking and propose recommendations for how to conduct pesticide epidemiological studies in order to improve and optimise the application in risk assessment. These recommendations should include harmonisation of exposure assessment (including use of biomonitoring data), vulnerable population sub-groups and/or health outcomes of interest (at biochemical, functional, morphological and clinical level) based on the gaps and limitations identified in point 1. Responses in Sections 4.2–4.5 pp. 27–33, Section 5.3 pp. 36: recommendations in Section 8.1 c) 1–4, pp. 56.
- 4) Discuss how to make appropriate use of epidemiological findings in risk assessment of pesticides during the peer review process of draft assessment reports, e.g. WoE as well as integrating the epidemiological information with data from experimental toxicology, AOPs, mechanism of actions, etc. Responses in Sections 6.2 and 6.3 pp. 37–45 and 7 pp. 45–54: Responses in Section 8.4 pp. 58.

As explained above, appropriate epidemiological data and post-approval surveillance may usefully contribute to the risk assessment framework by hazard identification, and – with methodological improvements – hazard characterisation. It can be improved by contributions from WoE analysis, Uncertainty analysis, and identification and estimation of biases. It is the responsibility of applicants to collect the available relevant literature, to consider its relevance and quality using relevant EFSA criteria including those for systematic review and to introduce discussion of the outcomes within the DAR, RAR and post-approval frameworks that are prescribed under EU law.

The definition of appropriate quality will require analysis of sample size, statistical procedures, estimates of effect size inflation, assessment of biases and their contribution to the conclusions drawn.

The nature of the studies will require consideration at all relevant points in the risk assessment process so that for example epidemiological data on reproductive topics will be considered alongside laboratory animal studies designed to reveal reproductive effects and in the context of recommendation for labelling for reproductive toxicity (for ECHA).

Unless there is history of use in countries outside the EU, the relevant epidemiological studies will be restricted in their effect on the DAR but the RAR and Surveillance framework is potentially able to benefit from epidemiology progressively as time after first approval passes and from prior use of Active Ingredients in other jurisdictions. It is recommended that RAR and surveillance protocols should reflect this difference.

The specific recommendations listed above follow from detailed arguments based on an analysis of present and foreseen **s**trengths **w**eaknesses **o**pportunities and **th**reats related to the use of epidemiological data in risk assessment. Broadly these are as follows:

#### Strengths. Include:

- The fact that the evidence concerns human specific risks.
- That health outcomes are integrated measures of the effects of all exposure to toxins.
- The ability to elicit subjective experience from potentially affected people.

#### Weaknesses. Include:

- The exposures to pesticides are usually complex; contribution of a specific active ingredient is not easily deciphered.
- · The exposures occur in various settings where precisely controlled conditions are lacking.
- Most data reflect the responses of mixed populations.
- Many data show low level associations that are inconsistently repeatable and require sophisticated analysis.

**Opportunities**. Despite the range of limitations described in this Opinion, which apply to many available published epidemiological studies, there are opportunities to benefit risk assessment of pesticides. These include:

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 The access to very large numbers of potentially exposed individuals for studies that may reveal subtle health effects and reveal the experience of sensitive sub-groups.

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回答 24-33 頁: セクション 8.1、8.2 及び 8.3 の推奨事項 54-58 頁。54-58)

3)情報及び/または基準が不十分または不足している分野を特定し、リスク評価における適用を改善し最適化する ために、農薬疫学的研究をどのように実施するかについての提言を行う。これらの推奨事項には、第1項で特定 されたギャップと限界に基づいて、ばく露評価(バイオモニタリングデータの使用を含む)、脆弱な集団のサブグル

ープ及び/または対象となる健康影響(生化学的、機能的、形態学的、臨床的レベルでの)の調和を含むべきで ある。(セクション 4.2-4.5 の回答 27-33 頁、セクション 5.3 36 頁:セクション 8.1 c) 1-4、56 頁の推奨事項)

4) WOE などの評価報告書草案のピアレビュープロセスにおいて、疫学的情報を実験毒性学、AOP、作用機序などのデータと統合しながら、農薬のリスク評価に疫学的知見を適切に活用する方法について議論する。(6.2 及び)

6.3節の回答 37-45 頁、7節 45-54 頁:8.4節の回答 58頁)

上記で説明したように、適切な疫学的データと承認後のサーベイランスは、ハザードの特定や、方法論の改善により ハザードの特徴を明らかにすることで、リスク評価の枠組みに有用に貢献することができる。WOE 解析、不確実性分析、 バイアスの同定と推定により改善することができる。利用可能な関連文献を収集し、システマティックレビューを含む関 連する EFSA 基準を用いてその妥当性と質を検討し、EU 法で規定されている DAR、RAR、承認後の枠組みの中で 結果の議論を導入することは申請者の責任である。

適切な品質の定義には、サンプルサイズの解析、統計的手続き、効果の大きさの推定値の浮動、バイアスの評価、 導き出された結論への寄与が必要である。研究の特性は、リスク評価プロセスのすべての関連ポイントで考慮する必要 があり、例えば、生殖に関する疫学的データは、生殖影響を明らかにするために計画された実験動物研究と一緒に考 慮され、生殖毒性(ECHAの場合)のための表示勧告の背景で考慮される。

EU 域外の国での使用実績がない限り、関連する疫学的研究は、DAR への影響が制限されるが、RAR とサーベイ ランスの枠組みは、最初の承認後の時間が経つにつれて、また他の法域での原薬の先行使用がある場合には、段階 的に疫学から恩恵を受けることができる可能性がある。RAR とサーベイランスのプロトコールはこの違いを反映させるこ とが推奨される。

上記の提言は、リスク評価における疫学的データの利用に関連した現在及び将来の強み・弱み・機会・脅威の解析 に基づく詳細な論拠に基づくものである。大まかには以下の通りである。

# 強み:

- ・その証拠は人間の特定のリスクに関するものであること。
- ・健康影響は、毒素へのすべてのばく露の影響を総合的に測定するものであること。
- 影響を受ける可能性のある人々から主観的な経験を引き出すことができること。

#### 弱み:

- ・農薬へのばく露は通常複雑である;特定の有効成分の寄与は容易に解読されない。
- ・ばく露は、正確に管理された条件が不足している様々な設定で発生する。
- ほとんどのデータは混合集団の反応を再現している。
- 多くのデータは低レベルの関連性を示しており、再現性がなく、高度な解析を必要とする。

機会:本意見書に記載されている限界は、公表されている多くの疫学研究に適用されるが、農薬のリスク評価に利益 をもたらす機会がある。これには以下が含まれる。

- ・軽微な健康影響を明らかにし、敏感な小集団の経験を明らかにする可能性のある研究のために、非常に多くの潜 在的にばく露された個人にアクセスできること。
- 潜在的な毒素とその残留物の組織負荷を確立するためのバイオモニタリングと新しい分子アプローチを用いたば く露推定の改善の見通し。
- 実験動物の反応に基づく従来のリスク評価にとトのデータを完全に統合する可能性。
- WOE, AOP, Expert judgement, Expert Knowledge Elicitation(EEKE)及び Uncertainty Analysis を利 用して、潜在的に関連性のあるデータの質の違いを評価する。
- ・専門の疫学者や統計学者と協力して、疫学的結果の解釈を再検討し、慢性ばく露リスクや複合ばく露リスク、用量 www.efsa.europa.eu/efsajourna
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- The prospect of improving exposure estimation using biomonitoring and new molecular approaches to establish tissue burdens of potential toxins and their residues.
- The possibility of fully integrating human data into the conventional risk assessment based on responses in laboratory animals.
- Utilising WoE, AOP, Expert judgement, Expert Knowledge Elicitation (EKE) and Uncertainty Analysis to evaluate differences in the quality of potentially relevant data.
- The opportunity to engage professional epidemiologists and statisticians to refine interpretation
  of epidemiological findings and to recommend improved designs to tackle difficult areas such
  as chronic and combined exposure risks and dose–response data.
- A major information technology opportunity exists in pooling data from a variety of national sources. Once the relevant legal, methodological and ethical issues are overcome much more valuable data can be collected. When this data is made available, in a form that can be used in a 'big data' setting for societal benefit there will be potential for significant improvements in epidemiological studies. First, however, it will be necessary to preserve individual privacy and essential commercial confidentiality. Once these obstacles are overcome the statistical power of epidemiological studies can be improved and applied to identify and possibly characterise hazards better. These aims can be realised effectively by agreed actions at a high EU level. Interstate approval for providing data and interactive platforms will need to be backed by harmonisation of population health information, food consumption data, active substance and co-formulant spatial and temporal application data. Such rich data can be expected to assist in increasing consistency, a criterion that strengthens evidence of causality and reliability. It promises larger sample sizes for epidemiological studies that will be better able to identify vulnerable groups that may require special protection from pesticide toxicity.

#### Threats. Include:

- Widespread perception of risk levels to the human population or to wildlife and the environment that are unrealistic and that cause negative consequences in societies.
- Poor experimental design yielding false positive or false negative conclusions that undermine data from other valid sources.
- Failure to respond to emerging risks as a result of ineffective surveillance or unwillingness to make appropriate anonymised data available for societal benefit.
- Waste of data through failure to collect appropriate information regarding exposure (specifically occupational exposure) by registries (cancer or congenital anomalies) or surveillance programmes which hinders linking health outcomes to exposure.
- Waste of data through failure to harmonise diagnostic criteria, failure to record data in a sufficiently detailed combinable form for integrated analysis, poor training of medical and paramedical staff in relevant toxidromes that will allow optimum quality of data entered into Health Statistics Databases.

# References

- Adami HO, Berry SC, Breckenridge CB, Smith LL, Swenberg JA, Trichopoulos D, Weiss NS and Pastoor TP, 2011. Toxicology and epidemiology: improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. Toxicology Sciences, 122, 223–234.
- Amler RW, Barone Jr S, Belger A, Berlin Jr CM, Cox C, Frank H, Goodman M, Harry J, Hooper SR, Ladda R, LaKind JS, Lipkin PH, Lipsitt LP, Lorber MN, Myers G, Mason AM, Needham LL, Sonawane B, Wachs TD and Yager JW, 2006. Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical exposure. Neurotoxicology, 27, 861–874.
- Bengtson AM, Westreich D, Musonda P, Pettifor A, Chibwesha C, Chi BH, Vwalika B, Pence BW, Stringer JS and Miller WC, 2016. Multiple overimputation to address missing data and measurement error: application to HIV treatment during pregnancy and pregnancy outcomes. Epidemiology, 27, 642–650.
- Bevan R, Brown T, Matthies F, Sams C, Jones K, Hanlon J and La Vedrine M, 2017. Human Biomonitoring data collection from occupational exposure to pesticides. EFSA supporting publication 2017:EN-1185, 207 pp.
- Bottai M, 2014. Lessons in biostatistics: inferences and conjectures about average and conditional treatment effects in randomized trials and observational studies. Journal of Internal Medicine, 276, 229–237.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2001. Benchmark dose calculation from epidemiological data. Biometrics, 57, 698–706.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2004. Effects of exposure imprecision on estimation of the benchmark dose. Risk Analysis, 24, 1689–1696.

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#### Epidemiological studies and pesticides

反応データなどの困難な分野に取り組むための改善案を提案する機会がある。

・様々な国の情報源からのデータを蓄積することには、大きな情報技術の機会が存在する。関連する法的、方法論 的、倫理的な問題が克服されれば、より多くの価値あるデータを収集することができる。このデータが社会的利益 のために「ビッグデータ」の設定で使用できる形で利用できるようになれば、疫学研究を大幅に改善できる可能性 がある。しかし、第一に、個人のプライバシーと本質的な商業的な機密性を守る必要がある。これらの障害が克服 されれば、疫学研究の統計的な力を向上させ、ハザードをより良く特定し、場合によっては特徴づけるために応用 することができる。これらの目的は、EUの高いレベルで合意された行動によって効果的に実現することができる。 データとインタラクティブなブラットフォームを提供するための相互間承認は、集団の健康情報、食品消費データ、 有効成分と共調合製剤の空間的・時間的な適用データの調和によって裏付けられる必要がある。このような豊富 なデータは、因果関係と信頼性のエビデンスを強化する基準である一貫性の向上を支援することが期待できる。そ れは、農薬毒性からの特別な保護を必要とするかもしれない脆弱なグループをよりよく特定できるようになる疫学研 究のためのより大きなサンプルサイズを約束している。

## 脅威:

- 非現実的であり、社会に否定的な結果をもたらすとト集団や野生生物、環境に対するリスクレベルの認識が広まっていること。
- ・他の有効な情報源からのデータを損なうような、誤った陽性または誤った陰性の結論をもたらす不十分な実験計 画。
- 効果的なサーベイランスが行われていなかったり、匿名化された適切なデータを社会的利益のために利用できる ようにする気がなかったりした結果、新たなリスクに対応できなかったこと。
- ・登録(がんや先天性異常)やサーベイランスプログラムによるばく露(特に職業上ばく露)に関する適切な情報収集の失敗によるデータの浪費。
- ・診断基準の調和の失敗、統合解析のための十分に詳細な組み合わせ可能な形でデータを記録しなかったことに よるデータの浪費、健康統計データベースに入力されるデータの最適な品質を可能にするための関連するトキシ ドロームについての医療や救急医療スタッフのトレーニング不足。

# 参考文献

- Adami HO, Berry SC, Breckenridge CB, Smith LL, Swenberg JA, Trichopoulos D, Weiss NS and Pastoor TP, 2011. Toxicology and epidemiology: improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. Toxicology Sciences, 122, 223-234.
- Amler RW, Barone Jr S, Belger A, Berlin Jr CM, Cox C, Frank H, Goodman M, Harry J, Hooper SR, Ladda R, LaKind JS, Lipkin PH, Lipsitt LP, Lorber MN, Myers G, Mason AM, Needham LL, Sonawane B, Wachs TD and Yager JW, 2006. Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical exposure. Neurotoxicology, 27, 861–874.
- Bengtson AM, Westreich D, Musonda P, Pettifor A, Chibwesha C, Chi BH, Vwalika B, Pence BW, Stringer JS and Miller WC, 2016. Multiple overimputation to address missing data and measurement error: application to HIV treatment during pregnancy and pregnancy outcomes. Epidemiology, 27, 642–650.
- Bevan R, Brown T, Matthies F, Sams C, Jones K, Hanlon J and La Vedrine M, 2017. Human Biomonitoring data collection from occupational exposure to pesticides. EFSA supporting publication 2017: EN-1185, 207 pp.
- 207 pp. Bottai M, 2014. Lessons in biostatistics: inferences and conjectures about average and conditional treatment effects in randomized trials and observational studies. Journal of Internal Medicine, 276, 229-237.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2001. Benchmark dose calculation from epidemiological data. Biometrics, 57, 698-706.
- Budtz-Jørgensen E, Keiding N and Grandjean P, 2004. Effects of exposure imprecision on estimation of the benchmark dose. Risk Analysis, 24, 1689-1696.
- Buonsante VA, Muilerman H, Santos T, Robinson C and Tweedale AC, 2014. Risk assessment's insensitive toxicity testing may cause it to fail. Environmental Research, 135, 139–147.
- Burton PR, Fortier I and Knoppers BM, 2010. The global emergence of epidemiological biobanks: opportunities and challenges. In: Khoury M, Bedrosian S, Gwinn M, Higgins J, Ioannidis J and Little J (eds.). Human Genome Epidemiology. Building the evidence for using genetic information to improve

- Buonsante VA, Muilerman H, Santos T, Robinson C and Tweedale AC, 2014. Risk assessment's insensitive toxicity testing may cause it to fail. Environmental Research, 135, 139-147.
- Burton PR, Fortier I and Knoppers BM, 2010. The global emergence of epidemiological biobanks: opportunities and challenges. In: Khoury M, Bedrosian S, Gwinn M, Higgins J, Ioannidis J and Little J (eds.). Human Genome Epidemiology. Building the evidence for using genetic information to improve health and prevent disease. 2nd Edition, Oxford University Press, Oxford, pp. 77–99.
- Choi J, Polcher A and Joas A, 2016. Systematic literature review on Parkinson's disease and Childhood Leukaemia and mode of actions for pesticides. EFSA supporting publication 2016:EN-955, 256 pp. Available online: http:// www.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-955/pdf
- Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Beane Freeman LE, Blair A, Sandler DP and Alavanja MC, 2011. An updated algorithm for estimation of pesticide exposure intensity in the agricultural health study. International Journal of Environmental Research and Public Health, 8, 4608–4622.
- Coggon D, 1995. Questionnaire based exposure assessment methods. Science of the Total Environment, 168, 175-178.
- Cornelis C, Schoeters G, Kellen E, Buntinx F and Zeegers M, 2009. Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case-control study on bladder cancer. International Journal of Hygiene and Environmental Health, 212, 172-185.
- la Cour JL, Brok J and Gøtzsche PC, 2010. Inconsistent reporting of surrogate outcomes in randomised clinical trials: cohort study. BMJ, 341, c3653.
- DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS and Yucesoy B, 2015. Systems biology and biomarkers of early effects for occupational exposure limit setting. The Journal of Occupational and Environmental Hygiene, 12(Suppl 1), S41-S54.
- Dionisio KL, Chang HH and Baxter LK, 2016. A simulation study to quantify the impacts of exposure measurement error on air pollution health risk estimates in copollutant time-series models. Environmental Health, 15, 114.
- DSE (Dutch Society for Epidemiology), 2017. Responsible Epidemiologic Research Practice (RERP). A quideline developed by the RERP working group of the Dutch Society for Epidemiology, 2017 (available at https:// www.epidemiologie.nl/home.html, https://epidemiologie.nl/fileadmin/Media/docs/Onderzoek/Responsible\_Epide miologic Research Practice.2017.pdf)
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2009, Framework for the Integration of Human and Animal Data in Chemical Risk Assessment. Technical Report No. 104. Brussels. Available online: http://www.ecetoc.org/uploads/Publications/documents/TR%20104.pdf
- ECHA/EFSA, 2014. Workshop on Mode of action and Human relevance framework in the context of classification and labelling (CLH) and regulatory assessment of biocides and pesticides. November 2014. Available online: https://echa.europa.eu/documents/10162/22816050/moaws\_workshop\_proceedings\_en.pdf/a656803e-4d97-438f-87ff-fc984cfe4836
- EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids. EFSA Journal 2004;81, 1-49 pp. https://doi. org/10.2903/j.efsa.2004.81
- EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;980, 1-139 pp. https:// doi.org/10.2903/i.efsa.2009.980
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2009b. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
- EFSA (European Food Safety Authority), 2010a. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/ i.efsa.2010.1637
- EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM), 2010b. Scientific Opinion on Lead in Food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
- EFSA (European Food Safety Authority), 2011a. Submission of scientific-peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092
- EFSA (European Food Safety Authority), 2011b. Statistical significance and biological relevance. EFSA Journal 2011;9(9):2372, 17 pp. https://doi.org/10.2903/j.efsa.2011.2372
- EFSA (European Food Safety Authority), 2012a, Scientific Opinion on risk assessment terminology, EFSA Journal 2012;10(5):2664, 43 pp. https://www.efsa.europa.eu/en/efsajournal/pub/2664
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2012b. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985
- EFSA (European Food Safety Authority), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293

#### Epidemiological studies and pesticides

- health and prevent disease. 2nd Edition, Oxford University Press, Oxford. pp. 77-99. Choi J, Polcher A and Joas A, 2016. Systematic literature review on Parkinson's disease and Childhood Leukaemia and mode of actions for pesticides. EFSA supporting publication 2016:EN-955, 256 pp. Available online: http:// www.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-955/pdf
- Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Beane Freeman LE, Blair A. Sandler DP and Alavania MC, 2011, An updated algorithm for estimation of pesticide exposure intensity in the agricultural health study. International Journal of Environmental Research and Public Health, 8, 4608-4622.
- Coggon D, 1995. Questionnaire based exposure assessment methods. Science of the Total Environment, 168, 175-178.
- Cornelis C, Schoeters G, Kellen E, Buntinx F and Zeegers M, 2009. Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case-control study on bladder
- cancer. International Journal of Hygiene and Environmental Health, 212, 172-185. la Cour JL, Brok J and Gøtzsche PC, 2010. Inconsistent reporting of surrogate outcomes in randomised clinical trials: cohort study. BMJ, 341, c3653.
- DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS and Yucesoy B, 2015. Systems biology and biomarkers of early effects for occupational exposure limit setting. The Journal of Occupational and Environmental Hygiene, 12(Suppl 1), \$41-S54.
- Dionisio KL, Chang HH and Baxter LK, 2016. A simulation study to quantify the impacts of exposure measurement error on air pollution health risk estimates in copollutant time series models. Environmental Health, 15, 114.
- DSE (Dutch Society for Epidemiology), 2017. Responsible Epidemiologic Research Practice (RERP). A guideline developed by the RERP working group of the Dutch Society for Epidemiology, 2017 (available at https:// www.epidemiologie.nl/home.html, https://epidemiologie.nl/fileadmin/Media/docs/ Onderzoek/ Responsible Epide miologic Research Practice.2017.pdf)
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), 2009. Framework for the Integration of Human and Animal Data in Chemical Risk Assessment. Technical Report No. 104. Brussels. Available online: http://www.ecetoc.org/uploads/Publications/documents/TR%20104.pdf
- ECHA/EFSA, 2014. Workshop on Mode of action and Human relevance framework in the context of classification and labelling (CLH) and regulatory assessment of biocides and pesticides. November 2014, Available online: https://echa.europa.eu/documents/10162/22816050/moaws\_workshop\_proceedings\_en.pdf/ a656803e-4d97-438f-87ff-fc984cfe4836
- EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of trans fatty acids in foods and the effect on human health of the consumption of trans fatty acids. EFSA Journal 2004;81, 1-49 pp. https://doi. org/10.2903/j.efsa.2004.81
- EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;980, 1-139 pp. https:// doi.org/10.2903/j.efsa.2009.980
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2009b. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
- EFSA (European Food Safety Authority), 2010a. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637
- EFSA (European Food Safety Authority) Panel on Contaminants in the Food Chain (CONTAM), 2010b. Scientific Opinion on Lead in Food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
- EFSA (European Food Safety Authority), 2011a. Submission of scientific peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal
- 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092 EFSA (European Food Safety Authority), 2011b. Statistical significance and biological relevance. EFSA Journal 2011;9(9):2372, 17 pp. https://doi.org/10.2903/j.efsa.2011.2372
- EFSA (European Food Safety Authority), 2012a. Scientific Opinion on risk assessment terminology. EFSA Journal 2012;10(5):2664, 43 pp. https://www.efsa.europa.eu/en/efsajournal/pub/2664
- EFSA (European Food Safety Authority Panel on Contaminants in the Food Chain CONTAM), 2012b. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985
- EFSA (European Food Safety Authority), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293 EFSA (European Food Safety Authority), 2013b. Scientific Opinion on the relevance of dissimilar mode of
- action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12):3472, 40 pp. https://doi.org/10.2903/j.efsa.2013.3472
- EFSA (European Food Safety Authority), 2014a, Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos. EFSA Journal 2014;12(4):3640, 34 pp. https://doi.org/ 10.2903/j.efsa.2014.3640
- EFSA (European Food Safety Authority), 2014b. Guidance on statistical reporting. EFSA Journal 2014;12(12): 3908, 18 pp. https://doi.org/10.2903/j.efsa.2014.3908
- EFSA (European Food Safety Authority), 2015a. Stakeholder Workshop on the use of epidemiological data

www.efsa.europa.eu/efsajournal

- ejusano
- EFSA (European Food Safety Authority), 2013b. Scientific Opinion on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12):3472, 40 pp. https://doi.org/10.2903/ji.efsa.2013.3472
- EFSA (European Food Safety Authority), 2014a. Conclusion on the peer review of the pesticide human health risk assessment of the active substance chlorpyrifos. EFSA Journal 2014;12(4):3640, 34 pp. https://doi.org/ 10.2903/j.efsa.2014.3640
- EFSA (European Food Safety Authority), 2014b. Guidance on statistical reporting. EFSA Journal 2014;12(12): 3908, 18 pp. https://doi.org/10.2903/j.efsa.2014.3908
- EFSA (European Food Safety Authority), 2015a. Stakeholder Workshop on the use of epidemiological data in pesticide risk assessment. EFSA supporting publication 2015:EN-798, 8 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/798e
- EFSA (European Food Safety Authority), 2015b. Increasing robustness, transparency and openness of scientific assessments – Report of the Workshop held on 29–30 June 2015 in Brussels. EFSA supporting publication 2015:EN-913. 29 pp. Available online: http://www.efsa.europa.eu/sites/default/files/corporate\_publications/ files/913e.pdf
- EFSA (European Food Safety Authority), 2015c. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302, 107 pp. https://doi.org/10.2903/j.efsa.2015.4302
- EFSA PPR Panel (European Food Safety Authority Panel on Plant Protection Products and their Residues), 2017. Scientific Opinion on the investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 2017;15(3):4691, 325 pp. https://doi.org/10.2903/j.efsa.2017.4691
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi. org/10.2903/j.efsa.2017.4971.
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017c. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1): 4658, 41 pp. https://doi. org/10.2903/j.efsa.2017.4658
- von Elm E, Altman DG, Egger M, Pocock SJ and Gøtzsche PC, Vandenbroucke JP and STROBE Initiative, 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ, 335, 806–808.
- Esch EW, Bahinski A and Huh D, 2015. Organs-on-chips at the frontiers of drug discovery. Nature Reviews. Drug Discovery, 14, 248–260.
- Fedak KM, Bernal A, Capshaw ZA and Gross S, 2015. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerging Themes in Epidemiology, 30, 14.
- Gibson SB, Downie JM, Tsetsou S, Feusier JE, Figueroa KP, Bromberg MB, Jorde LB and Pulst SM, 2017. The evolving genetic risk for sporadic ALS. Neurology, 89, 226–233.
- Gómez-Martín A, Hernández AF, Martínez-González LJ, González-Alzaga B, Rodríguez-Barranco M, López-Flores I, Aguilar-Garduno C and Lacasana M, 2015. Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities. Chemosphere, 139, 534–540.
- González-Alzaga B, Hernández AF, Rodríguez-Barranco M, Gómez I, Aguilar-Garduño C, López-Flores I, Parrón T and Lacasaña M, 2015. Pre- and postnatal exposures to pesticides and neurodevelopmental effects in children living in agricultural communities from South-Eastern Spain. Environment International, 85, 229–237.
- Greenland S and Longnecker MP, 1992. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. American Journal of Epidemiology, 135, 1301–1309.
- Greenland S and O'Rourke K, 2008. Meta-analysis. In: Rothman K, Greenland S and Lash T (eds). Modern Epidemiology. 3. Lippincott Williams & and Wilkins, Philadelphia. pp. 652–682.
- Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN and Altman DG, 2016. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. European Journal of Epidemiology, 31, 337–350.
- Grimes DA and Schulz KF, 2005. Surrogate end points in clinical research: hazardous to your health. Obstetrics and Gynecology, 105, 1114–1118.
- Gustafson P and McCandless LC, 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. International Journal of Environmental Research and Public Health, 7, 1520–1539.
- Hernández AF, González-Alzaga B, López-Flores I and Lacasaña M, 2016. Systematic reviews on neurodevelopmental and neurodegenerative disorders linked to pesticide exposure: methodological features and impact on risk assessment. Environment International, 92–93, 657–679.
- Higgins JP, 2008. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. International Journal of Epidemiology, 37, 1158–1160.

#### Epidemiological studies and pesticides

- in pesticide risk assessment. EFSA supporting publication 2015:EN-798, 8 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/798e
- EFSA (European Food Šafety Authority), 2015b. Increasing robustness, transparency and openness of scientific assessments Report of the Workshop held on 29–30 June 2015 in Brussels. EFSA supporting publication 2015: EN-913. 29 pp. Available online: http://www.efsa.europa.eu/sites/default/files/corporate
  - publications/ files/913e.pdf
- EFSA (European Food Safety Authority), 2015c. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015;13(11):4302, 107 pp. https://doi.org/10.2903/j.efsa.2015.4302
- EFSA PPR Panel (European Food Safety Authority Panel on Plant Protection Products and their Residues), 2017. Scientific Opinion on the investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia. EFSA Journal 2017;15(3):4691, 325 pp. https://doi.org/10.2903/j.efsa.2017.4691
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017a. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971.
- EFSA Scientific Committee (European Food Safety Authority Scientific Committee), 2017c. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1): 4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658
- von Élm E, Åltman DG, Egger M, Pocock SJ and Gøtzsche PC, Vandenbroucke JP and STROBE Initiative, 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ, 335, 806–808.
- Esch EW, Bahinski A and Huh D, 2015. Organs on chips at the frontiers of drug discovery. Nature Reviews. Drug Discovery, 14, 248-260.
- Fedak KM, Bernal A, Capshaw ZA and Gross S, 2015. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerging Themes in Epidemiology, 30, 14.
- Gibson SB, Downie JM, Tsetsou S, Feusier JE, Figueroa KP, Bromberg MB, Jorde LB and Pulst SM, 2017. The evolving genetic risk for sporadic ALS. Neurology, 89, 226–233.
- Gómez-Martín A, Hernández AF, Martínez-González LJ, González-Alzaga B, Rodríguez-Barranco M, Lopez-Flores I, Aguilar-Garduno C and Lacasana M, 2015. Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities. Chemosphere, 139, 534-540.
- González-Alzaga B, Hernández AF, Rodríguez-Barranco M, Gómez I, Aguilar-Garduño C, López-Flores I, Parrón T and Lacasaña M, 2015. Pre- and postnatal exposures to pesticides and neurodevelopmental effects in children living in agricultural communities from South-Eastern Spain. Environment International, 85, 229–237.
- Greenland S and Longnecker MP, 1992. Methods for trend estimation from summarized dose response data, with applications to meta-analysis. American Journal of Epidemiology, 135, 1301–1309.
- Greenland S and O Rourke K, 2008. Meta-analysis. In: Rothman K, Greenland S and Lash T (eds). Modern Epidemiology. 3. Lippincott Williams & and Wilkins, Philadelphia. pp. 652-682.
- Greenland S, Senn SJ, Rothman KJ, Carlin JB, Poole C, Goodman SN and Altman DG, 2016. Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. European Journal of Epidemiology, 31, 337-350.
- Grimes DA and Schulz KF, 2005. Surrogate end points in clinical research: hazardous to your health. Obstetrics and Gynecology, 105, 1114-1118. Gustafson P and McCandless LC, 2010. Probabilistic approaches to better quantifying the results of
- Gustafson P and McCandless LC, 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. International Journal of Environmental Research and Public Health, 7, 1520-1539.
- Hernández AF, González-Alzaga B, López-Flores I and Lacasaña M, 2016. Systematic reviews on neurodevelopmental and neurodegenerative disorders linked to pesticide exposure: methodological features and impact on risk assessment. Environment International, 92–93, 657–679.
- Higgins JP, 2008. Commentary: heterogeneity in meta-analysis should be expected and appropriately quantified. International Journal of Epidemiology, 37, 1158-1160.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295-300.
- Hines CJ, Deddens JA, Coble J, Kamel F and Alavanja MC, 2011. Determinants of captan air and dermal exposures among orchard pesticide applicators in the Agricultural Health Study. Annals of Occupational Hygiene, 55, 620–633.
- Hoffmann S, de Vries RBM, Stephens ML, Beck NB, Dirven HAAM, Fowle JR 3rd, Goodman JE, Hartung T, Kimber I, Lalu MM, Thayer K, Whaley P, Wikoff D and Tsaioun K, 2017. A primer on systematic reviews in toxicology. Archives of Toxicology, 91, 2551–2575.
- Höfler M, 2005. The Bradford Hill considerations on causality: a counterfactual perspective. Emerging Themes in Epidemiology, 2, 11.
- IEA (International Epidemiological Association), 2007. Good Epidemiological Practice (GEP) 2007. Available online: http://ieaweb.org/good-epidemiological-practice-gep/
- Imbens G and Rubin D, 2015. Causal Inference for Statistics, Social, and Biomedical Sciences: An

www.efsa.europa.eu/efsajournal



- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295-300.
- Hines CJ, Deddens JA, Coble J, Kamel F and Alavania MC, 2011, Determinants of captan air and dermal exposures among orchard pesticide applicators in the Agricultural Health Study. Annals of Occupational Hygiene, 55, 620-633.
- Hoffmann S, de Vries RBM, Stephens ML, Beck NB, Dirven HAAM, Fowle JR 3rd, Goodman JE, Hartung T, Kimber I, Lalu MM, Thayer K, Whaley P, Wikoff D and Tsaioun K, 2017. A primer on systematic reviews in toxicology. Archives of Toxicology, 91, 2551-2575.
- Höfler M, 2005. The Bradford Hill considerations on causality: a counterfactual perspective. Emerging Themes in Epidemiology, 2, 11.
- IEA (International Epidemiological Association), 2007. Good Epidemiological Practice (GEP) 2007. Available online: http://ieaweb.org/good-epidemiological-practice-gep/
- Imbens G and Rubin D, 2015. Causal Inference for Statistics, Social, and Biomedical Sciences: An Introduction. Cambridge University Press, New York, NY.
- INSERM, 2013. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.
- Ioannidis JP and Trikalinos TA, 2007. An exploratory test for an excess of significant findings. Clinical Trials, 4, 245-253.
- Jurek AM, Greenland S, Maldonado G and Church TR, 2005. Proper interpretation of non-differential misclassification effects: expectations vs observations. International Journal of Epidemiology, 34, 680–687.
- Kaltenhäuser J, Kneuer C, Marx-Stoelting P, Niemann L, Schubert J, Stein B and Solecki R, 2017. Relevance and reliability of experimental data in human health risk assessment of pesticides. Regulatory Toxicology and Pharmacology, 88, 227-237.
- Karabatsos G, Talbott E and Walker SG, 2015. A Bayesian nonparametric meta-analysis model. Research Synthesis Methods, 6, 28-44.
- Kavvoura FK, Liberopoulos G and Ioannidis JP, 2007. Selection in reported epidemiological risks: an empirical assessment. PLoS Medicine, 4, e79.
- Lachenmeier DW, Kanteres F and Rehm J, 2011. Epidemiology-based risk assessment using the benchmark dose/ margin of exposure approach: the example of ethanol and liver cirrhosis. International Journal of Epidemiology, 40, 210-218.
- LaKind JS, Sobus JR, Goodman M, Barr DB, Furst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teequarden J, Tornero-Velez R and Weisel CP, 2014. A proposal for assessing study quality: biomonitoring, environmental epidemiology, and short-lived chemicals (BEES-C) instrument. Environmental International, 73, 195-207.
- LaKind JS, Goodman M, Barr DB, Weisel CP and Schoeters G, 2015. Lessons learned from the application of BEES-C: systematic assessment of study quality of epidemiologic research on BPA, neurodevelopment, and respiratory health. Environment International, 80, 41–71.
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV and Alavanja MC, 2009. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study, Blood, 113, 6386-6391.
- Larsson MO, Nielsen VS, Brandt CØ, Bjerre N, Laporte F and Cedergreen N, 2017. Quantifying dietary exposure to pesticide residues using spraying journal data. Food and Chemical Toxicology, 105, 407-428.
- Lash TL, Fox MP and Fink AK, 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.
- Lavelle KS, Robert Schnatter A, Travis KZ, Swaen GM, Pallapies D, Money C, Priem P and Vriihof H, 2012. Framework for integrating human and animal data in chemical risk assessment. Regulatory Toxicology and Pharmacology, 62, 302-312.
- London L, Coggon D, Moretto A, Westerholm P, Wilks MF and Colosio C, 2010. The ethics of human volunteer studies involving experimental exposure to pesticides: unanswered dilemmas. Environmental Health, 18, 50.
- Maldonado G and Greenland S, 2002. Estimating causal effects. International Journal of Epidemiology, 31, 422-429. Marx-Stoelting P, Braeuning A, Buhrke T, Lampen A, Niemann L, Oelgeschlaeger M, Rieke S, Schmidt F, Heise T,
- Pfeil R and Solecki R, 2015. Application of omics data in regulatory toxicology: report of an international BfR expert workshop. Archives of Toxicology, 89, 2177-2184.
- McNamee R, 2003. Confounding and confounders. Occupational and Environmental Medicine, 60, 227-234.
- Monson R, 1990, Occupational Epidemiology, 2nd Edition, CRC Press, Boca Ration, FL,
- Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E and Vega C, 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology, 39, 158-168.
- Nachman KE, Fox MA, Sheehan MC, Burke TA, Rodricks JV and Woodruff TJ, 2011. Leveraging epidemiology to improve risk assessment. Open Epidemiology Journal, 4, 3-29.
- Nieuwenhuijsen MJ, 2015. Exposure assessment in environmental epidemiology. In: Vrijheid M (ed.). The Exposome-Concept and Implementation in Birth Cohorts Chapter 14. Oxford University Press.
- NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press.
- NRC (National Research Council), 2009. Science and Decisions: Advancing Risk Assessment. The National Academies Press, Washington, DC.

www.efsa.europa.eu/efsajournal

EFSA Journal 2017;15(10):5007

#### Epidemiological studies and pesticides

- Introduction. Cambridge University Press, New York, NY.
- INSERM, 2013. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.
- Ioannidis JP and Trikalinos TA, 2007. An exploratory test for an excess of significant findings. Clinical Trials, 4, 245–253
- Jurek AM, Greenland S, Maldonado G and Church TR, 2005. Proper interpretation of non-differential misclassification effects: expectations vs observations. International Journal of Epidemiology, 34, 680-687
- Kaltenhäuser J, Kneuer C, Marx-Stoelting P, Niemann L, Schubert J, Stein B and Solecki R, 2017. Relevance and reliability of experimental data in human health risk assessment of pesticides. Regulatory Toxicology and Pharmacology, 88, 227-237
- Karabatsos G, Talbott E and Walker SG, 2015. A Bayesian nonparametric meta-analysis model. Research Synthesis Methods, 6, 28–44.
- Kavvoura FK, Liberopoulos G and Ioannidis JP, 2007. Selection in reported epidemiological risks: an empirical assessment, PLoS Medicine, 4, e79.
- Lachenmeier DW, Kanteres F and Rehm J, 2011. Epidemiology-based risk assessment using the benchmark dose/margin of exposure approach: the example of ethanol and liver cirrhosis. International Journal of Epidemiology, 40, 210-218.
- LaKind JS, Sobus JR, Goodman M, Barr DB, Furst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teequarden J. Tornero-Velez R and Weisel CP. 2014. A proposal for assessing study quality biomonitoring. environmental epidemiology, and short-lived chemicals (BEES-C) instrument. Environmental International, 73, 195-207.
- LaKind JS, Goodman M, Barr DB, Weisel CP and Schoeters G, 2015. Lessons learned from the application of BEES-C: systematic assessment of study quality of epidemiologic research on BPA, neurodevelopment, and respiratory health. Environment International, 80, 41-71.
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV and Alavanja MČ, 2009. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. Blood, 113, 6386-6391.
- Larsson MO, Nielsen VS, Brandt CØ, Bjerre N, Laporte F and Cedergreen N, 2017. Quantifying dietary exposure to pesticide residues using spraying journal data. Food and Chemical Toxicology, 105, 407-428.
- Lash TL, Fox MP and Fink AK, 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York
- Lavelle KS, Robert Schnatter A, Travis KZ, Swaen GM, Pallapies D, Money C, Priem P and Vrijhof H, 2012. Framework for integrating human and animal data in chemical risk assessment. Regulatory Toxicology and Pharmacology, 2012; 62, 302-312.
- London L, Coggon D, Moretto A, Westerholm P, Wilks MF and Colosio C, 2010. The ethics of human volunteer studies involving experimental exposure to pesticides: unanswered dilemmas. Environmental Health, 18, 50.
- Maldonado G and Greenland S, 2002. Estimating causal effects. International Journal of Epidemiology, 31, 422 - 429.
- Marx-Stoelting P, Braeuning A, Buhrke T, Lampen A, Niemann L, Oelgeschlaeger M, Rieke S, Schmidt F. Heise T. Pfeil R and Solecki R, 2015. Application of omics data in regulatory toxicology: report of an international BfR expert workshop. Archives of Toxicology, 89, 2177-2184.
- McNamee R, 2003. Confounding and confounders. Occupational and Environmental Medicine, 60, 227-
- Monson R, 1990. *Occupational Epidemiology*, 2nd Edition. CRC Press, Boca Ration, FL. Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E and Vega C, 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology, 39, 158-168
- Nachman KE, Fox MA, Sheehan MC, Burke TA, Rodricks JV and Woodruff TJ, 2011. Leveraging epidemiology to improve risk assessment. Open Epidemiology Journal, 4, 3-29
- Nieuwenhuijsen MJ, 2015. Exposure assessment in environmental epidemiology. In: Vrijheid M (ed.). The Exposome-Concept and Implementation in Birth Cohorts Chapter 14. Oxford University Press.
- NRC (National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, DC: The National Academies Press.
- NRC (National Research Council), 2009. Science and Decisions: Advancing Risk Assessment. The National Academies Press, Washington, DC.
- Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E and Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013: EN-497, 159 pp.
- OECD (Organisation for Economic Co-operation and Development), 2013. Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment, No. 184. Paris. Avilable online: http:// search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282013% 296&doclanguage=en
- Orford R, Crabbe H, Hague C, Schaper A and Duarte-Davidson R, 2014. EU alerting and reporting systems for potential chemical public health threats and hazards. Environment International, 72, 15-25
- Orford R, Hague C, Duarte-Davidson R, Settimi L, Davanzo F, Desel H, Pelclova D, Dragelyte G, Mathieu-Nolf M, Jackson G and Adams R, 2015. Detecting, alerting and monitoring emerging chemical health threats: ASHTIII. European Journal of Public Health, 25(supp 3), 218.
- Orsini N, Li R, Wolk A, Khudyakov P and Spiegelman D, 2012. Meta-analysis for linear and nonlinear doseresponse relations: examples, an evaluation of approximations, and software. American Journal of

EPSA Inch

Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E and Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013:EN-497, 159 pp.

OECD (Organisation for Economic Co-operation and Development), 2013, Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment, No. 184. Paris. Avilable online: http:// search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/im/mono%282013%296&doclanguage=en

- Orford R. Crabbe H. Hague C. Schaper A and Duarte-Davidson R. 2014. EU alerting and reporting systems for potential chemical public health threats and hazards. Environment International, 72, 15-25.
- Orford R, Hague C, Duarte-Davidson R, Settimi L, Davanzo F, Desel H, Pelclova D, Dragelyte G, Mathieu-Nolf M, Jackson G and Adams R, 2015. Detecting, alerting and monitoring emerging chemical health threats: ASHTIII. European Journal of Public Health, 25(supp 3), 218.
- Orsini N, Li R, Wolk A, Khudyakov P and Spiegelman D, 2012. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. American Journal of Epidemiology, 175, 66-73.
- Oulhote Y and Bouchard MF, 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environmental Health Perspectives, 121, 1378–1384.
- Pearce N, 2011. Registration of protocols for observational research is unnecessary and would do more harm than good. Occupational and Environmental Medicine, 68, 86-88.

Pearce N, 2012. Classification of epidemiological study designs. International Journal of Epidemiology, 41, 393–397.

- Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA, Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Hemminki K, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, Lebailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, 't Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Saniose S. Schernhammer E. Costantini AS, Seixas N, Shy C, Siemiatycki J, 2015. Silverman DT, Simonato L. Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stayner LT, Steenland K, Stenzel M, Stewart BW, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. IARC monographs: 40 years of evaluating carcinogenic hazards to humans. Environmental Health Perspectives, 123, 507-514.
- Raffaele KC, Vulimiri SV and Bateson TF, 2011. Benefits and barriers to using epidemiology data in environmental risk. The Journal of Epidemiology, 4, 99-105.
- Raphael K, 1987. Recall bias: a proposal for assessment and control. International Journal of Epidemiology, 16, 167 - 170
- Rappaport SM, 2012. Biomarkers intersect with the exposome. Biomarkers, 17, 483-489.
- Reich CG, Ryan PB and Schuemie MJ, 2013. Alternative outcome definitions and their effect on the performance of methods for observational outcome studies. Drug Safety, 36(Suppl 1), S181-S193.
- Rothman KJ, 2002. Epidemiology An Introduction. Oxford University Press, Oxford.
- Rothman KJ and Greenland S, 1998, Modern Epidemiology, 2, Philadelphia: Lippincott Williams & Wilkins, 27 pp. Rothman KJ, Greenland S and Lash TL, 2008. Modern Epidemiology, 3rd Edition. Lippincott Williams & Wilkins, Philadelphia, PA, USA.
- Rushton L, 2011. Should protocols for observational research be registered? Occupational and Environmental Medicine, 68, 84-86.
- Salerno J, Knoppers BM, Lee LM, Hlaing WW and Goodman KW, 2017. Ethics, big data and computing in epidemiology and public health. Annals of Epidemiology, 27, 297-301. https://doi.org/10.1016/j.annepidem. 2017.05.002
- Santacatterina M and Bottai M, 2015. Inferences and conjectures in clinical trials: a systematic review of generalizability of study findings, Journal of Internal Medicine, 279, 123-126, https://doi.org/10.1111/joim.12389
- SCENIHR, 2012. Memorandum on the use of the scientific literature for human health risk assessment purposes weighing of evidence and expression of uncertainty.
- Simera I, Moher D, Hoey J, Schulz KF and Altman DG, 2010. A catalogue of reporting guidelines for health research, European Journal of Clinical Investigation, 40, 35-53.
- Skelly AC, 2011. Probability, proof, and clinical significance. Evidence-Based Spine-Care Journal, 2, 9-11.
- Spiegelman D, 2016. Evaluating Public Health Interventions: 4. the nurses' health study and methods for eliminating bias attributable to measurement error and misclassification. American Journal of Public Health, 106, 1563-1566.
- Stang PE, Ryan PB, Dusetzina SB, Hartzema AG, Reich C, Overhage JM and Racoosin JA, 2012. Health outcomes of interest in observational data: issues in identifying definitions in the literature. Health Outcomes Research in Medicine, 3, e37-e44.

64

Thomas DC, 2009. Statistical Methods in Environmental Epidemiology. Oxford University Press, Oxford, UK.

www.efsa.europa.eu/efsajournal

EFSA Journal 2017;15(10):5007

Epidemiological studies and pesticides

#### Epidemiology, 175, 66-73.

- Oulhote Y and Bouchard MF, 2013. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environmental Health Perspectives, 121, 1378-1384.
- Pearce N, 2011. Registration of protocols for observational research is unnecessary and would do more harm than good. Occupational and Environmental Medicine, 68, 86-88.
- Pearce N, 2012. Classification of epidemiological study designs. International Journal of Epidemiology, 41, 393-397. Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA, Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Hemminki K, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, Lebailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, t Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Sanjose S, Schernhammer E, Costantini AS, Seixas N, Shy C, Siemiatycki J,2015. Silverman DT, Simonato L, Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stavner LT, Steenland K, Stenzel M, Stewart BW, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. IARC monographs: 40 years of evaluating carcinogenic hazards to humans. Environmental Health Perspectives, 123, 507-514.
- Raffaele KC, Vulimiri SV and Bateson TF, 2011. Benefits and barriers to using epidemiology data in environmental risk. The Journal of Epidemiology, 4, 99-105.
- Raphael K, 1987. Recall bias: a proposal for assessment and control. International Journal of Epidemiology,  $\hat{16}, 167-170$
- Rappaport SM, 2012, Biomarkers intersect with the exposome, Biomarkers, 17, 483–489.
- Reich CG, Ryan PB and Schuemie MJ, 2013. Alternative outcome definitions and their effect on the performance of methods for observational outcome studies. Drug Safety, 36(Suppl 1), S181–S193.
- Rothman KJ, 2002. *Epidemiology An Introduction*. Oxford University Press, Oxford. Rothman KJ and Greenland S, 1998. *Modern Epidemiology. 2*. Philadelphia: Lippincott Williams & Wilkins, 27 pp.
- Rothman KJ, Greenland S and Lash TL, 2008. Modern Epidemiology, 3rd Edition. Lippincott Williams & Wilkins, Philadelphia, PA, USA.
- Rushton L. 2011. Should protocols for observational research be registered? Occupational and Environmental Medicine, 68, 84-86.
- Salerno J, Knoppers BM, Lee LM, Hlaing WW and Goodman KW, 2017. Ethics, big data and computing in epidemiology and public health. Annals of Epidemiology, 27, 297-301. https://doi.org/10.1016/ j. annepidem, 2017.05.002
- Santacatterina M and Bottai M, 2015. Inferences and conjectures in clinical trials: a systematic review of generalizability of study findings. Journal of Internal Medicine, 279, 123-126. https://doi.org/10.1111/ joim.12389
- SCENIHR, 2012. Memorandum on the use of the scientific literature for human health risk assessment purposes -weighing of evidence and expression of uncertainty.
- Simera I, Moher D, Hoey J, Schulz KF and Altman DG, 2010. A catalogue of reporting guidelines for health research. European Journal of Clinical Investigation, 40, 35-53.
- Skelly AC, 2011. Probability, proof, and clinical significance. Evidence-Based Spine-Care Journal, 2, 9-11.
- Spiegelman D, 2016. Evaluating Public Health Interventions: 4. the nurses' health study and methods for eliminating bias attributable to measurement error and misclassification. American Journal of Public Health, 106, 1563-1566.
- Stang PE, Rvan PB, Dusetzina SB, Hartzema AG, Reich C, Overhage JM and Racoosin JA, 2012. Health outcomes of interest in observational data: issues in identifying definitions in the literature. Health Outcomes Research in Medicine, 3, e37-e44.
- Thomas DC, 2009. Statistical Methods in Environmental Epidemiology. Oxford University Press, Oxford, UK.
- Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G, Croghan CW, Jones PA, Knott CE, Lynch CF, Sandler DP, Blair AE and Alavanja MC, 2010. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. Journal of Exposure Science & Environmental Epidemiology, 20, 559-569.
- Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod MR and Ioannidis JP, 2013. Evaluation of excess significance bias in animal studies of neurological diseases. PLoS Biology, 11, e1001609.
- Turner MC, Wigle DT and Krewski D, 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis.
- US EPA (United States Environmental Protection Agency), 2011. Chlorpyrifos: preliminary human health risk assessment for registration review, 30 June 2011, 159 pp. US-EPA (U.S. Environmental Protection Agency), 2010a. Framework for incorporating human
- epidemiologic & incident data in health risk assessment (draft). Office of Pesticide Programs. Washington, DC, 2010.



- Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G, Croghan CW, Jones PA, Knott CE, Lynch CF, Sandler DP. Blair AE and Alavania MC. 2010. Assessment of a pesticide exposure intensity algorithm in the agricultural health study. Journal of Exposure Science & Environmental Epidemiology, 20, 559-569.
- Tsilidis KK, Panagiotou OA, Sena ES, Aretouli E, Evangelou E, Howells DW, Al-Shahi Salman R, Macleod MR and Ioannidis JP, 2013. Evaluation of excess significance bias in animal studies of neurological diseases. PLoS Biology, 11, e1001609.
- Turner MC, Wigle DT and Krewski D, 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis.
- US EPA (United States Environmental Protection Agency), 2011. Chlorpyrifos: preliminary human health risk assessment for registration review, 30 June 2011, 159 pp.
- US-EPA (U.S. Environmental Protection Agency), 2010a. Framework for incorporating human epidemiologic & incident data in health risk assessment (draft). Office of Pesticide Programs. Washington, DC, 2010.
- US-EPA (U.S. Environmental Protection Agency), 2010b. Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. Arlington, Virginia, USA, April 22, 2010b, Available online: https://archive.epa.gov/scipoly/sap/meetings/web/ pdf/020210minutes.pdf
- US-EPA (U.S. Environmental Protection Agency), 2012. Guidance for considering and using open literature toxicity studies to support human health risk assessment. Office of Pesticide Programs. Washington, DC, 2012. Available online: http://www.epa.gov/pesticides/science/lit-studies.pdf
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides December 28, 2016. Avilable online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf
- Vandenberg LN, Ågerstrand M, Beronius A, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS, Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ and Rudén C, 2016. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environmental Health, 15, 74.
- van den Brandt P. Voorrips L. Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A and Goldbohm A, 2002. The contribution of epidemiology. Food and Chemical Toxicology, 40, 387-424.
- Vinken M, 2013. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology, 312, 158-165. Vlaanderen J, Moore LE, Smith MT, Lan O, Zhang L, Skibola CF, Rothman N and Vermeulen R, 2010. Application of OMICS technologies in occupational and environmental health research: current status and projections.
- Occupational and Environmental Medicine, 67, 136-43. WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. EHC 240: principles
- and methods for the risk assessment of chemicals in food.
- Wilson SJ and Tanner-Smith EE, 2014. Meta-analysis in prevention science. In: Sloboda Z and Petras H (eds.). Defining prevention science. Advances in Prevention Science (vol. 1): Defining Prevention Science Springer, New York. pp. 431-452.
- Youngstrom E, Kenworthy L, Lipkin PH, Goodman M, Squibb K, Mattison DR, Anthony LG, Makris SL, Bale AS, Raffaele KC and LaKind JS, 2011. A proposal to facilitate weight-of-evidence assessments: harmonization of Neurodevelopmental Environmental Epidemiology Studies (HONEES), Neurotoxicology and Teratology, 33, 354–359.
- Zingone A and Kuehl WM, 2011. Pathogenesis of monoclonal gammopathy of undetermined significance and progression to multiple myeloma. Seminars in Hematology, 48, 4-12.

#### Glossarv and Abbreviations

ADI		ure of the amount of a pesticide in food or sted (orally) on a daily basis over a lifetime sk.
ADME	Abbreviation used in pharmacolo	ogy (and toxicology) for absorption, distribution, chemical o pharmaceutical compound and
AOP	Adverse Outcome Pathway. A st leading to adverse effects releva	ructured representation of biological events ant to risk assessment.
ARfD	Acute Reference Dose. An estim water (normally expressed on a	nate of the amount a pesticide in food or drinking body weight basis) that can be ingested in a but appreciable health risks to the consumer on
Biomarker	and evaluated as an indication of	r'. A characteristic that is objectively measured of normal biologic processes, pathogenic ponses to a therapeutic intervention
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www.efsa.europa.eu/efsajournal
```

#### Epidemiological studies and pesticides

- US·EPA (U.S. Environmental Protection Agency), 2010b. Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. Arlington, Virginia, USA, April 22, 2010b. Available online: https://archive.epa.gov/scipoly/sap/meetings/web/pdf/020210minutes.pdf
- US-EPA (U.S. Environmental Protection Agency), 2012. Guidance for considering and using open literature toxicity studies to support human health risk assessment. Office of Pesticide Programs. Washington, DC, 2012. Available online: http://www.epa.gov/pesticides/science/lit-studies.pdf
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides December 28, 2016. Avilable online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf Vandenberg LN, Ågerstrand M, Beronius Å, Beausoleil C, Bergman Å, Bero LA, Bornehag CG, Boyer CS,
- Cooper GS, Cotgreave I, Gee D, Grandjean P, Guyton KZ, Hass U, Heindel JJ, Jobling S, Kidd KA, Kortenkamp A, Macleod MR, Martin OV, Norinder U, Scheringer M, Thayer KA, Toppari J, Whaley P, Woodruff TJ and Rude, n C, 2016. A proposed framework for the systematic review and integrated assessment (SYRINA) of endocrine disrupting chemicals. Environmental Health, 15, 74. van den Brandt P. Voorrips L. Hertz-Picciotto I. Shuker D. Boeing H. Speijers G. Guittard C. Kleiner J.
- Knowles M, Wolk A and Goldbohm A, 2002. The contribution of epidemiology. Food and Chemical Toxicology, 40, 387-424.
- Vinken M, 2013. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology, 312, 158 - 165.
- Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, Rothman N and Vermeulen R, 2010. Application of OMICS technologies in occupational and environmental health research: current status and projections. Occupational and Environmental Medicine, 67, 136-43.
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. EHC 240: principles and methods for the risk assessment of chemicals in food.
- Wilson SJ and Tanner Smith EE, 2014. Meta-analysis in prevention science. In: Sloboda Z and Petras H (eds.). Defining prevention science. Advances in Prevention Science (vol. 1): Defining Prevention Science Springer, New York. pp. 431-452.
- Youngstrom E. Kenworthy L. Lipkin PH. Goodman M. Squibb K. Mattison DR. Anthony LG, Makris SL. Bale AS, Raffaele KC and LaKind JS, 2011. A proposal to facilitate weight-of-evidence assessments: harmonization of Neurodevelopmental Environmental Epidemiology Studies (HONEES). Neurotoxicology and Teratology, 33, 354–359. Zingone A and Kuehl WM, 2011. Pathogenesis of monoclonal gammopathy of undetermined significance
- and progression to multiple myeloma. Seminars in Hematology, 48, 4–12.

# 用語集と略語

- ADI 一日の許容摂取量。食品または飲料水に含まれる農薬の量の尺度で、相当な健康リスクを伴わず に生涯にわたって日常的に(経口的に)摂取することができる。
- ADME 薬理学(及び毒物学)で使用される略語(体内動態)で、化学物質の吸収、分布、代謝及び排泄 のために使用され、生物体内でのその処理を示す。
- AOP Adverse Outcome Pathway(有害性転帰経路)。リスク評価に関連する有害な影響につながる生物学的事象 を構造的に表現したもの。
- ARfD 急性参照用量(Acute Reference Dose)。食品または飲料水に含まれる農薬の量(通常は体重べ ースで表される)の推定値で、評価時に知られているすべての事実に基づいて、24時間以内に消 費者が健康上のリスクを認めずに摂取できる量。
- バイオマーカー 「生物学的マーカー」とも呼ばれる。正常な生物学的プロセス、病原性プロセス、または治療的介 入に対する薬理学的反応の指標として客観的に測定され、評価される特性。
- BMD ベンチマークドーズ。バックグラウンドと比較して有害な影響の反応率(ベンチマーク反応または BMR)に所定の変化をもたらす閾値の用量または濃度。95%の下限値(BMDL)が計算され、健 康に基づいた参照値を導き出すための出発点としてさらに使用される。
- HBM ヒューマンバイオモニタリング(Human biomonitoring)。ヒトの生物学的体液または組織におけ る化学物質及び/またはその代謝物の測定。また、すべてのばく露経路からの総合的なばく露か ら得られる化学物質の内部ばく露量とも呼ばれる。
- ヒトデータ 研究者が研究参加者に働きかけることなく、要因と健康影響との間の自然な関係を観察する観察



BMD	Benchmark Dose. A threshold dose or concentration that produces a predetermined change in response rate of an adverse effect (the benchmark response or BMR) compared to background. The lower 95% confidence limit is calculated (BMDL) to be further used as a point of departure to derive health-
НВМ	based reference values. Human biomonitoring. The measurement of a chemical and/or its metabolites in human biological fluids or tissues. Also referred as to the internal dose of a chemical resulting from integrated exposures from all exposure routes.
Human data	They include observational studies (also called epidemiological studies) where the researcher is observing natural relationships between factors and health outcomes without acting upon study participants. Vigilance data also fall under this concept. In contrast, interventional studies (also called experimental studies or randomised clinical trials), where the researcher intercedes as part of the study design, are outside the scope of this opinion.
IARC	International Agency for Research on Cancer. An agency of the World Health Organization whose role is to conduct and coordinate research into the causes and occurrence of cancer worldwide.
LOAEL	Lowest-observed-adverse-effect level. The lowest concentration or amount of a chemical stressor evaluated in a toxicity test that shows harmful effects (e.g. an adverse alteration of morphology, biochemistry, function, or lifespan of a target organism).
NOAEL	No observed-adverse-effect level. Highest dose at which there was not an observed toxic or adverse effect.
OR	Odds ratio. A measure of association between an exposure and an outcome. The
PBTK-TD	OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Physiologically based toxicokinetic/toxicodynamic modelling is a mathematical modelling approach aimed at integrating <i>a priori</i> knowledge of physiological processes with other known/observed information to mimic the fates and effects of compounds in the bodies of humans, preclinical species and/or other
PPP	organisms. Plant Protection Product. The term 'pesticide' is often used interchangeably with 'plant protection product', however, pesticide is a broader term that also covers
RR	non plant/crop uses, for example biocides. Relative risk. Ratio of the probability of an event (e.g. developing a disease) occurring in an exposed group to the probability of the event occurring in a
RMS	comparison, non-exposed group. Rapporteur member state. The member state of the European Union initially in charge of assessing and evaluating a dossier on a pesticide active substance
Sensitivity	toxicological assessment. The ability of a test to correctly classify an individual as 'diseased'. Probability of
Specificity	being test positive when disease present. The ability of a test to correctly classify an individual as disease-free. Probability
Surrogate endpoint	of being test negative when disease absent. A biomarker intended to substitute for a clinical endpoint
AHS ASHTIII BEES-C DAR DDE DDT EMA EPA US EQUATOR EU-OSHA EWAS GIS	Agricultural Health Study Alerting and Reporting System for Chemical Health Threats, Phase III Biomonitoring, Environmental Epidemiology, and Short-Lived Chemicals draft assessment report dichlorodiphenyldichloroethylene dichlorodiphenyltrichloroethane European Medicines Agency Environmental Protection Agency Enhancing the QUAlity and Transparency Of health Research European Agency for Safety and Health at Work Exposome-wide association studies Geographical information systems

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	による研究(疫学研究とも呼ばれる)が含まれる。 警戒データもこの概念に該当する。 対照的に、研
	究者が研究デザインの一部として介入する介入研究(実験研究または無作為化臨床試験とも呼
	ばれる)は、本意見書の範囲外である。
IARC Interna	tional Agency for Research on Cancer(国際がん研究機関)。世界のがんの原因と発生に関する
	研究を実施し、調整することを役割とする世界保健機関(WHO)の機関。
LOAEL (LOA	EL) 最小中毒量(Lowestobserved-adverse-effect level)。毒性試験で評価され、有害な影響(対
	象生物の形態、生化学、機能、または生涯への有害な変化など)を示す化学的ストレス因子の最
	低濃度または用量。
NOAEL	無毒性量。毒性または毒性影響が観察されなかった最高用量。
OR	オッズ比。ばく露と結果との関連性を示す尺度。OR は、特定のばく露を受けた場合に転帰が起こ
	る確率を、ばく露がなかった場合に転帰が起こる確率と比較して表している。
PBTK-TD	Physiologically based toxicokinetic/toxicodynamic modelling (PBTK-TD) Physiologically
	based toxicokinetic/toxicodynamic modellingとは、生理学的プロセスに関する先端的な知識
	を他の既知/観察された情報と統合して、ヒト、前臨床試験動物種及び/または他の生物の体内で
	の化合物の転帰と影響を模倣することを目的とした数学的モデル化手法である。
PPP	植物防疫製品(農薬)。用語「pesticide(殺虫剤)」はしばしば「plant protection product(植物
	防疫製品)」と互換的に使用されるが、pesticide(殺虫剤)は植物/作物以外の用途、例えば
	biocide(殺生物剤)などもカバーするより広い用語(農薬)である。
RR	相対リスク(Relative risk)。ある事象(病気の発生など)がばく露したグループで発生する確率と、
	比較対照の非ばく露グループで発生する確率との比。
RMS	Rapporteur(ラポーター)の加盟国。農薬有効成分の毒性評価に関する書類の評価及び評価を
	最初に担当する欧州連合の加盟国。
感度	ある検査で個人を正しく「疾病」と同定する能力。疾患が存在する場合に検査が陽性となる可能性。
特異性	個人を疾患無しと正しく同定する検査の能力。疾患がない場合に検査が陰性である可能性。
代替エンドポイ	ント(surrogate endpoint)臨床エンドポイントの代わりとなることを目的としたバイオマーカー。
AHS	農業健康調査
ASHTIII	化学物質による健康影響の脅威に対する警告と報告システム、フェーズ III
BEES-C	バイオモニタリング、環境疫学、短命化学物質
DAR	評価報告書草案
DDE	ジクロロジフェニルジクロロエチレン
DDT	ジクロロジフェニルトリクロロエタン
EMA	欧州医薬品庁
EPA	米国環境保護庁
EQUATOR	健康研究の質と明白性を高める
EU-OSHA	欧州労働安全衛生機関
EWAS Expose	ome-wide association studies エキスポソームワイド関連研究
GIS	地理情報システム

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GPSglotHWEheaIATAInteICDInteIRRInteINSERMFreeLOQlimitMGUSmorMIEmolMoAmootNIOSHNatiNOSNevOECDOrgOPPOffiPCCPoisPPEpersRARRenRASFFrapisSTREGASTRSTRCOBESTrTORTerrUFuncWHOWor	d laboratory practice bal positioning system lithy worker effect ggrated Approach on Testing and Assessment ernational Classification of Diseases ernational Health Regulations nch National Institute of Health and Medical Research t of quantification noclonal gammopathy of undetermined significance lecular initiating event de of action n-Hodgkin's lymphoma ional Institute for Occupational Safety and Health wcastle-Ottawa scale janisation for Economic Co-operation and Development ice of Pesticide Programs son Control Centre sonal protective equipment ewal Assessment Report id alert system covering food and feed search Triangle Institute icture-activity relationship ROBE Extension to Genetic Association studies engthening the Reporting of OBservational studies in Epidemiology m of Reference sertainty factor rld Health Organization ight-of-Evidence
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GLP	優良試験所規範
GPS	全地球測位システム
HWE	健康労働者効果
IATA	試験と評価に関する統合的アプローチ
ICD	国際疾病分類
IHR	国際保健規則
INSERM	フランス国立保健医療研究所
LOQ	定量化限界
MGUS	単クローン性ガンマグロブリン血症
MIE	標的分子への作用
MOA	作用機序
NIHL	非ホジキンリンパ腫
NIOSH	国立労働安全衛生研究所
NOS	ニューカッスルオタワスケール
OECD	経済協力開発機構
OPP	農薬プログラム局
PCC	毒物管理センター
PPE	個人用保護具
RAR	更新評価報告書
RASFF	食品と飼料をカバーする迅速警報システム
RTI	リサーチトライアングル研究所
SAR	構造活性相関
STREGA	遺伝学的関連研究への STROBE 拡張
STROBE	疫学における観察研究の報告の強化
UF	不確実性因子
WHO	世界保健機関
WOE	エビデンスの重み付け

# Annex A – Pesticide epidemiological studies reviewed in the EFSA External Scientific Report and other reviews

The extensive evidence gathered by the EFSA External Scientific Report (Ntzani et al., 2013) highlights that there is a considerable amount of information available on pesticide exposure and health outcomes from epidemiological studies. Nonetheless, the quality of this evidence is usually low and many biases are likely to affect the results to an extent that firm conclusions cannot be made. In particular, exposure epidemiology has long suffered from poor measurement and definition and in particular for pesticides this has always been exceptionally difficult to assess and define.

# A.1. The EFSA External scientific report

### A.1.1. Methodological quality assessment

The External Scientific Report consists of a comprehensive systematic review of all the epidemiological studies published between 1 January 2006 and 30 September 2012, investigating the association between pesticide exposure and the occurrence of any human health-related outcomes.

The methodological assessment of eligible studies (to evaluate risk of bias associated with each study) was focused on: study design, study population, level of details in exposure definition and the methods of exposure measurement and the specificity of the measurement. Efforts undertaken to account for confounders through matching or multivariable models, blinded exposure assessment and well-defined and valid outcome assessment were considered.

The elements of the methodological appraisal were considered from the Research Triangle Institute (RTI; Research Triangle Park, NC, USA) item bank, a practical and validated tool for evaluating the risk of bias and precision of observational studies. Those elements are described below (Table A.1).

Table A.1:	Elements from the Research Triangle Institute (RTI; Research Triangle Park, NC, USA)
	item bank for methodological appraisal of epidemiological studies

Question	High risk	Low risk
Study design (prospective, retrospective, mixed, NA)	Retrospective, mixed, NA	Prospective
Inclusion/exclusion criteria clearly stated (yes, partially, no)	No	Yes
Authors mention power calculations (yes, no)		Yes
Level of detail in describing exposure (high, medium, low)	Low	High
Robust measurement of exposure. (biomarker (yes): small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	No	Yes
Were measures of exposure specific? yes; based on broader, chemically- related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	No	Yes
Attempt to balance the allocation between the groups (e.g., through stratification, matching)	No	Yes
Adjustment performed for potential confounders (yes, some, no)	No	Yes
Assessors blinded to exposure status (for cohort studies)	No	Yes
Outcomes assessed using valid and reliable measures, implemented	ed fr	
consistently across all study participants?	No	Yes
Sample size	Low	Тор
Rough quality assessment	>6 answers high risk	>6 asnwers low risk

Quantitative synthesis of the results was attempted when there were 5 or more eligible studies per examined outcome and when there was no substantial heterogeneity among the published evidence. Publication bias was assessed using funnel plots which allowed to visually inspect asymmetry when more than 10 studies were included in the meta-analysis.

Toxicological data was not reviewed or discussed in the External Scientific Report.

#### A.1.2. Inclusion/exclusion criteria

All types of pesticides, including those banned in the EU, were considered to enhance the totality of the epidemiological evidence available at the time of the review.

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# 付属書A-EFSAの外部科学報告書でレビューされた農薬疫学研究及びその他のレビュー

EFSA 外部科学報告書(Ntzani ら、2013 年)によって収集された広範なエビデンスは、疫学研究からの農薬ばく 露と健康影響に関するかなりの量の情報が利用可能であることを強調している。それにもかかわらず、このエビデンス の質は通常低く、多くのバイアスが結果に影響を与え、結論を出すことができない可能性が高い。特に、ばく露疫学は 長い間、測定と定義の貧弱さに悩まされてきたが、特に農薬に関しては、これは常に評価と定義が非常に難しいもので あった。

# A.1. EFSA の外部研究報告書 A.1.1. 方法論的品質評価

外部研究報告書は、2006年1月1日から2012年9月30日までに発表されたすべての疫学研究の包括的なシ ステマティックレビューから構成されており、農薬ばく露ととト健康関連影響の発生との関連性を調査している。

対象となる研究の方法論的評価(各研究に関連するバイアスのリスクを評価する)は、研究デザイン、研究対象集団、 ばく露の定義の詳細度、ばく露の測定方法、測定の特殊性に焦点を当てた。マッチングモデルや多変量モデル、盲検 化されたばく露評価、十分に説明された有効な結果評価を通じた交絡因子の説明などの取り組みが検討された。

方法論的評価の要素は、Research Triangle Institute (RTI; Research Triangle Park, NC, USA)の項目バンク で検討されたもので、観察による研究の偏りのリスクと精度を評価するための実用的で検証済みのツールである。これ らの要素を以下に示す(表 A.1)。

# 表 A.1: Research Triangle Institute (RTI; Research Triangle Park, NC, USA)の疫学研究の方法論的評価のための項目バンクの要素

賞問	高リスク	低リスク
研究デザイン(有望、回顧的、混合、断面的)	回顧的、混合、該当なし	有望
除外基準が明確に記載されている(はい、部分的に、いいえ)	いいえ	はい
著者は電力計算について言及しています(はい、いいえ)		はい
暴露の記述の詳細レベル(高、中、低)	低	高
暴露のロバストな測定(バイオマーカー(有);小面積生態学的尺度、職 種、アンケート(部分的);大面積生態学的尺度(無)に基づいている。)	いいえ	はい
曝露の尺度は特定のものだったか?はい:より広範な化学的に関連した グループに基づいて(部分的)、多様な化学的および毒性学的特性の広範 なグループに基づいて(いいえ)。	いいえ	はい
グループ間の配分のバランスを図る(層別化、マッチングなど)。	いいえ	はい
潜在的な交絡因子の調整を行った(はい、いくつか、いいえ)。	いいえ	はい
被ばく状態に盲検化された評価者(コホート研究の場合)	いいえ	はい
有効かつ信頼性の高い尺度を用いて評価された結果は、すべての研究参加	いいえ	はい
者に一貫して実施されているか?		
サンプルサイズ	低	最大
ラフな品質評価	6 以上の回答で高リスク	6 以上の回答で低リスク

結果の定量的な統合は、対象となる結果ごとに 5 件以上の適格な研究があり、発表されたエビデンス間に実質的な 異質性がない場合に試みられた。出版バイアスは、10 件以上の研究がメタアナリシスに含まれている場合に、非対称 性を視覚的に確認できる漏斗プロットを用いて評価した。

毒性学的データは、外部科学研究報告書ではレビューまたは議論されなかった。

# A.1.2. 除外基準

レビューの時点で入手可能な疫学的証拠の総合的に評価するために、EU で禁止されているものを含むすべての 種類の農薬を検討した。

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除外基準。

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#### Exclusion criteria:

- Studies without control populations (case reports, case series) and ecological studies
- Pesticide poisoning or accidental high dose exposure
- Studies with no quantitative information on effect estimates
- Studies with different follow-up periods and examining the same outcome, only the one with the longest follow-up was retained to avoid data duplication.
- Studies referred to the adverse effects of substances used as therapy for various medical conditions (e.g. warfarin-based anticoagulants)
- Studies on solvents and other non-active ingredients (e.g. co-formulants) in pesticides
- Studies examining the association between exposure and biomarkers of exposure were not considered eligible as they do not examine health outcomes
- Studies/analyses investigating exposure to pesticides: arsenic, hexachlorocyclohexane (HCH)  $\alpha$  or  $\beta$ , lead, dioxins and dioxin-like compounds including polychlorinated biphenyls (PCBs) were not considered
- Narrative reviews were excluded but not systematic reviews or meta-analyses.

Publications reporting series of acute poisonings or clinical cases, biomonitoring studies unrelated to health effects, or studies conducted on animals or human cell systems were not included; only epidemiological studies addressing human health effects were selected. Publications that lacked quantitative data for measuring associations were also excluded.

Cohort studies, case-control studies and cross-sectional studies were included. Each study underwent an assessment of its eligibility based on a method including 12 criteria such as study design, precise description of the inclusion/exclusion criteria, level of detail in describing exposure, robustness in the measurement of exposure, adjustment for potential confounding factors, method of assessment of the health outcome, sample size, etc. Among these 12 criteria, three were related to the degree of precision in the description/measurement of exposure, which may explain why a large number of epidemiological studies were not selected.

#### A.1.3. Results

Overall, 602 individual publications were included in the scientific review. These 602 publications corresponded to 6,479 different analyses. The overwhelming majority of evidence comes from retrospective or cross-sectional studies (38% and 32%, respectively) and only 30% of studies had a prospective design. Exposure assessment varied widely between studies and overall 46% measured biomarkers of pesticides exposure and another 46% used questionnaires to estimate exposure to pesticides. Almost half of the studies (49%) were based in America. Most studies examined associations between occupational exposure to pesticides and health effects. The entire spectrum of diseases associated with pesticides has not been studies before. The report examined a wide variety of outcomes (Figure A.1). The largest proportion of studies pertains to cancer outcomes (N = 164) and outcomes related to child health (N = 84).

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#### Epidemiological studies and pesticides

- ・ 対照集団のない研究(症例報告、症例シリーズ)及び生態学的研究
- ・ 農薬中毒または偶発的な高用量ばく露
- 影響の推定に関する定量的な情報がない研究
- データの重複を避けるために、追跡期間が異なり、同じ健康影響(アウトカム)を調査している研究については、
   追跡期間が最も長いもののみを残した。
- ・様々な病状の治療に使用される物質の有害影響について言及した研究(例:ワルファリンをベースとした抗凝固 薬)。
- ・ 農薬中の溶剤やその他の非有効成分(補助剤など)の研究
- ・ ばく露とばく露のバイオマーカーとの関連性を検討した研究は、健康影響を調べないため対象外とされた。
- ・ 農薬へのばく露を調査した研究/解析:ヒ素、ヘキサクロロシクロヘキサン(HCH)aまたはb、鉛、ダイオキシン 類及びポリ塩化ビフェニル(PCB)を含むダイオキシン様化合物は考慮されなかった。
- ・ナラティブレビューは除外したが、システマティックレビューやメタアナリシスは除外しなかった。

急性中毒または臨床症例のシリーズである出版物、健康影響とは無関係のバイオモニタリング研究、または動物また はヒトの細胞システムで実施された研究は含まれず、ヒトの健康影響を扱った疫学的研究のみが選ばれた。また、関連 性を測定するための定量的データを欠く出版物も除外した。

コホート研究、症例対照研究及び横断研究が含まれた。各研究は、研究デザイン、除外・包含基準の正確な記述、 ばく露の記述の詳細度、ばく露の測定の強固性、潜在的な交絡因子の調整、健康影響の評価方法、サンプルサイズ 等の12の基準を含む方法に基づいて適格性の評価を受けた。これら12の基準のうち、3つの基準はばく露の記述・ 測定の精度に関連しており、多くの疫学研究が選ばれなかった理由を説明することができるかもしれない。

## A.1.3. 結果

全体では、602 の個別の論文が科学的レビューに含まれている。これら 602 の出版物は、6,479 の異なる解析に対応していた。エビデンスの圧倒的多数は後ろ向きまたは横断研究(それぞれ 38%と 32%)であり、前向きな研究は 30%のみであった。ばく露評価は研究によって大きく異なり、全体の 46%が農薬ばく露のバイオマーカーを測定し、さらに46%が農薬ばく露を推定するためにアンケートを使用していた。研究のほぼ半数(49%)がアメリカを拠点としていた。ほとんどの研究では、農薬への職業性ばく露と健康影響との関連性が調査されていた。農薬に関連する疾患の全領域を対象とした研究はこれまで行われていなかった。報告書では、さまざまなアウトカムを調査している(図 A.1)。最も多いのは、がんのアウトカム(N=164)と子どもの健康に関するアウトカム(N=84)である。

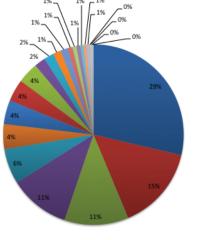






Figure A.1: Major outcome categories and corresponding percentage of studies examining those outcomes among the publications reviewed by the EFSA external scientific report (Ntzani et al., 2013)

Despite the large volume of available data and the large number (> 6.000) of analyses available. firm conclusions were not made for the majority of the outcomes studied. This was due to several limitations of the data collected as well as to inherent limitations of the review itself. As mentioned above, the review studied the whole range of outcomes examined in relation to pesticides during an approximately 5 years' period. Thus, only recent evidence was reviewed and the results of the metaanalyses performed should be cautiously interpreted as they do not include all the available evidence. It is therefore capable of highlighting outcomes which merit further in-depth analysis in relation to pesticides by looking at the entire literature (beyond 5 years) and by focusing on appraising the credibility of evidence selected. The limitations of the studies itself are in line with other field of environmental epidemiology and focus around the exposure assessment, the study design, the statistical analysis and reporting. In particular:

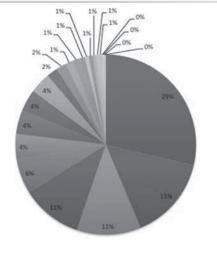
a) Exposure assessment: The assessment of exposure is perhaps the most important methodological limitation of the studies reviewed in the ESR. Studies used different methods for exposure assessment and assignment. Most studies were based on self-reported exposure to pesticides, defined as 'ever versus never' use or as 'regular versus non-regular' use. Such methods suffer from high misclassification rates and do not allow for dose-response analysis. This is especially the case for retrospective studies where misclassification would be differential with higher exposures reported in participants with disease (recall bias) (Raphael, 1987). While questionnaires might be capable of differentiating subjects with very high and very low exposure levels, they are not capable of valid exposure classification across an exposure gradient, thus not allowing the study of dose-response relationships. Also, questionnaire for exposure assessment need to be validated for use in epidemiological studies. Nonetheless, a vast proportion of studies use in house version of non-validated questionnaires which may suffer from content (the questionnaire does not cover all sources of exposure to the hazard of interest) or criterion validity (e.g. through inaccurate recall or misunderstanding of questions) (Coggon, 1995).

Although the range of categories of pesticide studied is wide, studies very often concentrate on a broadly defined pesticide category, so that it is difficult to know what type of pesticide the population is exposed to.

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Cancer outcomes Child health ■ Reproductive diseases Neurological diseases Endocrine diseases Respiratory diseases ■ Neuropsychiatric diseases Diabetes (type I and II) Cardiovascular diseases ■ Mortality Immune/Autcimmune diseases ■ Allergic diseases Gastrointestiral diseases Symptoms and general health # Gynecological diseases Skin diseases ■ Bone diseases Hematological diseases

### 図 A.1:EFSA の外部科学研究報告書でレビューされた出版物のうち、主要な健康影響のカテゴリーと、その健康影 響を調査した研究の割合(Ntzaniら、2013年)

利用可能な大量のデータと大量の分析(>6,000 件)にもかかわらず、研究されたアウトカムの大部分については確 固たる結論が出ていない。これは、収集したデータのいくつかの限界と、レビュー自体の固有の限界によるものである。 上述したように、レビューでは約5年間に農薬に関連して検討されたアウトカムの全範囲が調査された。したがって、最 近のエビデンスのみがレビューされ、実施されたメタアナリシスの結果は、利用可能なすべてのエビデンスを含んでい るわけではないため、慎重に解釈されるべきである。したがって、文献全体(5年以上)をみて、選択したエビデンスの 信頼性を評価することに焦点を当てることで、農薬に関連してさらに詳細な解析を行う価値のある結果を浮き彫りにす ることができる。研究自体の限界は、環境疫学の他の分野と一致しており、ばく露評価、研究デザイン、統計解析、報 告に焦点を当てている。特に以下の点が挙げられる:

a) ばく (標評価: ばく 露の評価は、おそらく ESR でレビューされた研究の中で最も重要な方法論的限界である。 研究 では、ばく露の評価と割り付けにさまざまな方法が用いられている。ほとんどの研究では、「これまでに使用したこと があるかないか」または「定期的に使用したことがあるかないか」という自己申告による農薬へのばく露に基づいて いた。このような方法では、高い誤分類率に悩まされ、用量反応分析を行うことができない。これは特に後ろ向き研 究の場合で、病気のある参加者で報告されるより高いばく露量の差が誤分類を生じる(想起バイアス)(Raphael、 1987年)。アンケートは非常に高いばく露レベルと非常に低いばく露レベルの被験者を区別することができるかも しれないが、ばく露濃度による有効な分類を行うことはできず、その結果、用量反応関係の研究を行うことができな い。また、ばく露評価のためのアンケートは、疫学研究で使用するために検証される必要がある。それにもかかわ らず、多くの研究では検証されていないアンケートを使用しているが、これには内容(アンケートが対象とする有害 なばく露源をすべて網羅していない)や基準妥当性(例えば、不正確な想起や質問の誤解)に問題があるかもしれ ない(Coggon、1995年)。

調査対象とした農薬のカテゴリーの範囲は広いが、研究では多くの場合、広く定義された農薬のカテゴリーに集中し ているため、対象集団がどのような種類の農薬にばく露されているのかを知ることは困難である。

農薬へのばく露は、研究参加者による農薬の使用報告または政府の登録データとして定義された。これらのデータ EESA Journal 2017:15(10):5007 www.efsa.europa.eu/efsajourna 70

Exposure to pesticides was defined as reported use of pesticides by the study participant or by government registry data. These derive from self-administered questionnaires, interviewer administrated questionnaires, job exposure matrices (JEM), by residential status (proximity to pesticide exposure), by detecting biomarkers associated with pesticide exposure or by other means as defined by each study.

Studies often examine pesticides that have already been banned in western populations and the EU. The use of biomarkers as means of exposure assessment is infrequent, but still available in almost half of the studies.

b) Study design: As mentioned above, the majority of evidence comes form case-control studies and cross-sectional studies. Cross-sectional, and in part also case-control studies, cannot fully assess the temporal relationships and thus are less able to provide support regarding the causality of associations.

c) Outcomes examined: The definition of clinical outcomes displayed large variability in eligible epidemiological studies, which can further cause the variability in results. Perhaps most important in this setting is the use of a great number of surrogate outcomes examined. Surrogate outcomes are biomarkers or physical measures that are generally accepted as substitutes for, or predictors of, specific clinical outcomes. However, often these surrogate outcomes are not validated and do not meet the strict definitions of surrogate outcomes. Such outcomes can be defined as possible predictors of clinical outcomes but do not fulfil the criteria for a surrogate outcome. It is essential to appraise the evidence around non-validated surrogate outcomes by taking into account the implicit assumptions of these outcomes.

A great variety of assessed outcomes covering a wide range of pathophysiologies was observed. 'Hard' clinical outcomes as well as many surrogate outcomes included in the database reflect the different methodologies endorsed to approach the assessed clinical research questions. The different outcomes were divided into 23 major disease categories, with the largest proportion of studies addressing cancer and child health outcomes.

The adverse health effects assessed included:

- a) major clinical outcomes, such as cancer, respiratory (allergy), reproductive (decreased fertility, birth defects) and neurodegenerative (Parkinson's disease);
- b) clinical surrogate outcomes, e.g. neurodevelopmental impairment (assessed by neurocognitive scales);
- c) laboratory surrogate outcomes (e.g. liver enzyme changes).

For many adverse health effects attributed to pesticide exposure, there exist contradictory or ambiguous studies. Whether this results from lack of consistency or real heterogeneity warrants further clarification.

#### d) Statistical analysis:

Simultaneous exposure to multiple agents (heavy metals, solvents, suspended particulate matter etc.) from different sources is common. It may introduce further bias in the results as all of them may produce adverse health outcomes. Thus, it is essential to account for confounding from exposure to multiple agents in order to delineate true associations but this has not been possible in the overwhelming majority of evidence assessed in the EFSA external scientific report.

In addition, the evidence collected and appraised in the EFSA external scientific report (Ntzani et al., 2013) is likely to suffer from selective reporting and multiple testing. The studies reported a very wide range of analyses; 602 publications resulted in 6,000 analyses. The amount of multiple hypothesis testing is enormous. These analyses need to be adjusted for multiple hypothesis testing else, otherwise the results suffer from high false positive rate. Even when studies present only one analysis, selective reporting is always a possibility as has been shown in other epidemiological fields as well. In addition, when interpreting results one should also take into account that, especially for certain outcomes (e.g. cancers), the majority of evidence comes from single study populations and the Agricultural Health Study in particular.

### A.1.4. Conclusion of the EFSA External Scientific Report

Regardless of the limitations highlighted above, the External Scientific Report (Ntzani et al., 2013) showed consistent evidence of a link between exposure to pesticides and Parkinson's disease and childhood leukaemia, which was also supported by previous meta-analyses. In addition, an increased risk was also found for diverse health outcomes less well studied to date, such as liver cancer, breast cancer and type II diabetes. Effects on other outcomes, such as endocrine disorders, asthma and allergies, diabetes and obesity showed increased risks and should be explored further.

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は、自己記入式アンケート、質問者が管理するアンケート、職業性ばく露マトリックス(JEM)、居住状況(農薬ばく露に 近接しているかどうか)、農薬ばく露に関連するバイオマーカーの検出、または各研究によって定められたその他の方 法から得られたものである。

研究では、欧米の集団や EU ですでに禁止されている農薬を調査することが多い。ばく露評価の手段としてバイオ マーカーを使用することはまれであるが、ほぼ半数の研究ではまだ利用可能である。

- b) 研究デザイン:上述したように、エビデンスの大部分は症例対照研究と横断研究から得られている。横断研究や一部の症例対照研究では、時間的関係を完全に評価することができないため、関連性の因果関係に関する裏付けを提供することができない。
- c)調査された健康影響:臨床健康影響の定義は、適格な疫学研究において大きなばらつきを示しており、これが結果のばらつきの原因となっている。おそらくこのような状況において最も重要なのは、調査された多数の代替健康影響の使用である。代替健康影響とは、特定の臨床転帰の代替または予測因子として一般的に受け入れられているバイオマーカーまたは身体測定値のことである。しかし、多くの場合、これらの代替健康影響は検証されておらず、代替健康影響の厳密な定義を満たしていない。このような健康影響は、臨床健康影響の予測因子の可能性があるとされているが、代替健康影響の基準を満たしていない。これらの健康影響の暗黙の仮定を考慮に入れることにより、検証されていない代替健康影響に関するエビデンスを評価することが不可欠である。

広範囲の病態生理をカバーすると評価されたアウトカムは非常に多様だった。データベースに含まれる多くの代替 健康影響と同様に「ハード」な臨床健康影響は、評価された臨床研究の課題にアプローチするために支持された異な る方法論を反映している。さまざまな健康影響は 23 の主要な疾患カテゴリーに分類されており、がんと小児の健康影 響を扱った研究が最も多くを占めている。

評価された健康への悪影響には以下のものが含まれる。

- a)がん、呼吸器(アレルギー)、生殖(受精率低下、先天性疾患)、神経変性(パーキンソン病)などの主要な臨床転帰;
- b)神経発達障害(神経認知スケールで評価)などの臨床代替健康影響;

c)検査での代替健康影響(例:肝酵素の変化)。

農薬ばく露に起因する多くの有害な健康影響については、矛盾した、あるいは曖昧な研究が存在する。この結果が 一貫性の欠如からくるものなのか、真の不均一性からくるものなのかは、さらなる解明が必要である。

#### d) 統計的解析。

異なる供給源からの複数の物質(重金属、溶剤、浮遊粒子状物質など)への同時ばく露は一般的である。それらの すべてが有害な健康影響をもたらす可能性があるため、結果にさらなるバイアスがかかる可能性がある。したがって、 真の関連性を明らかにするためには、複数の物質へのばく露による交絡を考慮することが不可欠であるが、EFSAの 外部科学研究報告書で評価された圧倒的多数のエビデンスでは、これは不可能であった。

さらに、EFSA の外部科学研究報告書(Ntzani ら、2013 年)で収集・評価されたエビデンスは、都合の良い報告と 複数の試験に悩まされる可能性が高い。研究は非常に広範な解析を報告しており、602 の出版物で 6,000 の解析が 行われた。多重仮説検定の量は膨大である。これらの解析は、複数の仮説検定のために調整されていなければならず、 そうでなければ結果は高い偽陽性率に悩まされる。研究が1つの解析しか行われていない場合でも、他の疫学的研究 でも示されているように、都合の良い報告の可能性が常にある。さらに、結果を解釈する際には、特に特定の健康影響 (がんなど)については、エビデンスの大部分が単一の研究集団そして特に農業健康調査から得られていることも考慮 に入れるべきである。

### A.1.4. EFSA 外部科学研究報告書の結論

上記で強調された限界にもかかわらず、外部科学研究報告書(Ntzani 6、2013 年)では農薬ばく露とパーキンソン 病及び小児の白血病との関連、これらは先行するメタアナリシスでも裏付けられている、について首尾一貫したエビデ ンスを示した。さらに、肝臓がん、乳がん、Ⅱ型糖尿病など、これまであまり研究されてこなかった多様な健康影響につ

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Childhood leukaemia and Parkinson's disease are the two outcomes for which a meta-analysis after 2006 was found consistently showing an increased risk associated with pesticide exposure. Nonetheless, the exposure needs to be better studied to disentangle the effect of specific pesticide classes or even individual pesticides. Significant summary estimates have also been reported for other outcomes (summarised in Table A.2). However, as they represent studies from 2006 onwards results should be regarded as suggestive of associations only and limitations especially regarding the heterogeneity of exposure should always been taken into consideration. Data synthesis and statistical tools should be applied to these data in relation to specific outcomes, after the update of the results to include publications before 2006, in order to quantify the amount of bias that could exist and isolate outcomes where the association with pesticides is well supported even when estimates of bias are taken into account. Similarly, outcomes where further evidence is needed to draw firm conclusions need to be highlighted.

#### **Table A.2:** Summary of meta-analyses performed in the report

Health outcome	N studies	Meta-analysis results	I <sup>2</sup>
Leukaemia	6	1.26 (0.93; 1.71)	59.4%
Hodgkin lymphoma	7	1.29 (0.81-2.06)	81.6%
Childhood leukaemia (exposure to pesticides during pregnancy)	6	1.67 (1.25-2.23)	81.2%
Childhood leukaemia (exposure to insecticides during pregnancy)	5	1.55 (1.14–2.11)	65%
Childhood leukaemia (exposure to insecticides during pregnancy $-$ update Turner, 2010)	9	1.69 (1.35–2.11)	49.8%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy)	5	2.00 (1.73–2.30)	39.6%
Childhood leukaemia (exposure to unspecified pesticides during pregnancy – update Turner, 2010)	11	1.30 (1.06–1.26)	26.5%
Childhood leukaemia (exposure to pesticides during childhood)	7	1.27 (0.96-1.69)	61.1%
Childhood leukaemia (exposure to insecticides during childhood – update Turner, 2010)	8	1.51 (1.28–1.78)	0%
Childhood leukaemia (exposure to unspecified pesticides during childhood – update Turner, 2010)	11	1.36 (1.19–1.55)	0%
Breast cancer (DDE exposure)	5	1.13 (0.81–1.57)	0%
Breast cancer	11	1.24 (1.08–1.43)	0%
Testicular cancer (DDE exposure)	5	1.40 (0.82–2.39)	59.5%
Stomach cancer	6	1.79 (1.30–2.47)	0%
Liver cancer	5	2.50 (1.57–3.98)	25.4%
Cryptorchidism	8	1.19 (0.96–1.49)	23.9%
Cryptorchidism (DDT exposure)	4	1.47 (0.98-2.20)	51%
Hypospadias (general pesticide exposure)	6	1.01 (0.74–1.39)	71.5%
Hypospadias (exposure to specific pesticides)	9	1.00 (0.84–1.18)	65.9%
Abortion	6	1.52 (1.09–2.13)	63.1%
Parkinson's disease	26	1.49 (1.28–1.73)	54.6%
Parkinson's disease (DDT exposure)	5	1.01 (0.78-1.30)	0%
Parkinson's disease (paraquat exposure)	9	1.32 (1.09–1.60)	34.1%
Amyotrophic lateral sclerosis	6	1.58 (1.31-1.90)	10%
Asthma (DDT exposure)	5	1.29 (1.14–1.45)	0%
Asthma (paraquat exposure)	6	1.40 (0.95-2.06)	53.3%
Asthma (chlorpyrifos exposure)	5	1.03 (0.82–1.28)	0%
Type 1 diabetes (DDE exposure)	8	1.89 (1.25–2.86)	49%
Type 1 diabetes (DDT exposure)	6	1.76 (1.20–2.59)	76.3%
Type 2 diabetes (DDE exposure)	4	1.29 (1.13–1.48)	0%

N = number of studies considered for the meta-analysis; in the column of meta-analysis results, the numbers represent the statistical estimate for the size of effect (odds ratio (OR), or relative risk (RR)) with the corresponding 95% confidence interval (CI). I<sup>2</sup> represents the percentage of total variation across studies that is due to heterogeneity.

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#### Epidemiological studies and pesticides

いてもリスクの増加が認められた。内分泌疾患、喘息、アレルギー、糖尿病、肥満などの他の健康影響への影響は、リ スクの増加を示しており、今後さらに調査が必要である。

小児白血病とパーキンソン病は、2006 年以降のメタアナリシスで一貫して農薬ばく露に関連したリスクの増加を示した2 つの健康影響である。それにもかかわらず、特定の農薬クラスや個々の農薬の影響を解きほどくためには、ばく露をよりよく研究する必要がある。他の健康影響についても、有意な要約推定値が報告されている(表 A.2 に要約)。しかし、これらは2006 年以降の研究であるため、結果は関連性を示唆するものとみなすべきであり、特にばく露の不均一性に関する限界は常に考慮されるべきである。存在しうるバイアスの量を定量化し、バイアスの推定値を考慮に入れても農薬との関連性が十分に支持される結果を分離するために、2006 年以前の出版物を含むように結果を更新した後、特定の健康影響に関連してこれらのデータにデータ統合と統計ツールを適用すべきである。同様に、結論を出すためにさらなるエビデンスが必要な健康影響は、強調される必要がある。

#### 表 A.2:報告書で実施されたメタアナリシスの概要

健康面での成果		メタアナリシスの	<sup>2</sup>
	studies	結果	
白血病	6	1.26 (0.93; 1.71)	59.40%
ホジキンリンパ腫	7	1.29 (0.81-2.06)	81.60%
小児白血病(妊娠中の農薬ばく露)	6	1.67 (1.25-2.23)	81.20%
小児白血病(妊娠中の殺虫剤ばく露)	5	1.55 (1.14-2.11)	65%
小児白血病(妊娠中に殺虫剤にばく露された場合-ターナー,2010 年の更新)	9	1.69 (1.35-2.11)	49.80%
小児白血病(妊娠中に不特定多数の農薬にばく露された場合	5	2.00 (1.73-2.30)	39.60%
小児白血病 (妊娠中の特定されていない農薬へのばく露 - ターナー、2010 年の更新)	11	1.30 (1.06-1.26)	26.50%
小児白血病(小児期の農薬ばく露)	7	1.27 (0.96-1.69)	61.10%
小児白血病 (小児期の殺虫剤ばく露-小児期の殺虫剤ばく露,更新 ターナー,2010年 の更新)	8	1.51 (1.28–1.78)	0%
小児白血病 (小児期に特定されていない農薬へのばく露 - ターナー、2010 年の更新)	11	1.36 (1.19-1.55)	0%
乳がん(DDE ばく露)	5	1.13 (0.81-1.57)	0%
乳がん	11	1.24 (1.08-1.43)	0%
精巣がん(DDE ばく露)	5	1.40 (0.82-2.39)	59.50%
胃がん	6	1.79 (1.30-2.47)	0%
肝臓がん	5	2.50 (1.57-3.98)	25.40%
停留精巣	8	1.19 (0.96-1.49)	23.90%
停留精巣(DDT ばく露)	4	1.47 (0.98-2.20)	51%
尿道下裂(一般的な農薬ばく露)	6	1.01 (0.74-1.39)	71.50%
尿道下裂(特定の農薬へのばく露)	9	1.00 (0.84-1.18)	65.90%
流産	6	1.52 (1.09-2.13)	63.10%
パーキンソン病	26	1.49 (1.28-1.73)	54.60%
パーキンソン病(DDT ばく露)	5	1.01 (0.78-1.30)	0%
パーキンソン病(パラコートばく露)	9	1.32 (1.09-1.60)	34.10%
筋萎縮性側索硬化症	6	1.58 (1.31-1.90)	10%
喘息(DDT ばく露)	5	1.29 (1.14-1.45)	0%
喘息(パラコートばく露)	6	1.40 (0.95-2.06)	53.30%
喘息(クロルピリホスばく露)	5	1.03 (0.82-1.28)	0%
1 型糖尿病(DDE ばく露)	8	1.89 (1.25-2.86)	49%
1 型糖尿病(DDT ばく露)	6	1.76 (1.20-2.59)	76.30%
2 型糖尿病(DDE ばく露)	4	1.29 (1.13-1.48)	0%

N=メタアナリシスのために検討された研究の数;メタアナリシス結果の列では、数字は効果の大きさ(オッズ比(OR)または相対リ スク(RR))の統計的推定値を、対応する 95%信頼区間(CI)とともに表している。

l<sup>2</sup>は、研究間の総変動のうち、不均一性に起因する割合を示す。

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#### A.2. The INSERM report

In September 2013, the French National Institute of Health and Medical Research (INSERM) released a literature review carried out with a group of experts on the human health effects of exposure to pesticides.<sup>22</sup> Epidemiological or experimental data published in the scientific literature up to June 2012 were analysed. The report was accompanied by a summary outlining the literature analysis and highlighting the main findings and policy lines, as well as the recommendations.

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The INSERM report is composed of four parts: (1) exposure assessment, with a detailed description of direct and indirect methods to assess exposure in epidemiological studies; (2) epidemiology, with an inventory and analysis of epidemiological studies available in the literature up to 2012, and a scoring system to assess the strength of presumed association; (3) toxicology, with a review of toxicological data (metabolism, mode of action and molecular pathway) of some substances and assessment of biological plausibility; (4) recommendations.

The vast majority of substances identified by the INSERM report as having a presumed moderate or strong association with the occurrence of health effects are chemicals that are now prohibited. This is mainly driven by the fact that the majority of the diseases examined are diseases of the elderly; therefore, the studies performed to date are based on persons who were old at the time of the study and exposed many years ago. By definition, it is not yet possible to investigate the potential long term effects of many of the more recent products.

These substances belong to the group of organochlorine insecticides, such as DDT or toxaphene, or insecticides with cholinesterase-inhibiting properties, such as terbufos or propoxur.

Of the seven approved active substances identified by the INSERM expert appraisal report (the herbicides 2,4-D, MCPA, mecoprop, glyphosate, the insecticide chlorpyrifos, and the foliar fungicides mancozeb and maneb), all had a presumed moderate or weak association with haematopoietic cancers. Two of them (the foliar fungicides mancozeb and maneb) had a presumed weak association with Parkinson's disease and two (chlorpyrifos and glyphosate) had a presumed association with developmental impairment identified as weak or moderate in the expert appraisal.

#### A.2.1. Description of methods to assess exposure in epidemiological studies

Different methods (direct and indirect) have been developed to assess exposure, such as biological or environmental monitoring data, ad hoc questionnaires, job- or crop-exposure matrices, analysis of professional calendars, sales data, land use data, etc. According to the authors, these various tools can be combined with each other but, to date none has been validated as a reference method for estimating exposure in the context of occupational pesticide exposure assessment.

#### A.2.2. Epidemiology

The group of experts from INSERM carried out an inventory and analysis of epidemiological studies available in the literature, examining the possible association between pesticide exposure and health outcomes: eight cancer sites (non-Hodgkin lymphoma, leukaemia, lymphoma, multiple myeloma, prostate, testis, brain, melanoma), three neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis), cognitive or depressive disorders, effects on reproductive function (fertility, pregnancy and child development) and childhood cancers. These are health outcomes that have been identified in previous studies as potentially related to pesticide exposure.

Epidemiological studies addressing primarily farmers, pesticide applicators and workers of the pesticide manufacturing industries, as well as the general population when it was relevant, were selected.

The INSERM group of experts established a hierarchy in the relevance of the studies, placing the meta-analysis at the top, then the systematic review, then the cohort study, and finally, the case-control study. Based on this hierarchy, a scoring system was defined to assess the strength of presumption of the association between exposure and the occurrence of health outcomes from the analysis of the study results; for each disease or pathological condition investigated, this score may vary depending on the quality, type and number of available studies, as, for example:

(++): strong presumption: based on the results of a meta-analysis, or several cohort studies or at least one cohort study and two case–control studies, or more than two case–control studies;

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#### A.2. INSERIM レポート

2013 年 9 月、フランス国立保健医療研究所(INSERM)は、農薬へのばく露によるとト健康影響について専門家グ ループと共に実施した文献レビューを発表した22。2012 年 6 月までの科学文献に発表された疫学的または実験的デ ータが解析された。報告書には、文献的解析を概説し、主要な結論と政策及び勧告を強調した要約が添付されている。

INSERM の報告書は 4 つの部分から構成されている。(1) ばく露評価、疫学研究におけるばく露を評価するための 直接的及び間接的な方法の詳細な説明、(2) 疫学、2012 年までの文献で利用可能な疫学研究のインベントリと解析 及び推定される関連性の強さを評価するためのスコアリングシステム、(3) 毒性学、いくつかの物質の毒性学的データ (代謝、作用機序、分子経路)のレビューと生物学的妥当性の評価、(4) 推奨事項。

健康影響の発生と推定される中等度または強い関連性を持つと INSERM の報告書で特定された物質の大部分は、 現在禁止されている化学物質である。これは主に、調査された疾患の大部分が高齢者の疾患であるという事実によっ て推進されている。したがって、これまでに実施された研究は、研究の時点で高齢であり、何年も前にばく露された人に 基づいている。結論から言うと、最近の製品の多くの潜在的な長期的影響を調査することはまだ可能ではない。

これらの物質は、DDT やトキサフェンのような有機塩素系殺虫剤や、テルブフォスやプロポキサーのようなコリンエス テラーゼ阻害作用を持つ殺虫剤のグループに属している。

INSERM の専門家評価報告書で確認された 7 つの承認された有効成分(除草剤 2,4-D、MCPA、メコプロップ、グ リホサート、殺虫剤クロルビリホス、葉の殺菌剤マンコゼブとマネブ)のうち、すべてが造血器がんとの中等度または弱い 関連性があると推定されていた。そのうち 2 つ(葉状殺菌剤マンコゼブとマネブ)はパーキンソン病との関連性が弱いと 推定され、2 つ(クロルビリホスとグリホサート)は専門家の評価で弱い、または中等度とされた発達障害との関連性があ ると推定された。

### A.2.1. 疫学研究におけるばく露評価方法の説明

ばく露を評価するために、生物学的または環境モニタリングデータ、その場しのぎのアンケート、職業別または作物 別のばく露マトリクス、専門家のカレンダーの分析、販売データ、土地利用データなど、さまざまな方法(直接的及び間 接的)が開発されてきた。著者らによると、これらの様々なツールは互いに組み合わせることができるが、現在までのとこ ろ、職業性農薬ばく露評価の背景でばく露を推定するための基準となる方法として有効性が確認されていない。

#### A.2.2. 疫学

INSERMの専門家グループは、文献で入手可能な疫学研究の目録作成と解析を行い、農薬ばく露と健康影響との 関連性の可能性を検討した。8 つのがん部位(非ホジキンリンパ腫、白血病、リンパ腫、多発性骨髄腫、前立腺、精巣、 脳、メラノーマ)、3 つの神経変性疾患(パーキンソン病、アルツハイマー病、筋萎縮性側索硬化症)、認知・抑うつ障害、 生殖能への影響(受胎性、胎児と出生児の発生)、小児がんである。これらは、以前の研究で農薬ばく露に関連する可 能性があるとして同定されている健康影響である。

主に農家、農薬散布者、農薬製造業の労働者を対象とした疫学的研究と、関連性がある場合には一般集団を対象 とした研究が選ばれた。

INSERM の専門家グループは、研究の関連性における階層を設定し、メタアナリシスを最上位に置き、次にシステ マティックレビュー、コホート研究、そして最終的には症例対照研究とした。この階層に基づいて、研究結果の解析から、 ばく露と健康影響の発生との間の関連性の推定の強さを評価するためにスコアリングシステムが定義された;調査され た各疾患や病態について、このスコアは、例えば、利用可能な研究の質、種類、数によって異なる。

(++):強い推定:メタアナリシスの結果に基づく、または複数のコホート研究、または少なくとも 1 つのコホート研究と 2 つの症例対照研究、または 2 つ以上の症例対照研究。

(+):中程度の推定:コホート研究または入れ子になった症例対照研究または2つの症例対照研究の結果に基づ

<sup>&</sup>lt;sup>22</sup> INSERM. Pesticides. Effets sur la santé. Collection expertise collective, Inserm, Paris, 2013.

<sup>&</sup>lt;sup>22</sup> INSERM. 農薬。 sante への影響。 Collection expertise collective, Inserm, Paris, 2013.



(+): moderate presumption: based on the results of a cohort study or a nested case–control study or two case–control studies;

 $(\pm):$  weak presumption: based on the results of one case–control study. This synthesis takes the work beyond the status of a simple mapping exercise.

#### A.2.3. Toxicological data

Toxicological data that were considered in the literature review were mainly those regarding metabolism, mode of action and molecular pathways. None of the studies provided as part of the procedures for placing products on the market were considered except if they were published in the open literature.

When substances were clearly identified in the epidemiological studies, a scoring system was defined to assess the biological plausibility from the study results: coherence with pathophysiological data and occurrence of health outcome.

(++): hypothesis supported by 3 mechanisms of toxicity;

(+): hypothesis supported by at least one mechanism of toxicity.

#### A.2.4. Findings

The major results of the INSERM report are summarised in Tables A.3-A.6.

Table A.3:	Statistically significant associations between occupational exposure to pesticides and	
	health outcomes in adults (health outcomes that were analysed in the review)	

Health outcome	Type of population with significant risk excess	Strength of presumption <sup>(a)</sup>
NHL	Farmers, operators, manufacturing plant personnel	++
Prostate cancer	Farmers, operators, manufacturing plant personnel	++
Multiple myeloma	Farmers, operators	++
Parkinson's disease	Occupational and non-occupational exposure	++
Leukaemia	Farmers, operators, manufacturing plant personnel	+
Alzheimer's disease	Farmers	+
Cognitive disorders <sup>(b)</sup>	Farmers	+
Fertility and fecundability disorders	Occupational exposure	+
Hodgkin lymphoma	Agricultural workers	±
Testicular cancer	Agricultural workers	±
Brain cancer (glioma, meningioma)	Agricultural workers	±
Melanoma	Agricultural workers	±
Amyotrophic lateral sclerosis	Farmers	±
Anxiety, depression <sup>(b)</sup>	Farmers, farmers with a history of acute poisoning, operators	±

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(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ). (b): Almost all pesticides were organophosphates.

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### $<_{\circ}$

(±):弱い推定:1件の症例対照研究の結果に基づく。この統合により、この作業は単純なマッピング作業の状態を 超えたものとなった。

### A.2.3. 毒性学的データ

文献レビューで検討した毒性データは、主に代謝、作用機序、分子経路に関するものであった。製品を上市するための手続きの一部として提供された研究は、公表された文献で発表された場合を除き、考慮されなかった。

疫学研究で物質が明らかに同定された場合には、研究結果から生物学的に妥当であるかどうかを評価するためのス コアリングシステムを設定した:病態生理学的データとの整合性と健康影響の発生。

(++):3つの毒性メカニズムで支持された仮説。

(+):少なくとも1つの毒性メカニズムによって支持された仮説。

#### A.2.4. 所見

INSERM 報告書の主な結果は、表 A.3-A.6 にまとめられている。

#### 表 A.3:農薬への職業上ばく露と成人の健康影響との間の統計的に有意な関連(レビューで解析された健康影響)

健康面での成果	リスク過剰が顕著な集団のタイプ	推定の強さ <sup>(a)</sup>
エヌエイチエル	農家、農薬散布者、製造工場関係者	++
前立腺がん	農家、農薬散布者、製造工場関係者	++
多発性骨髄腫	農家、農薬散布者	++
パーキンソン病	職業上ばく露と非職業上ばく露	++
白血病	農家、農薬散布者、製造工場関係者	+
アルツハイマー病	農家	+
認知障害 <sup>(b)</sup>	農家	+
妊孕性・胎動性障害	職業上ばく露	+
ホジキンリンパ腫	農業従事者	±
精巣がん	農業従事者	±
脳腫瘍(神経膠腫、髄膜腫	農業従事者	±
メラノーマ	農業従事者	±
筋萎縮性側索硬化症	農家	±
不安、うつ病 <sup>(b)</sup>	農家、急性中毒の既往歴のある農家、農薬散布者	±

(a):スコアリングシステム:強い推定(++)、中程度の推定(+)、弱い推定(±)。
 (b):ほとんどの農薬が有機リン酸塩であった。

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Table A.4: Associations between occupational or home use exposure to pesticides and cancers or developmental impairment in children (health outcomes that were analysed in the review) (only statistically significant associations are shown)

Health outcome	Type of exposure and population with significant risk excess	Strength of presumption <sup>(a)</sup>
Leukaemia	Occupational exposure during pregnancy, prenatal exposure (residential)	++
Brain cancer	Occupational exposure during pregnancy	++
Congenital malformation	Occupational exposure during pregnancy; Residential exposure during pregnancy (agricultural area, home use)	++ +
Fetal death	Occupational exposure during pregnancy	+
Neurodevelopment	Residential exposure during pregnancy (agricultural area, home use, food) <sup>(b)</sup> ; Occupational exposure during pregnancy	++ ±

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ).

(b): Organophosphates.

Table A.5: Findings related to approved active substances: epidemiological assessment and biological plausibility

Active substance	Classification	Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>
Organophosphates Insecticide			
Chlorpyrifos	Acute Tox cat 3	Leukaemia (+) Neurodevelopment (+) NHL (±)	Yes (++) Yes (++) Yes (++)
Dithiocarbamates Fungicide			
Mancozeb/Maneb	Repro cat 2	Leukaemia (+) Melanoma (+) Parkinson's disease (in combination with paraquat) (±)	? ? Yes (+)
Phenoxy herbicides Herbicide	1		
2,4-D MCPA Mecoprop	Acute Tox cat 4 Acute Tox cat 4 Acute Tox cat 4	NHL (+) NHL (±) NHL (±)	? ? ?
Aminophosphonate Herbicide	glycine		
Glyphosate		NHL (+) Fetal death (±)	? ?

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ). (b): Scoring system: (++): hypothesis supported by 3 different known mechanisms of toxicity, (+): hypothesis supported by at least one mechanism of toxicity.

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#### 表 A.4:職業または家庭生活での農薬ばく露と小児のがんまたは発達障害(レビューで解析された健康影響)との間の 関連(統計的に有意な関連のみを示す) .

健康面での成果	リスク超過が顕著なばく露の種類と集団	推定の強さ <sup>(a)</sup>
白血病	妊娠中の職業上ばく露、出生前ばく露(住居)	++
脳腫瘍	妊娠中の職業上ばく露	++
先天性奇形	妊娠中の職業上ばく露。	++
	妊娠中の住居ばく露(農地、家庭生活での使用)	+
胎児の死	妊娠中の職業上ばく露	+
神経発達	妊娠中の住居ばく露(農地、家庭生活、食品) <sup>(b)</sup> 。	++
	妊娠中の職業上ばく露	±

(a):スコアリングシステム:強い推定 (++)、中程度の推定 (+)、弱い推定 (±)。 (b):有機リン酸系。

#### 表 A.5:承認された有効成分に関する知見:疫学的評価と生物学的妥当性

有効成分	分類	推定の強さ <sup>(a)</sup>	生物学的妥当性 (b)
有機リン酸系殺虫剤			
クロルピリホス	急性毒性 CAT3	白血病 (+)	Yes (++)
		神経発達 (+)	Yes (++)
		NHL (±)	Yes (++)
ジチオカルバメート	系殺菌剤		
マンコゼブ/マネブ	生殖毒性 CAT2	白血病 (+)	?
		メラノーマ (+)	?
		パーキンソン病	Yes (+)
		(パラコートと併用して)(±)	
フェノキシ系除草剤			
2,4-D	急性毒性 CAT4	NHL (+)	?
MCPA	急性毒性 CAT4	NHL (±)	?
メコプロップ	急性毒性 CAT4	NHL (±)	?
アミノホスホン酸グ	リシン除草剤		

グリホサート	NHL (+)	2
y y a y 1		:
	胎児死亡 (±)	?

(a):スコアリングシステム:強い推定 (++)、中程度の推定 (+)、弱い推定 (±)。

(b):スコアリングシステム。(++):毒性の3つの異なる既知のメカニズムによって支持された仮説、(+):毒性の少なくとも1つ のメカニズムによって支持された仮説。



# Table A.6: Findings related to non-approved active substances: epidemiological assessment and biological plausibility

Active substance	Ban in the EU	IARC classification	Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>
Dieldrin	rin 1978 3 or 2 (US-EPA) NHL <sup>(c)</sup> (土) Prostate cancer (土) Parkinson's disease (ニ			Yes (+) Yes (+)
DDT/DDE	1978 2B NHL (++) Testicular cancer (+) Child growth (++) Neurodevelopment (±) Impaired sperm parameters (+)		Yes (+) ? ?	
Chlordane	1978	2B	NHL (±) Leukaemia (+) Prostate cancer (±) Testicular cancer (+)	Yes (+) Yes (+) Yes (+) ?
Lindane (γ-HCH)	2002/2004/2006/2007	2B <sup>(d)</sup>	NHL (++) Leukaemia (+)	Yes (++) Yes (++)
β-ΗϹΗ	2002/2004/2006/2007	2B <sup>(d)</sup>	Prostate cancer (±)	?
, Toxaphene	2004	2B	NHL <sup>(c)</sup> (±) Leukaemia (+) Melanoma (+)	Yes (++) Yes (++) Yes (+)
Chlordecone	2004	2В	Cancer prostate (++) Impaired sperm parameters (+) Neurodevelopment (+)	Yes (+) ? ?
Heptachlor	1978	2B	Leukaemia (+)	Yes (+)
Endosulfan	2005	Not classified	?	Yes (+)
Hexachlorobenzene (HCB)	1978	2B	Child growth (+)	?
Terbufos	2003/2007		NHL (+) Leukaemia (+)	? ?
Diazinon	2008		NHL (+) Leukaemia (+)	? ?
Malathion	2008	3	NHL (++) Leukaemia (+) Neurodevelopment (+) Impaired sperm parameters (+)	Yes (+) Yes (+) ? ?
Fonofos	2003		NHL (±) Leukaemia (+) Prostate cancer (+)	? ? ?
Parathion	2002	3	Melanoma (+)	?
Coumaphos	Never notified and authorised in the EU		Prostate cancer (+)	?
Carbaryl	2008	3	NHL (±) Melanoma (+) Impaired sperm parameters (+)	? ? ?
Propoxur	2002		Neurodevelopment (+) Fetal growth (+)	? ?
Carbofuran	2008		NHL (±) Prostate cancer (+)	? ?
Butylate	2003		NHL (+) Prostate cancer (+)	? ?
EPTC	2003		Leukaemia (+)	?

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有効成分	Ban in the EU	IARC classfication	推定の強さ <sup>(a)</sup>	生物学的妥当性
ディルドリン	1978	3or2 (US- EPA)	NHL (c) 前立腺がん(±) パーキンソン病(±)	Yes (+) Yes (+) ?
DDT/DDE 1978 2B		NHL (++) 精巣がん(+) 子供の成長 (++) 神経発達 (±) 精子パラメータ異常 (+)	Yes (+) ? ? ? ?	
クロルデン	1978	2В	NHL 白血病(+) 前立腺がん(±) 精巣がん(+)	Yes (+) Yes (+) Yes (+) ?
リンデン(c-HCH	2002/2004/2006/2007	2B(d)	NHL (++) 白血病 (+)	Yes (++) Yes (++)
b-HCH	2002/2004/2006/2007	2B(d)	前立腺がん (±)	?
トキサフェン	2004	2B	NHL (c) 白血病 (+) メラノーマ (+)	Yes (++) Yes (++) Yes (+)
クロルデコン	2004	2B	前立腺がん(++) 精子パラメータ異常(+) 神経発達(+)	Yes (+) ? ?
ヘプタクロル	1978	2B	白血病 (+)	Yes (+)
エンドスルファン	2005	Not classified	?	Yes (+)
ヘキサクロロベン ゼン(HCB)	1978	2B	子供の成長 (+)	?
テルブフォス	2003/2007		NHL(+) 白血病(+)	? ?
ダイアジノン	2008		NHL(+) 白血病(+)	? ?
マラチオン	2008	3	NHL (++) 白血病 (+) 神経発達 (+) 精子パラメータ異常 (+)	Yes (+) Yes (+) ? ?
フォノフォス	2003		NHL (±) 白血病 (+) 前立腺がん (+)	? ? ?
パラチオン	2002	3	メラノーマ (+)	?
クマフォス	EU では届出認可されてい	ない	前立腺がん (+)	?
カルバリル	2008	3	NHL (±) メラノーマ (+) 精子パラメータ異常 (+)	? ? ?
プロポキサー	2002		神経発達(+) 胎児発育(+)	? ?
カルボフラン	2008		NHL (±) 前立腺がん (+)	? ?
ブチル酸塩	2003		NHL (+) 前立腺がん (+)	? ?
EPTC	2003		白血病(+)	?
アトラジン	2005	3	NHL 胎児発育(+)	Yes (+) ?
シアニジン	2002/2007		NHL (c)	?
ペルメトリン	2002	3	前立腺がん (+)	Yes (+)

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Active substance	Ban in the EU	IARC classification	Strength of presumption <sup>(a)</sup>	Biological plausibility <sup>(b)</sup>
Atrazine	2005	3	NHL (±) Fetal growth (+)	Yes (+) ?
Cyanizine	2002/2007		NHL <sup>(c)</sup> (±)	?
Permethrin	2002	3	Prostate cancer (+)	Yes (+)
Fenvalerate	1998	Not classified	Impaired sperm parameters (+)	?
Methyl bromide	2010	3	Testicular cancer (+)	?
Dibromoethane	Banned	2A	Impaired sperm parameters (+)	?
Dibromochloropropane (DBCP)	Banned	2В	Impaired sperm parameters/impaired fertility (+++) (causal association)	Yes (+++) (mode of action elucidated)
Paraquat	2007		Parkinson's disease (+)	Yes (++)
Rotenone	2011		Parkinson's disease (+)	Yes (++)
Alachlor	2008		Leukaemia (+)	Yes (++)

(a): Scoring system: strong presumption (++), moderate presumption (+), weak presumption ( $\pm$ ).

(b): Scoring system: (++): hypothesis supported by 3 mechanisms of toxicity, (+): hypothesis supported by at least one mechanism of toxicity.

(c): Population with t(14,18) translocation. only. (d): Technical mixture ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH).

#### A.2.5. Recommendations

The analysis of the available epidemiological and mechanistic data on some active substances suggests several recommendations for developing further research:

a) Knowledge on population exposure to pesticides should be improved

- 1) Collect information about use of active substances by farmers
- 2) Conduct field studies to measure actual levels of exposure
- 3) Monitor exposure during the full occupational life span
- 4) Measure exposure levels in air (outdoor and indoor), water, food, soil
- 5) Collect information on acute poisonings
- 6) Improve analytical methods for biomonitoring and external measurements
- 7) Allow researchers to have access to extensive formulation data (solvents, co-formulants, etc.).

b) Research potential links between exposure and health outcomes

- 1) Characterise substances or groups of substances causing health outcomes
- 2) Focus on susceptible individuals or groups of individuals (gene polymorphism of enzymes, etc.)
- 3) Focus on exposure windows and susceptibility (pregnancy, development)
- 4) Bridge the gap between epidemiology and toxicology (mode of action)
- 5) Improve knowledge on mixture toxicity

6) Foster new approaches of research (in vitro and in silico models, omics, etc.).

#### A.3. Similarities and differences between the EFSA External Scientific Report and the INSERM report

The two reports discussed herein have used different methodologies. Yet, their results and conclusions in many cases agree. The INSERM report is limited to predefined outcomes and it attempted to investigate the biological plausibility of epidemiological studies by reviewing toxicological data as well, meanwhile the EFSA report is a comprehensive systematic review of all available epidemiological studies that were published during an approximately 5 year window.

The differences between the reports are shown in Table A.7 and are related to the time period of search (i.e. both reports did not assess the same body of published data), different criteria for eligibility of studies and different approaches to summarising the evidence across and within outcomes.

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フェンバレレート	1998	Not classified	精子パラメータ異常(+)	?
臭化メチル	2010	3	精巣がん (+)	?
ジブロモエタン	Banned	2A	精子パラメータ異常(+)	?
ジブロモクロロプ ロパン(DBCP)	Banned	2B	精子パラメータ異常/不妊 (++++)(因果関係)	Yes (+++) (作用機 序解明)
パライバット	2007		パーキンソン病(+)	Yes (++)
ロテノン	2011		パーキンソン病(+)	Yes (++)
アラクロール	2008		白血病(+)	Yes (++)

(a):スコアリングシステム: 強い推定 (++)、中程度の推定 (+)、弱い推定 (±)。

(b):スコアリングシステム。(++):3つの毒性メカニズムに支持された仮説。(+):少なくとも1つの毒性メカニズムに支持され ≁-仮説

(c):t(14,18)転座を有する母集団のみ。(d):技術的混合物(α-、β-及びγ-HCH)。

### A.2.5. 推奨事項

いくつかの有効成分に関する利用可能な疫学的・機序学的データを解析した結果、さらなる研究開発のためのいく つかの推奨事項が示唆された。

a) 農薬への集団ばく露に関する知識は改善されるべきである。

- 1) 農家の有効成分使用に関する情報収集
- 2)実際のばく露レベルを測定するための農地での研究の実施
- 3) 生涯労働期間のばく露を監視すること
- 4)空気(屋外・屋内)、水、食品、土壌中のばく露レベルの測定
- 5) 急性中毒に関する情報収集
- 6) バイオモニタリングや外部ばく露量測定のための解析方法の改善
- 7)研究者が広範な製剤データ(溶剤、共配合製剤など)にアクセスできるようにする。
- b)ばく露と健康影響との間の潜在的な関連性を研究する。
- 1)健康影響をもたらす物質または物質群の特性を把握する
- 2) 影響を受けやすい個人または集団に焦点を当てる(酵素の遺伝子多型など)
- 3) ばく露枠と感受性(妊娠期間、発育期間)に焦点を当てた研究
- 4) 疫学と毒物学のギャップを埋める(作用機序)
- 5) 混合物の毒性に関する知識の向上
- 6)研究の新しいアプローチ(in vitro や in silico モデル、オミクスなど)を育成する。

#### A.3. EFSA 外部科学研究報告書と INSERM 報告書の類似点と相違点

ここで議論されている2つの報告書は、異なる方法論を使用している。しかし、多くの場合、それらの結果と結論は一 致している。INSERMの報告書は、事前に調査された結果に限定されており、毒物学的データもレビューすることで疫 学研究の生物学的妥当性を調査しようとしているのに対し、EFSA の報告書は、約5年の期間に発表されたすべての 利用可能な疫学研究の包括的なシステマティックレビューである。

両報告書の違いは表A.7に示されており、検索期間(すなわち、両報告書は同じ出版データを評価していない)、研 究の適格性の基準の違い、健康影響全体と健康影響内のエビデンスを要約するアプローチの違いに関連している。

全体的に、INSERM の報告書は EFSA の報告書よりも多くの健康被害との関連を特定している。しかし、同じ健康 影響(小児白血病、パーキンソン病)については、両方の報告書で農薬ばく露との関連性が十分に証明されていると主 張されている。

Overall, the INSERM report identified a greater number of associations with adverse health effects than the EFSA report. However, a well-documented association with pesticide exposure was claimed by both reports for the same health outcomes (childhood leukaemia, Parkinson's disease).

Table A.7:	Comparison	between	methods	used	in	the	EFSA	External	Scientific	Report	and	the
	INSERM Rep	ort										

	EFSA External report	INSERM report
Articles reviewed	602/43,000	NR
Language	Yes	NR
Search strategy (key words, MeSH)	Yes	NR
Search database	Yes (4)	NR
Years of publication	2006–2012 (Sep)	? to 2012 (Jun)
Type of epi studies assessed	Cross-sectional	Cross-sectional
	Case-control	Case-control
	Cohort	Cohort
Inclusion criteria	Yes	NR
Exclusion criteria	Yes	NR
Methodological quality assessment	Yes (12 criteria)	NR
Exposure groups <sup>(a)</sup>	Yes	Yes
Exposure assessment	Yes	Yes
Quantitative synthesis (meta-analysis)	Yes	No
Qualitative synthesis <sup>(c)</sup>	Yes	Yes
Supporting Toxicological data	NI	Yes
Associations with individual pesticides	Yes	Yes
Health outcomes studied		
Haematological cancer	Yes	Yes
Solid tumours	Yes	Yes
Childhood cancer	Yes	Yes
Neurodegenerative disorders	Yes	Yes
Neurodevelopmental outcomes	Yes	Yes
Neuropsychiatric disturbances <sup>(b)</sup>	No	Yes
Reproductive and developmental	Yes	Yes
Endocrine	Yes	NI
Metabolism	Yes	Yes
Immunological	Yes	NI
Respiratory	Yes	NI

NR: not reported; NI: not investigated.

(a): Exposure type (environmental, occupational, etc.) and period (general population, children, etc.).

(b): E.g. depressive disorders. (c): Add explanation.

A.4. The Ontario College of Family Physicians Literature review (OCFPLR)

In 2004, the Ontario College of Family Physicians (Ontario, Canada) reviewed the literature published between 1992 and 2003 on major health effects associated with pesticide exposure. The authors concluded that positive associations exist between solid tumours and pesticide exposures as shown in Table A.8. They noted that in large well-designed cohort studies these associations were consistently statistically significant, and the relationships were most consistent for high exposure levels. They also noted that dose–response relationships were often observed, and they considered the quality of studies to be generally good.

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	EFSA External report	INSERM report
レビューされた論文	602/43,000	NR
言語	Yes	NR
検索戦略(キーワード、MeSH)	Yes	NR
検索データベース	Yes (4)	NR
出版年	2006-2012 (Sep)	? to 2012 (Jun)
評価された疫学研究の種類	Cross-sectional Case-control Cohort	Cross-sectional Case–control Cohort
含有基準	Yes	NR
除外基準	Yes	NR
方法論的品質評価	Yes (12 criteria)	NR
ばく露グループ(a)	Yes	Yes
ばく露評価	Yes	Yes
定量的統合(メタアナリシス)	Yes	No
質的統合(c)	Yes	Yes
毒物学的データのサポート	NI	Yes
個々の農薬との関連性	Yes	Yes
健康影響研究		
血管がん	Yes	Yes
充実性腫瘍	Yes	Yes
小児がん	Yes	Yes
神経変性疾患	Yes	Yes
神経発達影響	Yes	Yes
精神障害(b)	No	Yes
生殖と発生	Yes	Yes
内分泌	Yes	NI
代謝	Yes	Yes
免疫学的	Yes	NI
呼吸器	Yes	NI

NR:報告されていない、NI:調査されていない。

(a):ばく露の種類(環境、職業など)及び期間(一般集団、児童など)。

(b):例:うつ病性障害。

(c):説明を追加する。

#### A.4. The Ontario College of Family Physicians の文献レビュー(OCFPLR)

2004 年、カナダの The Ontario College of Family Physicians は、1992 年から 2003 年の間に発表された、農 薬ばく露に関連した主要な健康影響に関する文献をレビューした。著者らは、表 A.8 に示すように充実性腫瘍と農薬 ばく露との間には正の関連が存在すると結論づけた。著者らは、よく計画された大規模コホート研究では、これらの関 連性は一貫して統計的に有意であり、その関係は高ばく露レベルで最も一貫していたと指摘している。また、用量反応 関係がしばしば観察され、研究の質は概ね良好であるとした。

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### Table A.8: Health Effects considered in the Ontario College of Family Physicians review, 2004

Endpoint Associations identified by the Ontario College, pesticide differentiated), study type, (no. of studies/total no. of s						
A) Cancer						
1. Lung	-ve cohort (1/1) +ve case_control (1/1) +ve carbamate, phenoxy acid, case_control (1/1)					
2. Breast	+ve case-control (2/4) +ve ecological (1/1) +ve triazine, ecological (1/1) -ve atrazine, ecological (1/1)					
3. Colorectal						
4. Pancreas	+ve cohort (1/1) +ve case_control (2/2)					
5. Non-Hodgkin's lymphoma	+ve cohort (9/11) +ve case-control (12/14) +ve ecological (2/2)					
6. Leukaemia	+ve cohort (5/6) +ve case_control (8/8) -ve ecological (1/1) +ve lab study (1/1)					
7. Brain	+ve cohort (5), similar case_control (5)					
8. Prostate	+ve cohort (5/5) case-control (2/2) ecological (1/1)					
9. Stomach						
10. Ovary						
11. Kidney	+ve pentachlorophenol cohort (1/1) +ve cohort (1/1) +ve case_control (4/4)					
12. Testicular						
B) Non-Cancer						
1) Reproductive effects	+ve glyphosate					
Congenital malformations	+ve pyridyl derivatives					
Fecundity/time to pregnancy	Suggest impaired					
Fertility						
Altered growth	Possible +ve association, but further study required					
Fetal death	Suggested association					
Mixed outcomes						
2) Genotoxic/immunotoxic Chromosome aberrations	+ve Synthetic pyrethroids (1) +ve organophosphates (1) +ve fumigant and insecticide applicators					
NHL rearrangements	+ve fumigant and herbicide applicators					
3) Dermatologic						
4) Neurotoxic Mental & emotional impact	+ve					
Functional nervous system impact	+ ve organophosphate/carbamate poisoning					
Neurodegenerative impacts (PD)	+ve cohort (4/4) +ve case_control (2/2) +ve ecological (1/1)					

+ve: positive; -ve: negative.

The report concluded that there was compelling evidence of a link between pesticide exposure and the development of non-Hodgkin's lymphoma (NHL), and also clear evidence of a positive association between pesticide exposure and leukaemia. The authors also claimed to have found consistent findings of a number of nervous system effects, arising from a range of exposure time courses.

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エンドポイント	オンタリオ大学で同定された関連性、農薬(差異化されている場合)、研究の
	種類、(研究数/総研究数)
A)がん	
1. 肺	-ve cohort (1/1)
	+ve case-control (1/1)
	+ve carbamate, phenoxy acid, case-control (1/1)
2. 乳房	+ve case-control (2/4)
	+ve ecological (1/1)
	+ve triazine, ecological $(1/1)$
2 1 四 末 四	—ve atrazine, ecological (1/1)
3. 大腸直腸	
4. 膵臓	+ve cohort $(1/1)$
	+ve case-control (2/2)
5. 非ホジキンリンパ腫	+ve cohort (9/11)
	+ve case-control (12/14) +ve ecological (2/2)
6. 白血病	+ve ecological (2/2) +ve cohort (5/6)
0. 口皿/內	+ve conort (5/6) +ve case-control (8/8)
	-ve ecological (1/1)
	+ve lab study (1/1)
7. 脳	+ve cohort (5), similar case-control (5)
8. 前立腺	+ve cohort (5/5) case-control (2/2) ecological (1/1)
9. 胃	
10. 卵巣	
10. 卯未 11. 腎臓	+ve pentachlorophenol cohort (1/1)
11. 戶別戰	+ve pentachiorophenoi conort $(1/1)$ +ve cohort $(1/1)$
	+ve case-control (4/4)
12. 精巣	
12. 信来 B)非がん	
1) 生殖影響	+ve glyphosate
	+ve pyridyl derivatives
分娩/妊娠までの期間	Suggest impaired
受胎性	
発育影響	Possible +ve association, but further study required
胎児死亡	Suggested association
混合した健康影響	005500000000000
<ul><li>2)遺伝毒性・免疫毒性</li></ul>	+ve Synthetic pyrethroids (1)
2/週 <b>ム毎日・光没毎日</b> 染色体異常	+ve organophosphates (1)
NHL 再編成	+ve fumigant and insecticide applicators
	+ve fumigant and herbicide applicators
3)皮膚科学的	
4)神経毒性の精神及び感情への影響	+ve
機能的神経系の影響	+ ve organophosphate/carbamate poisoning
神経変性の影響 (PD)	+ve cohort (4/4)
	+ve case-control (2/2)
	+ve ecological (1/1)

+ve: positive; -ve: negative.

報告書は、農薬ばく露と非ホジキンリンパ腫(NHL)の発症との関連性を示す説得力のあるエビデンスがあり、また農 薬ばく露と白血病との間に正の関連性があるという明確なエビデンスがあると結論づけている。著者らはまた、様々な ばく露時間の経過から生じる多くの神経系への影響についても一貫した結果が得られたと主張している。

このような断定された結論は、非政府組織(NGO)の支持を得て、いくつかの規制当局の間で疑問が生じた。当時の



Such strong conclusions found favour with Non-Governmental organisations (NGOs) and raised questions among some Regulatory Authorities. The Advisory Committee on Pesticides (ACP), at that time an UK government independent advisory committee, was asked to provide an evaluation of the outcome of the Ontario College review. The committee membership included one epidemiologist and the committee consulted five other epidemiologists involved in providing independent advice to other government committees. They all agreed that the review had major shortcomings (e.g. exact search strategy and selection criteria not specified, selective reporting of results, inadequate understanding and consideration of relevant toxicology, insufficient attention to routes and levels of exposure, not justified conclusions, etc.). Overall, the conclusions of the Ontario College review were considered not to be supported by the analysis presented. In 2012, the Ontario review authors published an update of their evaluation; in their second report they used a very similar approach but offered more detail concerning the inclusion criteria used. This example is a reminder of the risk of over interpretation of epidemiological studies. In particular, a causal inference between exposure and the occurrence of adverse health effects is often made, but this represents an association that should be further assessed.

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#### Epidemiological studies and pesticides

英国政府の独立諮問委員会である農薬諮問委員会(ACP)は、オンタリオ大学レビューの結果の評価を依頼された。 委員会のメンバーには1人の疫学者が含まれており、委員会は、他の政府の委員会に独立した助言を提供することに 関与している他の5人の疫学者に相談した。彼らはすべてのレビューが主要な欠点を持っていたことに同意した(例え ば、正確な検索戦略と特定されていない選択基準、結果の選択的な報告、不適切な理解と関連する毒性学の考慮、 ばく露のルートとレベルへの不十分な注意、正当化された結論、等)。全体的に、オンタリオ大学レビューの結論は、提 示された解析によってサポートされていないと考えられた。2012年には、オンタリオ大学レビューの著者は、彼らの評 価の更新を発表した;彼らの2番目の報告書では、彼らは非常に似たようなアプローチを使用したが、使用される包含 基準に関するより詳細を提供した。この例は、疫学研究の過剰解釈のリスクを思い起こさせるものである。特に、ばく露 と有害な性健康影響の発生との間の因果関係を推論することはよくあるが、これはさらに評価されるべき関連性を示し ている。

### Annex B – Human biomonitoring project outsourced by EFSA<sup>23</sup>

In 2015, EFSA outsourced a project to further investigate the role of HBM in occupational health and safety strategies as a tool for refined exposure assessment in epidemiological studies and to contribute to the evaluation of potential health risks from occupational exposure to pesticides. It was in fact recognised that exposure assessment is a key part of all epidemiological studies and misclassification of exposure and use of simple categorical methods are known to weaken the ability of a study to determine whether an association between contact and ill-health outcome exists; at present, this limits integration of epidemiological findings into regulatory risk assessment.

The consortium formed by Risk & Policy Analysts Limited (RPA), IEH Consulting Limited (IEH) and the Health&Safety Laboratory (HSL) carried out a systematic literature review for the period 1990–2015 with the aim to provide an overview on the use of HBM as a tool for occupational exposure assessment refinement, identifying advantages, disadvantages and needs for further development (first objective). The search identified 2096 publications relating to the use of HBM to assess occupational exposure to pesticides (or metabolites). The outcome of the search (Bevan et al., 2017) indicated that over the past 10–20 years there has been an expansion in the use of HBM, especially into the field of environmental and consumer exposure analysis. However, further improvement of the use of HBM for pesticide exposure assessment is needed, in particular with regards to: development of strategies to improve or standardise analytical quality, improvement of the availability of reference material for metabolites, integration of HBM data into mathematical modelling, exposure reconstruction, improvements in analytical instrumentation and increased availability of human toxicology data.

The contractors performed a review of available HBM studies/surveillance programmes conducted in EU/US occupational settings to identify pesticides (or metabolites) both persistent and not persistent, for which biomarkers of exposure (and possibly effect) were available and validated (second objective). A two-tiered screening process that included quality scoring for HBM, epidemiological and toxicological aspects, was utilised to identify the most relevant studies, resulting in 178 studies for critical review. In parallel with the screening of identified studies, a Master Spreadsheet was designed to collate data from these papers, which contained information relating to: study type; study participants; chemicals under investigation; biomarker quality check; analytical methodology; exposure assessment; health outcome/toxicological endpoint; period of follow-up; narrative of results; risk of bias and other comments.

HBM has been extensively used for monitoring worker exposure to a variety of pesticides. Epidemiological studies of occupational pesticide use were seen to be limited by inadequate or retrospective exposure information, typically obtained through self-reported questionnaires, which can potentially lead to exposure misclassification. Some examples of the use of job exposure or crop exposure matrices were reported. However, little validation of these matrix studies against actual exposure data had been carried out. Very limited data was identified that examined seasonal exposures and the impact of PPE, and many of the studies used HBM to only assess one or two specific compounds. A wide variety of exposure models are currently employed for health risk assessments and biomarkers have also often been used to evaluate exposure estimates predicted by a model.

From the 178 publications identified to be of relevance, 41 individual studies included herbicides, and of these, 34 separate herbicides were identified, 15 of which currently have approved for use in the EU. Similarly, of the 90 individual studies that included insecticides, 79 separate insecticides were identified, of which 18 currently have approved for use in the EU. Twenty individual studies studies that EU. Twenty individual studies in the EU. The most studied herbicides (in order) were shown to be: 2,4-D > atrazine > metolachlor = MCPA > alachlor = glyphosate. Similarly, the most studied insecticides (in order) were: chlorpyrifos > permethrin > cypermethrin = deltamethrin > malathion, and the most studied fungicides were: captan > mancozeb > folpet.

Current limitations comprised the limited number of kinetic data from humans, particularly with respect to the ADME of individual pesticides in human subjects, which would allow more accurate HBM sampling for all routes of exposure. A wider impact of this is on the development of PBPK models for the risk assessment of pesticides, which rely on toxicokinetic data, and on validation of currently used exposure assessment models. Further limitations currently impacting on the use of HBM in this field are a lack of large prospective cohort studies to assess long term exposure to currently used pesticides.

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### 付属書 B-EFSA が委託したヒト・バイオモニタリング・プロジェクト<sup>23</sup>

2015 年、EFSA は、疫学研究におけるばく露評価のためのツールとして、また、農薬への職業上ばく露による潜在 的な健康リスク評価に貢献するために、労働安全衛生戦略における HBM の役割をさらに調査するためのプロジェクト を外部委託した。実際、ばく露評価はすべての疫学研究の重要な部分であり、ばく露の誤分類や単純な分類法の使用 は、接触と健康被害の結果との間に関連性があるかどうかを判断する研究の能力を弱めてしまうことが知られている。

Risk & Policy Analysts Limited (RPA)、IEH Consulting Limited (IEH)、Health & Safety Laboratory (HSL)からなるコンソーシアムは、1990 年から 2015 年までの期間、系統的な文献レビューを実施した。その目的は、 作業ばく露評価の再開発のためのツールとしての HBM の使用に関する概要を提供し、長所、短所、さらなる開発の 必要性を特定することであった (第一の目的)。検索では、農薬(またはその代謝物)への職業上ばく露を評価するため の HBM の使用に関連する 2096 の文献を特定した。検索の結果(Bevan 6、2017 年)は、過去 10~20 年の間に HBM の使用が拡大してきたこと、特に環境や消費者のばく露分析の分野にまで拡大してきたことを示している。しかし、 農薬ばく露評価のための HBM の使用については、特に、分析品質の向上や標準化のための戦略の開発、代謝物の ための標準物質の利用可能性の向上、数学的モデリングへの HBM データの統合、ばく露の再構築、分析機器の改 善、ヒト毒性データの利用可能性の向上など、さらなる改善が必要とされている。

請負業者は、EU/米国の作業環境で実施された利用可能な HBM 研究/サーベイランスプログラムのレビューを 実施し、残留性のあるものと残留性のないものの両方の農薬(または代謝物)を特定した。最も関連性の高い研究を特 定するために、HBM、疫学的、毒性学的側面の品質スコアリングを含む 2 段階のスクリーニングプロセスを利用し、 178 件の研究をクリティカルレビューの対象とした。特定された研究のスクリーニングと並行して、これらの文献からのデ ータを照合するためにマスタースプレッドシートが計画され、その中には、研究タイプ、研究参加者、調査対象の化学 物質、バイオマーカーの品質チェック、分析方法、ばく露評価、健康影響/毒性エンドポイント、追跡期間、結果の説 明、バイアスのリスク、その他のコメントに関する情報が含まれている。

HBM は、様々な農薬への作業者のばく露を監視するために広く使用されている。職業上の農薬の使用に関する疫 学的研究では、不十分なばく露情報や後ろ向きなばく露情報が制限されていることが見受けられる。職業別または作 物別のばく露マトリックスの使用例も報告されている。しかし、実際のばく露データに対するこれらのマトリックス研究の 検証はほとんど行われていない。季節的なばく露と PPE の影響を調査したデータは非常に限られており、多くの研究 では、1 つまたは 2 つの特定化合物のみを評価するために HBM を使用している。現在、健康リスク評価には多種多 様なばく露モデルが採用されており、モデルによって予測されたばく露推定値を評価するためにバイオマーカーもしば しば使用されている。

関連性があると判断された 178 の出版物から、41 の個別研究が除草剤を含み、そのうち 34 の個別除草剤が同定さ れ、そのうちの 15 が現在 EU での使用が承認されている。同様に、殺虫剤を含む 90 件の個別研究のうち、79 件の殺 虫剤が同定され、そのうち 18 件は現在 EU での使用が承認されている。20 の個別研究には殺菌剤が含まれており、 34 種類の殺菌剤が確認され、そのうち 22 種類が現在 EU での使用が承認されている。最も研究された除草剤は(順 に)、2,4-D>アトラジン>メトラクロール=MCPA>アラクロール=グリホサートであることが示されている。同様に、最も研究 された殺虫剤(順に)は、クロルビリホス>ペルメトリン>シペルメトリン=デルタメトリン>マラチオンであり、最も研究された 殺菌剤は、キャブタン>マンコゼプ>フォルペットであった。

現在の限界は、特にとトを対象とした個々の農薬の ADME に関して、とトからの動特性データの数が限られているこ とに起因しており、これにより、すべてのばく露経路についてより正確な HBM サンプリングが可能になる。このことは、 毒物動態データに依存する農薬のリスク評価のための PBPK モデルの開発や、現在使用されているばく露評価モデ ルのパリデーションにも影響を及ぼす。現在、この分野での HBM の使用に影響を与えているさらなる限界は、現在使 用されている農薬への長期ばく露を評価するための大規模な前向きコホート研究が不足していることである。

特定されたエビデンスは、ヨーロッパにおける農薬の労働衛生サーベイランスの一環としての HBM の実施に関する

<sup>&</sup>lt;sup>23</sup> Bevan et al. (2017).

<sup>23</sup> Bevan S



The evidence identified has been used to help formulate recommendations on the implementation of HBM as part of the occupational health surveillance for pesticides in Europe. Some key issues were considered that would need to be overcome to enable implementation. These included the setting of priorities for the development of new specific and sensitive biomarkers, the derivation and adoption of health-based guidance values, development of QA schemes to validate inter-laboratory measurements, good practice in field work and questionnaire design, extension of the use of biobanking and the use of HBM for post-approval monitoring of pesticide safety.

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推奨事項を策定するために使用されている。実施を可能にするために克服しなければならないいくつかの重要な問題 が検討された。その中には、新しいスペックや感度の高いバイオマーカーの開発の優先順位の設定、健康に基づいた ガイダンス値の導入と採用、研究間の計測値を検証するための QA スキームの開発、野外作業やアンケートの作成に おける良好な実施、バイオバンキングの使用範囲の拡大、農薬の安全性の承認後のモニタリングにおける HBM の使 用などが含まれている。

Annex C – Experience of international regulatory agencies in regards to the integration of epidemiological studies for hazard identification

#### C.1. WHO-International Agency for Research on Cancer (IARC)

The IARC Monographs on the Evaluation of Carcinogenic Risks to Humans of the International Agency for Research on Cancer (IARC) is a programme established four decades ago to assess environmental exposures that can increase the risk of human cancer. These include individual chemicals and chemical mixtures, occupational exposures, physical agents, biological agents and lifestyle factors.

IARC assembles international interdisciplinary Working Groups of scientists to review and assess the quality and strength of evidence from scientific publications and perform a hazard evaluation to assess the likelihood that the agents of concern pose a cancer risk to humans. In particular, the tasks of IARC Working Group Members include the evaluation of the results of epidemiological and other experimental studies on cancer, to evaluate data on the mechanisms of carcinogenesis and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The Monographs are widely used and referenced by governments, organisations, and the public around the world to set preventive and control public health measures.

The Preamble<sup>24</sup> to the IARC Monographs explains the scope of the programme, the scientific principles and procedures used in developing a Monograph, the types of evidence considered and the scientific criteria that guide the evaluations. The scope of the monographs broadened to include not only single chemicals but also groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. Thus, the title of the monographs reads 'Evaluation of carcinogenic risks to humans'.

Relevant epidemiological studies, cancer bioassays in experimental animals, mechanistic data, as well as exposure data are critically reviewed. Only reports that have been published or accepted for publication in the openly available scientific literature are included. However, the inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results. Qualitative aspects of the available studies are carefully scrutinised.

Although the Monographs have emphasised hazard identification, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose-response relationship. A Monograph may undertake to estimate dose-response relationships within the range of the available epidemiological data, or it may compare the dose-response information from experimental and epidemiological studies.

The structure of a Monograph includes the following sections:

- 1) Exposure data
- 2) Studies of cancer in humans
- 3) Studies of cancer in experimental animals
- 4) Mechanistic and other relevant data
- 5) Summary
- 6) Evaluation and rationale.

Human epidemiological data are addressed in point 2, where all pertinent epidemiological studies are assessed. Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

The IARC evaluation of epidemiological studies includes an assessment of the following criteria: types of studies considered (e.g. cohort studies, case-control studies, correlation (or ecological) studies and intervention studies, case reports), quality of the study (e.g. bias, confounding, biological variability and the influence of sample size on the precision of estimates of effect), meta analysis and pooled analyses, temporal effects (e.g. temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure), use of biomarkers in epidemiological studies (e.g. evidence of exposure, of early effects, of cellular, tissue or organism responses), and criteria for causality.

With specific reference to causality, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group

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### 付属書 C-ハザードの特定のための疫学研究の統合に関する国際規制機関の経験 C.1. WHO-国際がん研究機関(IARC)

国際がん研究機関(IARC)の「ヒトに対する発がん性リスク評価に関する IARC モノグラフ」は、ヒトのがんリスクを増加させる可能性のある環境ばく露を評価するために 40 年前に設立されたプログラムである。これらには、個々の化学物質や化学物質の混合物、職業上ばく露、物理的要因、生物学的要因、生活様式の要因が含まれる。

IARC は、科学者からなる国際的な学際的作業部会を組織し、科学的出版物からのエビデンスの質と強度をレビューして評価し、懸念される物質がヒトに発がんリスクをもたらす可能性を評価するためのハザード評価を実施している。 特に、IARC ワーキンググループのメンバーの役割は、がんに関する疫学的研究やその他の実験的研究の結果の評価、発がんのメカニズムに関するデータの評価、ヒトへのばく露による発がん性の総合的な評価を行うことである。

モノグラフは、世界中の政府、組織、公衆衛生の予防・管理措置を設定するために広く利用され、参照されている。

IARC モノグラフの前文<sup>24</sup>は、プログラムの範囲、モノグラフの開発に使用される科学的原理と手順、考慮されるエビ デンスの種類、評価の指針となる科学的基準を説明している。モノグラフの範囲は、単一の化学物質だけでなく、関連 する化学物質のグループ、複雑な混合物、職業上ばく露、物理的・生物学的物質、生活様式の要因を含むように拡大 された。そのため、モノグラフのタイトルは「ヒトに対する発がん性リスクの評価」となっている。

関連する疫学研究、実験動物を用いた発がんバイオアッセイ、メカニズムデータ、ばく露データなどが批判的にレビューされている。公表されている科学文献に掲載された、または掲載が認められた報告書のみが含まれる。しかし、研究を含めることは、研究デザインの妥当性や結果の分析と解釈を受け入れることを意味するものではない。利用可能な研究の質的側面は慎重に精査されている。

モノグラフではハザードの特定を強調しているが、がんのハザードを評価するために用いられた疫学研究や実験研 究と同じものを、用量反応関係を推定するためにも用いることができる。モノグラフは、利用可能な疫学データの範囲内 で用量反応関係を推定することもあれば、実験研究と疫学研究の用量反応情報を比較することもある。

モノグラフの構成は以下のようになっている。

- 1)ばく露データ
- 2) ヒトにおけるがんの研究
- 3)実験動物を用いたがんの研究
- 4)メカニズム等の関連データ
- 5)まとめ
- 6)評価と根拠

ヒトの疫学的データは、関連するすべての疫学的研究が評価されている 2)に記載されている。バイオマーカーの研究は、ヒトに対する発がん性の評価に関連する場合に含まれる。

疫学研究の IARC 評価には、以下の基準の評価が含まれる:検討された研究の種類(例:コホート研究、症例対照 研究、相関(または生態学的)研究及び介入研究、症例報告)、研究の質(例:バイアス、交絡、生物学的変動及び影響の推定精度に対するサンプルサイズの影響)、メタアナリシス及びプール分析、時間的影響。例えば、最初のばく露 時の年齢、最初のばく露からの時間、ばく露の期間、累積ばく露、ピークばく露などの時間的変数)、疫学研究におけ るバイオマーカーの使用(例えば、ばく露のエビデンス、初期効果のエビデンス、細胞、組織または生物の反応のエビ デンス)、因果関係の基準。

因果関係に関する特定の基準では、問題の薬剤がヒトに対して発がん性があるというエビデンスの強さに関して判断 がなされる。

判断を下す際に、作業部会は因果関係についていくつかの基準を考慮している(Hill、1965年)。強い関連性(例 えば、大きな相対リスク)は因果関係を示す可能性が高い。しかし、疾患やばく露が一般的な場合には、弱い関連性が 重要であることが認識されている。異なるばく露条件で計画の異なる複数の研究で再現された関連性は、単一の研究

<sup>24</sup> http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf

www.efsa.europa.eu/efsajournal

<sup>&</sup>lt;sup>24</sup> http://monographs.iarc.fr/ENG/Preamble/CurrentPreamble.pdf

considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality. However, it is recognised that weak associations may be important when the disease or exposure is common. Associations that are replicated in several studies of different design under different exposure conditions are more likely to represent a causal relationship than isolated observations from single studies. In case of inconsistent results among different investigations, possible reasons (e.g. differences in exposure) are sought, and high quality studies are given more weight compared to less methodologically sound ones. Risk increasing with the exposure is considered to be a strong indication of causality, although the absence of a clear dose-response effect is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure also supports a causal interpretation of the findings. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall data are considered. Biomarkers information may be used in an assessment of the biological plausibility of epidemiological observations. Randomised trials showing different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement of lack of carcinogenicity can be made. In those cases, studies are scrutinised to assess the standards of design and analysis described above, including the possibility of bias, confounding or misclassification of exposure. In addition, methodologically sound studies should be consistent with an estimate of effect of unity for any observed level of exposure, provide a pooled estimate of relative risk near to unity, and have a narrow confidence interval. Moreover, no individual study nor the pooled results of all the studies should show any increasing risk with increasing level of exposure. Evidence of lack of carcinogenicity can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years, and latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation which summarises the results of epidemiological studies, the target organs or tissues, dose-response associations, evaluations of the strength of the evidence for human and animal data, and the strength of the mechanistic evidence.

At the end of the overall evaluation, the agent is assigned to one of the following groups: Group 1, the agent is carcinogenic to humans; Group 2A, the agent is probably carcinogenic to humans; Group 2B, the agent is possibly carcinogenic to humans; Group 3, the agent is not classifiable as to its carcinogenicity to humans; Group 4, the agent is probably not carcinogenic to humans.

The categorisation of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data. These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency).

For example, Group 1: The agent is carcinogenic to humans. This category is used when there is sufficient evidence of carcinogenicity in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Although widely accepted internationally, there have been criticisms of the classification of particular agents in the past, and more recent criticisms have been directed at the general approach adopted by IARC for such evaluations possibly motivating publication of a rebuttal (Pearce et al., 2015).

#### C.2. The experience of US-EPA in regards to the integration of epidemiological studies in risk assessment

The US Environmental Protection Agency's Office of Pesticide Programs (OPP) is the governmental organisation in the US responsible for registering and regulating pesticide products.<sup>25</sup> As part of this activity and prior to any permitted use of a pesticide, OPP evaluates the effects of pesticides on human health and the environment. EPA receives extensive hazard and exposure information to characterise

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からの孤立した観察よりも因果関係を示す可能性が高い。異なる研究間で一貫性のない結果が得られた場合には、考 えられる理由(例えば、ばく露の違い)を探り、方法論的に健全でない研究よりも質の高い研究の方が重要視される。明 確な用量反応効果がないからといって必ずしも因果関係を否定するエビデンスにはならないが、ばく露に伴ってリスク が増加することは因果関係を示す強いエビデンスと考えられている。ばく露の停止または減少後にリスクが低下してい ることが示された場合も、結果の因果関係の解釈を支持するものである。時間性、影響の推定精度、生物学的妥当性、 全体的なデータの一貫性が考慮される。バイオマーカー情報は、疫学的観察の生物学的妥当性の評価に使用される ことがある。被験者と非被験者でがんの発生率が異なることを示している無作為試験は、特に因果関係を示す強力な エビデンスとなる。

疫学的研究でばく露とがんとの間の関連性がほとんど、または全く示されない場合には、発がん性がないと判断する ことができる。このような場合、研究は、バイアスの可能性、交絡の可能性、またはばく露の誤分類を含めて、上述の計 画と解析の基準を評価するために精査される。さらに、方法論的に健全な研究は、観察されたばく露レベルのどのよう なばく露についても、影響の推定値が一致していること、相対リスクのプール推定値がほぼ一致すること、そして狭い信 頼性間隔を持つことに一貫性があるべきである。さらに、個々の研究も、すべての研究のプール結果も、ばく露レベル の増加に伴うリスクの増加を示すべきではない。発がん性がないというエビデンスは、研究されたがんの種類、報告され たばく露量及びこれらの研究で観察された最初のばく露と疾患発症の間の期間にのみ適用できる。ヒトのがんの経験 から、最初のばく露から臨床症状のがんの発生までの期間が20年よりも長いことがあり、30年よりも実質的に短い潜伏 期間は、発がん性の欠如のエビデンスを提供できないことが示されている。

最後に、疫学研究の結果、標的臓器または組織、用量反応関連、ヒト及び動物のデータのエビデンスの強固さの評 価、メカニズムのエビデンスの強固さをまとめた総合評価に到達するために、エビデンスの全体像を検討する。

総合評価の最後に、以下のいずれかのグループに分類される。グループ 1、その薬剤はヒトに対して発がん性があ る:グループ 2A、その薬剤はおそらくヒトに対して発がん性がある:グループ 2B、その薬剤はヒトに対する発がん性が 疑われる:グループ 3、その薬剤はヒトに対する発がん性に関して分類できない:グループ 4、その薬剤はおそらくヒトに 対して発がん性がない。

薬剤の分類は、ヒト及び実験動物での研究、ならびにメカニズム及びその他の関連データから得られたエビデンスの 強固さを反映する科学的判断の問題である。これらの分類は、ばく露が発がん性であるというエビデンスの強固さにの み言及しており、発がん性(可能性)の程度には言及していない。

例えば、グループ 1:その薬剤はヒトに対して発がん性がある。このカテゴリーは、ヒトにおける発がん性の十分なエビ デンスがある場合に使用される。例外的に、ヒトにおける発がん性のエビデンスが十分ではないが、実験動物における 発がん性の十分なエビデンスがあり、ばく露されたヒトにおいて、その薬剤が発がん性の関連メカニズムを介して作用 するという強いエビデンスがある場合には、薬剤はこのカテゴリーに分類される。国際的に広く受け入れられているが、 過去には特定の薬剤の分類に対する批判があり、より最近の批判は、そのような評価のために IARC が採用した一般 的なアプローチに向けられており、反論の発表を動機づける可能性がある(Pearce ら、2015年)。

### C.2. リスク評価における疫学的研究の統合に関する US-EPA の経験

米国環境保護庁の農薬プログラム(OPP)は、農薬製品の登録と規制を担当する米国の政府機関である25。この活 動の一環として、また農薬の使用が許可される前に、OPP は農薬がヒト健康と環境に及ぼす影響を評価している。

EPAは、連邦殺虫剤・殺菌剤・殺鼠剤法(FIFRA)及び連邦食品・医薬品・化粧品法(FFDCA)を通じて、農薬製品 のリスクを特性評価するための広範なハザード及びばく露情報を入手している。農薬の毒性影響に関する情報は、一 般的に、農薬登録者が実施し、EPA に提出する実験動物を用いた研究から得られている。

これまでは、農薬へのばく露に関連する可能性のある潜在的なリスクを EPA が評価するための情報として、農薬に 関する十分に計画された疫学研究から得られた情報は一般的には得られていなかった。農薬へのばく露と健康影響と

<sup>&</sup>lt;sup>25</sup> See https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks for general information on pesticide science and assessing pesticide risks. 84

<sup>25</sup> 農薬科学及び農薬リスクの評価に関する一般的な情報については、https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks を参昭のこと

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the risks of pesticide products through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Information on the toxic effects of pesticides is generally derived from studies with laboratory animals conducted by pesticide registrants and submitted to EPA.

In the past, information from well-designed epidemiology studies on pesticides has not been typically available to inform EPA's evaluations of potential risks that might be associated with exposure to pesticides. With an increasing number of epidemiology studies entering the literature which explore the putative associations between pesticides exposure and health outcomes, EPA is putting additional emphases on this source of information. This is especially true for the wealth of studies deriving from the Agricultural Health Study<sup>26</sup> (AHS), a large, well-conducted prospective cohort study following close to 90,000 individuals over more than 20 years and from the Children's Environmental Health and Disease Prevention Research Centers.<sup>27</sup> EPA intends to make increasing use of these epidemiology studies in its human health risk assessment with the goal of using such epidemiological information in the most scientifically robust and transparent way.

#### C.2.1. OPP Epidemiological Framework Document

As an early first step in this process, EPA-OPP developed a proposed epidemiological framework document released as a draft in 2010, 'Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment' (US-EPA, 2010a). The 2010 draft framework was reviewed favourably by the FIFRA Scientific Advisory Panel (SAP) in February, 2010 (US-EPA, 2010b). This document was recently updated in 2016 to the 'Office of Pesticide Programs' Framework Document for Incorporating Human Epidemiology and Incident Data in Risk Assessments for Pesticides' (US-EPA, 2016). The revised and updated 2016 Framework document proposes that human information like that found in epidemiology studies (in addition to human incident databases, and biomonitoring studies) along with experimental toxicological information play a significant role in this new approach by providing insight into the effects caused by actual chemical exposures. In addition, epidemiological/ molecular epidemiological data can guide additional analyses, identify potentially susceptible populations and new health effects and potentially confirming existing toxicological observations. The concepts in the 2016 Framework are based on peer-reviewed robust principles and tools and rely on many existing guidance documents and frameworks (Table C.1) for reviewing and evaluating epidemiology data. It is also consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action (MoA)/human relevance framework which highlight the importance of problem formulation and the need to integrate information at different levels of biological organisation (Meek et al., 2014). Furthermore, it is consistent with recommendations by the National Academy of Sciences' National Research Council (NAS/NRC) in its 2009 report Science and Decisions (NRC, 2009) in that the framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage is envisioned as starting with a planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern, exposure pathways, and duration along with key gaps in data or scientific information.

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の間に考えられる関連性を調査する疫学研究が文献に掲載されることが増えてきたため、EPA はこの情報源をさらに 重視している。これは、20年以上にわたって9万人近くの個人を追跡した大規模でよく実施された前向きなコホート研 究である農業健康調査(Agricultural Health Study<sup>26</sup>(AHS)や、小児環境保健・疾病予防研究センターから得られ た豊富な研究に特に当てはまる。EPA27は、このような疫学的情報を最も科学的に強固で明白性の高い方法で利用す ることを目標に、ヒトの健康リスク評価においてこれらの疫学的研究をより多く利用することを意図している。

### C.2.1. OPP 疫学的フレームワーク文書

このプロセスの初期段階として、EPA-OPPは、2010年に「健康リスク評価にヒトの疫学的データ及びインシデントデ ータを組み込むためのフレームワーク(Framework for incorporated human Epidemiologic and Incident Data in Health Risk Assessment) (US-EPA、2010 年 a)という疫学的枠組み文書案を作成した。2010 年のフレームワ ーク草案は、2010年2月にFIFRA科学諮問委員会(SAP)によって好意的にレビューされた(US-EPA、2010年b)。 この文書は最近、2016 年に「Office of Pesticide Programs' Framework Document for Incorporating Human Epidemiology and Incident Data in Risk Assessments for Pesticides | (US-EPA, 2016 年)に更新された。改訂 及び更新された2016年のフレームワーク文書は、疫学研究(ヒト事例データベース、バイオモニタリング研究に加えて) でみられるようなヒトの情報が、実験的な毒性学的情報とともに、実際の化学物質ばく露によって引き起こされる影響に ついての予測を提供することで、この新しいアプローチにおいて重要な役割を果たすことが提案されている。さらに、疫 学的/分子疫学的データは、追加解析の指針となり、潜在的に影響を受けやすい集団や新たな健康影響を特定し、 既存の毒物学的観測を補完する可能性がある。2016年版フレームワークの概念は、専門家の査読を経た強固な原則 とツールに基づいており、疫学データのレビューと評価のための多くの既存のガイダンス文書とフレームワーク(表 C.1) に依存している。また、問題設定の重要性と生物学的組織の異なるレベルでの情報統合の必要性を強調した世界保 健機関/国際化学安全計画(MOA)/ヒト関連性フレームワークの更新とも一致している(Meek ら、2014 年)。さらに、 このフレームワークは、2009年の報告書「Science and Decisions (NRC、2009年)」の中で、複雑な科学的分析の最 初に問題の定式化を使用することの重要性を説明しているという点で、全米科学アカデミーの全米研究評議会(NAS /NRC)の勧告と一致している。問題の定式化の段階は、解析の目標と可能なリスク管理戦略を特定するためのリスク 管理者との計画的な対話から始まると想定されている。この最初の対話は、科学的解析のための規制の背景を提供し、 そのような解析の範囲を明確にするのに役立つ。問題設定の段階では、農薬の使用/使用、懸念される毒性学的影 響、ばく露経路、持続時間、データや科学的情報の主要なギャップに関する利用可能な情報を考慮することも含まれ ている。

<sup>&</sup>lt;sup>26</sup> See https://aghealth.nih.gov/

<sup>&</sup>lt;sup>27</sup> See https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers 85

<sup>26</sup> https://aghealth.nih.gov/を参照

<sup>27</sup> https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers を参照し てください。

www.efsa.europa.eu/efsajourna

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Table C.1:	Key guidance	documents and	frameworks use	d by OPP	(from US-EPA, 20:	16)
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	1983	Risk Assessment in the Federal Government. Managing the Process				
NAS	1994	Science and Judgement				
	2007	Toxicity testing in the 21st Century				
	2009	Science and Decisions: Advancing Risk Assessment				
WHO/	2001-2007	Mode of Action/Human Relevance Framework				
IPCS	2005	Chemical Specific Adjustment Factors (CSAF)				
	2014	New Development in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis				
EPA	1991–2005	Risk Assessment Forum Guidance for Risk Assessment (e.g. guidelines for carcinogen, reproductive, developmental, neurotoxicity, ecological, and exposure assessment, guidance for benchmark dose modelling, review of reference dose and reference concentration processes) http://www.epa.gov/risk_assessment/guidance.htm				
	2000	Science Policy Handbook on Risk Characterisation http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=4000006.txt				
	2006	Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data for Risk Assessment				
	2014	Framework for Human Health Risk Assessment to Inform Decision-making				
	2014	Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Inter-species and Intra-species Extrapolation				
	2001	Aggregate Risk Assessment https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf				
OPP	2001 and 2002	Cumulative Risk Assessment http://www.epa.gov/ncer/cra/				
OECD	2013	Organisation for Economic Co-operation and Development Guidance Document on Developing and Assessing Adverse Outcome Pathways				

Briefly, this EPA Framework document describes the scientific considerations that the Agency will weigh in evaluating how such epidemiological studies and scientific information can be integrated into risk assessments of pesticide chemicals and also in providing the foundation for evaluating multiple lines of scientific evidence in the context of the understanding of the adverse outcome pathway (or MoA). The framework relies on and espouses standard practices in epidemiology, toxicology and risk assessment, but allows for the flexibility to incorporate information from new or additional sources. One of the key components of the Agency's framework is the use the MoA framework/adverse outcome pathway concept as a tool for organising and integrating information from different sources to inform the causal nature of links observed in both experimental and observational studies. MoA (Boobis et al., 2008; Simon et al., 2014; Meek et al., 2014) and adverse outcome pathway (Ankley et al., 2010) provide important concepts in the integrative analysis discussed in the Framework document. Both a MoA and an adverse outcome pathway are based on the premise that an adverse effect caused by exposure to a compound can be described by a series of causally linked biological key events that result in an adverse human health outcome, and have as their goal a determination of how exposure to environmental agents can perturb these pathways, thereby causing a cascade of subsequent key events leading to adverse health effects.

A number of concepts in the Framework are taken from two reports from the National Academies, *Science and Decisions: Advancing Risk Assessment* (NAS 2009) and *Toxicity Testing on the 21st Century* (NAS 2007). These two NRC reports advocate substantial changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made. In particular, the 2007 report on 21st century toxicity testing advocates a decided shift away from the current focus of using apical toxicity endpoints to using toxicity pathways to better inform toxicity testing, risk assessment, and decision-making.

The MoA framework begins with the identification of the series of key events that are along the causal path and established on weight of evidence using criteria based on those described by Bradford Hill taking into account factors such as dose-response, temporal concordance, biological plausibility, coherence and consistency. Specifically, the modified Bradford Hill Criteria (Hill, 1965) are used to evaluate the experimental support that establishes key events within a MoA or an adverse outcome pathway, and explicitly considers such concepts as strength, consistency, dose response, temporal

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	1983	連邦政府におけるリスクアセスメントプロセスの管理
NAS	1994	科学と判断
	2007	21 世紀の毒性試験
	2009	科学と政策決定。リスク評価の推進
WHO/IPCS	2001-2007	行動様式/ヒトとの関連性のフレームワーク
	2005	化学物質調整係数(CSAF)
	2014	行動様式/種のコンコーダンス解析に関する WHO/IPCS フレームワークの 進化と応用における新展開
EPA	1991–2005	リスクアセスメントフォーラムリスクアセスメントのためのガイダンス (例:発がん性、生殖、発生、神経毒性、生態学的、ばく露評価のためのガ イドライン、ベンチマーク用量モデリングのためのガイダンス、参照用量と 参照濃度プロセスのレビュー
		http://www.epa.gov/risk_assessment/guidance.htm
	2000	リスク特性評価に関する科学政策ハンドブック http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=40000006.txt
	2006	生理的薬物動態(PBPK)モデルのリスク評価への応用のためのアプローチ とその裏付けとなるデータ
	2014	政策決定に役立つヒトの健康リスク評価の枠組み
	2014	種間・種族内の外挿のためのデータ由来の外挿係数を開発するための定量デ ータ適用の手引き
	2001	総合的なリスク評価
		https://www.epa.gov/sites/production/files/2015-
		07/documents/aggregate.pdf
OPP	2001 and 2002	累積リスク評価 http://www.epa.gov/ncer/cra/
OECD	2013	経済協力開発機構 (Organisation for Economic Co-operation and Development) 有害性転帰経路の開発と評価に関するガイダンス文書

EPA フレームワーク文書は、このような疫学的研究及び科学的情報を農薬化学物質のリスク評価にどのように組み 込むことができるかを評価する際に、また、有害性転帰経路(または MOA)の理解との関連で複数の科学的エビデン スを評価するための基盤を提供する際に、EPA が考慮する科学的考察を記述している。このフレームワークは、疫学、 毒性学、リスク評価の標準的な手法に依存し、それを支持しているが、新しい情報源や追加の情報源からの情報を取り 入れることも可能である。この機関のフレームワークの重要な構成要素の一つは、実験研究と観察研究の両方で観察 された因果関係の特性を知るために、異なる情報源からの情報を整理して統合するためのツールとして、MOA フレー ムワーク/有害性転帰経路の概念を使用することである。MOA(Boobis 6、2008 年;Simon 6、2014 年;Meek 6、 2014 年)と有害性転帰経路(Ankley 6、2010 年)は、フレームワーク文書で議論されている統合解析において重要な 概念を提供する。MOA と有害性転帰経路の両方とも、化合物へのばく露によって引き起こされる毒性影響は、ヒトの毒 性影響をもたらす一連の因果関係のある生物学的重要事象によって記述できるという前提に基づいており、その目的 は、環境物質へのばく露がこれらのパスウェイをどのようにかく乱させ、それによって毒性影響につながる後続の重要事 象のカスケードを引き起こすかを決定することである。

フレームワークの多くの概念は、全米科学アカデミー(National Academies, Science and Decisions)の2つの報 告書:「リスクアセスメントの進展」(Advancing Risk Assessment:NAS 2009 年)と「21 世紀の毒性試験」(Toxicity Testing on the 21st Century: NAS 2007 年)から引用されている。これら2つのNRC報告書は、毒性試験の実施 方法、データの解釈方法、そして最終的に規制上の政策決定の方法を大幅に変更することを提唱している。特に、21 世紀の毒性試験に関する2007 年の報告書では、毒性試験、リスク評価、政策決定をより良く伝えるために、現在の先 毒性エンドポイントの頂点の使用に焦点を当てたものから、毒性経路を使用することへの決定的な変化を提唱している。 MOA のフレームワークは、原因経路に沿って、そして用量反応、時間的一致、生物学的妥当性、一貫性 などの要素を考慮に入れ、Bradford Hill によって記述されたものに基づいた基準を使用して、エビデンスの重み付け concordance, and biological plausibility in a weight of evidence analysis. Using this analytic approach, epidemiological findings can be evaluated in the context of other human information and experimental studies to evaluate consistency, reproducibility, and biological plausibility of reported outcomes and to identify areas of uncertainty and future research. Figure C.1 below (adapted from NRC, 2007) suggests how different types of information relate to each other across multiple levels of biological organisation (ranging from the molecular level up to population-based surveillance) and is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function in humans.

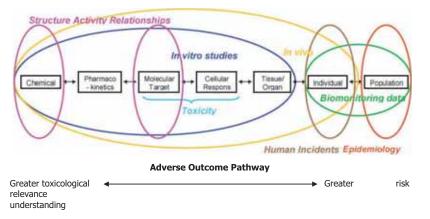


Figure C.1: Source to Outcome Pathway: Chemical effects across levels of biological organisation (adapted from NRC, 2007)

#### C.2.2. Systematic reviews: Fit for purpose

The National Academies' National Research Council (NRC) in its review of EPA's IRIS program defines systematic review as 'a scientific investigation that focuses on a specific question and uses explicit, prespecified scientific methods to identify, select, assess, and summarise the findings of similar but separate studies'.<sup>28</sup> In recent years, the NRC has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision-making.<sup>29</sup>

Consistent with NRC's recommendations, EPA-OPP employs fit-for-purpose systematic reviews that rely on transparent methods for collecting, evaluating and integrating the scientific data supporting its decisions. As such, the complexity and scope of each systematic review will vary among risk assessments. EPA-OPP starts with scoping/problem formulation followed by data collection, data evaluation, data integration and summary findings with critical data gaps identified.

Systematic reviews often use statistical (e.g. meta-analysis) and other quantitative techniques to combine results of the eligible studies, and can use a semi-quantitative scoring system to evaluate the levels of evidence available or the degree of bias that might be present. For EPA's Office of Pesticide Programs, such a Tier III (systematic review) assessment conducted as part of its regulatory review process would involve review of the pesticide chemical undergoing review and a specific associated suspected health outcome (as suggested by the initial Tier II assessment).

A number of federal and other organisations in the US are evaluating or have issued guidance documents for methods to conduct such systematic reviews and a number of frameworks have been

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に基づいて確立された一連の重要事象を特定することから始まる。特に、修正された Bradford Hill 基準(Hill、1965年)は、MOA または有害な影響の現経路内の重要事象を立証する実験的支持を評価するために使用され、エビデンスの重み付け分析において、強度、一貫性、用量反応、時間的一致、生物学的妥当性などの概念を明示的に考慮している。この分析的アプローチを用いることで、疫学的結果は、報告された結果の一貫性、再現性、生物学的妥当性を評価し、不確実性の領域と将来の研究を特定するために、他のヒト情報との関連で評価することができる。以下の図C.1(NRC、2007年より引用)は、異なるタイプの情報が生物学的組織の複数のレベル(分子レベルから集団ベースのサーベイランスに至るまで)でどのように相互に関連しているかを示唆しており、遺伝子、タンパク質、低分子がヒトの細胞機能を維持する分子経路を形成するためにどのように相互作用するかという急速に発展している科学的理解に基づいている。

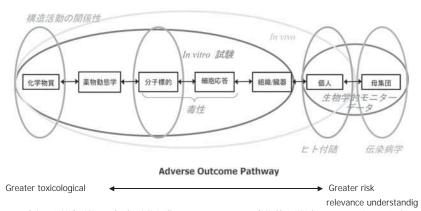


図 C.1:起源から健康影響への経路。生物組織のレベルにまたがる化学物質の影響(NRC、2007年より引用)

### C.2.2. システマティックレビュー:目的にかなった

全米アカデミーの全米研究評議会(NRC)は、EPA の IRIS プログラムのレビューにおいて、システマティックレビュ ーを「特定の問題に焦点を当て、明確かつ事前に特定された科学的方法を用いて、類似しているが別個の研究の結 果を特定、選択、評価、要約する科学的調査」と定義している<sup>28</sup>。近年、NRC は、規制上の政策決定に情報を提供す るために化学的特異性リスク評価をサポートする科学的な文献レビューの明白性を高めるシステマティックレビュープロ セスに移行することを EPA に勧めている<sup>6</sup>。

NRC の勧告に沿って、EPA-OPP は、政策決定を支える科学的データの収集、評価、統合のための明白性の高い 方法に依拠した、目的にかなったシステマティックレビューを採用している。そのため、各システマティックレビューの複 雑さと範囲はリスク評価ごとに異なる。EPA-OPP は、対象範囲/問題策定から始まり、データ収集、データ評価、デー タ統合及び重要なデータギャップが特定された結果の要約が行われる。

システマティックレビューでは、対象となる研究の結果をまとめるために統計的手法(メタアナリシスなど)やその他の 定量的手法を使用することが多く、利用可能なエビデンスのレベルや存在する可能性のあるバイアスの程度を評価す るために半定量的な採点システムを使用することができる。EPAの農薬プログラム管理局の場合、規制審査プロセスの 一環として実施されるこのような Tier III(システマティックレビュー)評価には、審査中の農薬化学物質と、(最初の Tier II評価で示唆されたように)特定の関連する健康影響が疑われる農薬化学物質の審査が含まれることになる。

米国の多くの連邦政府やその他の組織が、このようなシステマティックレビューの実施方法を評価したり、ガイダンス

<sup>&</sup>lt;sup>28</sup> http://dels.nas.edu/Report/Review-Integrated-Risk/18764

<sup>&</sup>lt;sup>29</sup> NRC, 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde' available for download at https://www.nap.edu/catalog/13142/review-of-the-environmental-protection-agency-s-draft-iris-assessment-of-formaldehyde; See also NRC, 2014. 'Review of EPA's Integrated Risk Information System (IRIS) Process' available for download at https:// www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process' available for download at https:// www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process'

<sup>&</sup>lt;sup>28</sup> http://dels.nas.edu/Report/Review-Integrated-Risk/18764

developed. These include the EPA IRIS programs' approach,<sup>30</sup> the National Toxicology Programs' Office of Health Assessment and Translation (NTP/OHAT) approach<sup>31</sup> the Cochran Collaboration's approach,<sup>32</sup> the Campbell Collaboration and the Navigation Guide,<sup>33</sup> with this latter described in a series of articles in the journal *Environmental Health Perspectives*. Each broadly shares four defined steps: data collection, data evaluation, data integration, and summary/update. For example, The Cochrane Collaboration in its Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine lists a number of the important key characteristics of a systematic review to be (from US-EPA, 2016):

- a clearly stated set of objectives with predefined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- · an assessment of the validity of the findings from the identified studies;
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

As described and elaborated in the following sections of this Annex, OPP's approach to review and integration of epidemiological data into pesticide risk assessments takes a tiered approach which each tier appropriately fit-for-purpose in the sense that is considers 'the usefulness of the assessment for its intended purpose, to ensure that the assessment produced is suitable and useful for informing the needed decisions (US-EPA, 2012) and that required resources are matched or balanced against any projected or anticipated information gain from further more in-depth research. A Tier 1 assessment is either a scoping exercise or an update to a scoping exercise in which a research and evaluation is limited to studies derived from the AHS. A Tier II assessment involves a broader search of the epidemiological literature, comprehensive data collection, and a deeper, more involved data evaluation and is more extensive but is generally limited in scope to epidemiology and stops short of multidisciplinary integration across epidemiology, human poisoning events, animal toxicology and adverse outcome pathways. A Tier III assessment is a complete systematic review with data integration and more extensive data evaluation and extraction and may involve more sophisticated epidemiological methods such as meta-analysis and meta-regression, causal inference/causal diagrams, and quantitative bias and sensitivity analyses, among others.

- C.2.3. Current and Anticipated Future EPA Epidemiology Review Practices
- C.2.3.1. Tier I (Scoping & Problem Formulation) and Tier II (more extensive literature search)

Currently at EPA, epidemiology review of pesticides is conducted in a tiered process as the risk assessment develops, as briefly described above. The purpose of this early Tier I/scoping epidemiology report is to ensure that highly relevant epidemiology studies are considered in the problem formulation/scoping phase of the process and, if appropriate, fully reviewed in the (later) risk assessment phase of the process. In Tier I, EPA-OPP focuses on well-known high quality cohort studies which focus on pesticide issues, particularly the Agricultural Health Study (AHS). The AHS is a federally funded study that evaluates associations between pesticide exposures and cancer and other health outcomes and represents a collaborative effort between the US National Institute of Occupational Safety and Health (NIOSH) and the US EPA. The AHS participant cohort includes more than 89,000 licensed commercial and private pesticide applicators and their spouses from Iowa and North Carolina. Enrolment occurred from 1993 to 1997, and data collection is ongoing. The AHS maintains on its website a list of publications associated with and using the AHS cohort (see https://aghealth.nih.gov/ news/publications.html).

If the pesticide of interest has been investigated as part of the AHS (www.aghealth.org), a preliminary (Tier I/scoping) review of these studies is performed early on in the evaluation as the

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文書を発行したりしており、多くのフレームワークが開発されている。これらには、EPA IRIS プログラムのアプローチ<sup>29</sup>、 National Toxicology Programs'Office of Health Assessment and Translation (NTP/OHAT) アプローチ<sup>30</sup>、 Cochran Collaboration のアプローチ<sup>31</sup>、Campbell Collaboration 及び Navigation Guide<sup>32</sup>が含まれ、後者につ いては Environmental Health Perspectives 誌の一連の記事で説明されている。それぞれのアプローチは、データ 収集、データ評価、データ統合、要約/更新という 4 つのステップを大まかに共有している。例えば、The Cochrane Collaboration は、The Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine の中で、システマティックレビューの重要な主要特性の多くをリストアップしている(US-EPA、2016 年)。

- 目的が明確に示されており、研究の適格性基準があらかじめ定義されていること
- 明示的で再現性のある方法論
- 適格性基準を満たすすべての研究を特定するための系統的な検索
- 特定された研究から得られた知見の妥当性の評価
- 収録された研究の特性と知見を体系的に提示し、総合的にまとめたもの。

この付属書の以下のセクションで説明・詳述されているように、農薬リスク評価への疫学的データのレビューと統合に 対する OPP のアプローチは、「作成された評価が必要な政策決定を報告するのに適しており有用であることを確認し (US-EPA、2012 年)、必要な供給源が、より詳細な研究から得られる予測または予測される情報と一致しているか、バ ランスが取れていることを確認する」という意味で、各段階が目的に応じて適切に実施される段階的なアプローチを採 用している。Tier I 評価は、調査及び評価が AHS に由来する研究に限定されている場合の、調査実施または調査実 施の更新のいずれかである。Tier II 評価では、疫学的文献の広範な検索、包括的なデータ収集、より深く、より関与し たデータ評価が行われ、より広範であるが、一般的には範囲が疫学に限定されており、疫学、ヒト中毒事例、動物毒物 学、有害な影響経路を横断した学際的な統合には至らない。Tier III 評価は、データ統合とより広範なデータ評価と抽 出を伴う完全なシステマティックレビューであり、メタアナリシスやメタ回帰、因果関係推論/因果関係図、定量的バイア ス解析や感度分析などのより高度な疫学的手法を含むことがある。

### C.2.3. 現在及び将来予想される EPA 疫学レビューの実施 C.2.3.1. Tier I (scoping と問題の定式化)と Tier II (より広範な文献検索)

現在 EPA では、農薬の疫学的レビューは、上述の通り、リスク評価の進展に応じて段階的なプロセスで実施されて いる。この初期段階の Tier I/scoping 疫学報告書の目的は、プロセスの問題の定式化/scoping 段階で関連性の高い 疫学研究が検討され、適切な場合には、プロセスの(後期の)リスク評価段階で十分に検討されるようにすることである。 Tier I 段階では、EPA-OPP は、農薬問題に焦点を当てた質の高い有名なコホート研究、特に農業健康調査 (Agricultural Health Study: AHS)に焦点を当てている。AHS は、農薬ばく露とがん及びその他の健康影響との関 連を評価する連邦政府出資の研究であり、米国国立がん研究所(NCI)、国立環境衛生科学研究所(NIEHS)、CDC の国立労働安全衛生研究所(NIOSH)及び米国環境保護庁との共同研究を代表するものである。AHS 参加者コホー トには、アイオワ州とノースカロライナ州の 89,000 人以上の免許を持つ商業及び民間の農薬散布者とその配偶者が含 まれている。登録は 1993 年から 1997 年まで行われ、データ収集は現在も継続中である。AHS は、AHS コホートに 関 連 し た 、ま た そ れ を 利 用 し た 出 版 物 の リ スト を ウ ェ ブ サ イト に 掲 載 し て い る (https://aghealth.nih.gov/news/publications.html を参照)。

対象となる農薬がAHS(www.aghealth.org)の一部として調査されている場合、EPAの「scoping」解析の一環として内容摘要(または「調査資料」)が公表されるため、評価の早い段階でこれらの研究の予備的な(Tier I/scoping)レビ

<sup>&</sup>lt;sup>30</sup> See https://www.epa.gov/iris/advancing-systematic-review-workshop-December-2015

<sup>&</sup>lt;sup>31</sup> See http://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html and NTP's 'Handbook for Conducting a Literature-based Assessment Using OHAT Approach for Systematic Review and Evidence Integration' at https://ntp.niehs.nih.gov/ntp/ohat/pub s/handbookian2015 508.pdf

<sup>32</sup> See http://handbook.cochrane.org/

<sup>33</sup> See http://ehp.niehs.nih.gov/1307175/

www.efsa.europa.eu/efsajournal

<sup>&</sup>lt;sup>29</sup> <u>https://www.epa.gov/iris/advancing-systematic-review-workshop-December-2015</u>年を参照。

<sup>30</sup> http://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html 及び NTP の「系統的レビューとエビデンス統合のための OHAT アプローチを 用いた文献ベースのアセスメントを実施するためのハンドブック」https://ntp.niehs.nih.gov/ntp/ohat/pub s/handbookjan2015\_508.pdf を参 照。

<sup>&</sup>lt;sup>31</sup> http://handbook.cochrane.org/を参照。

<sup>32</sup> http://ehp.niehs.nih.gov/1307175/を参照。

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docket (or 'dossier') is opened as part of EPA's 'Scoping' analysis. In this early Tier I/scoping phase, basic epidemiological findings and conclusions from the Agricultural Health Study are described in a Tier I/scoping document which is designed to simply summarise in brief form the pertinent conclusions of various AHS study authors if there are AHS findings relevant to a the pesticide undergoing review; this Tier I scoping review is not designed to offer detailed content, critical evaluation, or evidence synthesis, and may only touch on summarised highlights of the relevant AHS -related journal articles. If other high-quality non-AHS studies are available like those from the Children's Environmental Health and Disease Prevention Research Centres, these may be similarly summarised in this Tier I/scoping epidemiological review as well. Again, no critique or synthesis of the literature is offered. In some cases, the Tier I/scoping review may conclude that no additional epidemiological review of available evidence is further required. Alternatively, it may recommend that further review is necessary as part of a more involved Tier I/update or Tier II assessment.

A <u>Tier I/update assessment</u> is generally completed 1" to 3 years following the completion of the Tier I/scoping assessment and is issued, like the Tier II discussed below, along with and as part of the Draft Human Health Risk Assessment. Tier I/update assessments perform a thorough review of the available literature in the AHS. A Tier I/update assessment reviews, summarises and evaluates in a qualitative, narrative summary (including reported measures of association), the applicable studies that are listed on the AHS website.<sup>34</sup> Reviews are generally in the form of a narrative, focusing on the key aspects of studies and their conclusions and include EPA OPP commentary along with summary EPA OPP conclusions and recommendations for further study, if necessary.

#### C.2.3.2. Tier II (more extensive literature search)

A Tier II assessment is a more complete review of the available epidemiological evidence and is generally done only if the earlier Tier I/scoping document suggests a potential for a specific concern (e.g. a specific and credible exposure-disease hypothesis has been advanced and needs to be further evaluated as part of a more detailed assessment). A Tier II epidemiology assessment, similar to the Tier I/update, is generally completed 1" to 3 years following the completion of the Tier I assessment and is issued along with and as part of OPP's Draft Human Health Risk Assessment; the Tier II evaluation is considered to be a qualitative narrative review that incorporates certain elements of a systematic review. For example, a Tier II assessment will include a thorough and complete literature search that is broader than that of the Tier I/update, including not only the AHS database, but also such databases as PubMed, Web of Science, Google Scholar and Science Direct, and sometimes others using standardised, transparent and reproducible query language for which specialised professional library and information science support is obtained.<sup>35</sup> Evidence synthesis by EPA – albeit generally in a qualitative and narrative form - also occurs in a Tier II assessment, and overall conclusions regarding the body of epidemiological literature are made. In addition, the Tier II assessment may indicate areas in which further epidemiological data and studies with respect to specific hypothesised exposure-health outcome is of interest for future work. The Tier II assessment document will not generally attempt to integrate the epidemiological findings with other lines of evidence such as that from animal toxicology studies or information from MoAs/AOPs which may be done (separately) to some degree as part of the risk assessment. To the extent that the Tier II assessment identifies specific health outcomes putatively associated with a given pesticide, further investigation and integration across disciplines can subsequently be done as part of a more comprehensive Tier III assessment (see below).

#### C.2.3.3. Tier III (Full Systematic Review with Data Integration)

While a Tier II assessment examines a wide range of health outcomes appearing in the epidemiological literature that are hypothesised to be associated with a given pesticide chemical, a Tier III assessment might encompass a broader (multidisciplinary) and sometimes more quantitative/statistical evaluation of at the epidemiological evidence for the association of interest, and it attempts to more

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ューが実施される。この初期の段階では、AHS 研究の基本的な疫学的結果と結論は、審査中の農薬に関連するAHS 結果がある場合には、様々な AHS 研究の著者による適切な結論を簡潔に要約することを目的とした Tier I/scoping 文書に記載されている;この Tier I scoping レビューは詳細な内容、批判的な評価またはエビデンスの統合を提供す るようになっていなくて AHS に関連する学術論文の要約されたハイライトに触れる可能性があるだけである。子ども環 境保健・疾病予防研究センター (Children's Environmental Health and Disease Prevention Research Centres) の研究のように、AHS 以外の高品質な研究がある場合は、これらの研究も同様にこの Tier I/scoping 疫学レビューに 要約されているかもしれない。繰り返しになるが、文献の批評や統合は行われていない。場合によっては、Tier I/scoping レビューでは、利用可能なエビデンスの追加的な疫学的レビューはこれ以上必要ないと結論付けられること がある。あるいは、より詳細な Tier I/update または Tier II 評価の一部として、更なるレビューが必要であると勧告する 場合もある。

Tier I/update 評価は通常、Tier I/scoping 評価の完了から1年から3年後に完了し、後述するTier IIと同様に、 ヒト健康リスクアセスメント案(Draft Human Health Risk Assessment)と一緒に、またその一部として発行される。 Tier I/update アセスメントでは、AHS で利用可能な文献の徹底的なレビューが行われる。Tier I/update 評価では、 AHS のウェブサイトに掲載されている該当する研究を質的、叙述的な要約(報告された関連性の尺度を含む)でレビュ ーし、要約し、評価する<sup>33</sup>。レビューは一般的に叙述の形式で行われ、研究の主要な側面とその結論に焦点を当て、必 要に応じて EPA OPP の結論の要約及び更なる研究のための勧告に加えて EPA OPP の解説を含む。

#### C.2.3.2. Tier II(より広範な文献検索)

Tier II 評価は、利用可能な疫学的証拠のより完全なレビューであり、一般的には、初期の Tier I/scoping 文書が特定の懸念の可能性を示唆している場合にのみ実施される(例えば、特定で信頼できるばく露・疾病仮説が進められており、より詳細な評価の一部としてさらに評価する必要がある)。Tier II 疫学評価は、Tier I/update と同様に、一般的に Tier I 評価の完了から 1 年から 3 年後に完了し、OPP のヒトの健康リスク評価案(Draft Human Health Risk Assessment)と一緒に、またその一部として発行される;Tier II 評価はシステマティックレビューの特定の要素を組み込む定性的で叙述的なレビューだと考えられる。例えば、Tier II 評価では、AHS データベースだけでなく、PubMed、 Web of Science、Google Scholar、Science Direct などのデータベースや、場合によっては標準化された明白で再現性のある照会言語を使用して、専門の図書館や情報科学の支援を得て、Tier I 評価よりも広範囲に及ぶ徹底した完全な文献検索が含まれている<sup>34</sup>。EPA によるエビデンス統合(一般的には定性的かつ叙述的な形式で行われるが)も Tier II 評価では行われ、疫学的文献に関する全体的な結論が出されている。さらに、Tier II 評価は、特別な仮説としてばく露・健康影響に関する更なる疫学的データや研究が将来の研究のための興味深い分野を示す可能性がある。 Tier II 評価文書は一般的に、疫学的知見を、動物毒性試験や、リスク評価の一部としてある程度(別個にに)行われる MOAs/AOPs からの情報などの他のエビデンスと統合しようとはしない。特定の農薬に関連すると考えられる特定の健康影響が特定される範囲までの Tier II 評価に対して、その後のより包括的な Tier III 評価では、分野を超えた更なる調査及び統合を行うことができる(下記参照)。

### C.2.3.3. Tier 3(データ統合を伴う完全なシステマティックレビュー)

Tier II 評価では、ある農薬化学物質との関連性があると仮説が立てられている疫学的文献に現れる広範な健康影響を検討するが、Tier III 評価では、より広範な(学際的な)疫学的根拠に基づく、時にはより定量的/統計学的な評

<sup>34</sup> https://aghealth.nih.gov/news/publications.html

<sup>&</sup>lt;sup>35</sup> Additional searches conducted under the rubric of epidemiology and biomonitoring/exposure could be done using the NHANES Exposure Reports (http://www.cdc.gov/exposurereport/); TOXNET (http://toxnet.nlm.nih.gov/); CDC NBP Biomonitoring Summaries (http://www.ichcem.org/pages/cicad s.html); ATSDR Toxicological Profiles (http://www.atsdr.cdc.gov/toxprofiles/index.asp); IARC Monographs (http://monographs. iarc.fr/ENG/Monographs/PDF5/; EFSAS Draft Assessment Report Database (http://dar.efsa.europa.eu/dar-web/provision); and Biomonitoring Equivalents (https://blog.americanchemistry.com/2014/07/biomonitoring-equivalents-a-valuable-scientific-toolfor-making-better-chemical-safety-decisions/

<sup>33</sup> https://aghealth.nih.gov/news/publications.html

<sup>&</sup>lt;sup>34</sup> 疫学とバイオモニタリング/ばく霧の項目の下で行われた追加検索は、NHANES Exposure Reports (http://www.cdc.gov/exposurereport/); TOXNET (http://toxnet.nlm.nih.gov/); CDC NBP Biomonitoring Summaries

<sup>(</sup>http://www.ichem.org/pages/cicad s.html); ICICADS (http://www.inchem.org/pages/cicad s.html); ATSDR Toxicological Profiles (http://www.atsdr.cdc.gov/toxprofiles/index.asp), IARC モバグラフ (http://monographs. iarc.fr/ENG/Monographs/PDFs/; EFSA's Draft Assessment Report Database (http://dar.efsa.europa.eu/dar.web/provision); and Biomonitoring Equivalents (https://blog.americanchemistry.com/2014/07/biomonitoring-equivalents-a-valuable-scientific-tool- formaking-better-chemical-safety-decisions/)

formally integrate this with animal toxicology and MoA/AOP information. Such a Tier III assessment could take the form of a systematic review of the epidemiological literature which would be performed together with evaluation of toxicity and adverse outcome pathways. For pesticide chemicals from AHS, a Tier III analysis would also ideally incorporate the results of evaluations from other high-quality epidemiological investigations and incorporate 'Weight of the Evidence' to a greater degree to reflect a more diverse set of information sources. Results from these investigations would be used to evaluate replication and consistency with results from the AHS. Early AHS findings in a number of cases were based on only a small number of participants that had developed specific outcomes or a relatively few number of years over which the participants have been followed. As the AHS cohort ages, the release of second evaluations of some chemicals from AHS will be based on additional years of follow-up and a greater number of cases that are expected to provide a more robust basis for interpreting positive and negative associations between exposure and outcome. In addition, the AHS is increasingly generating a substantial amount of biochemical, genetic marker, and molecular data to help interpret results from the epidemiological studies. Such results may further clarify AHS findings, provide evidence for a biological basis linking exposures to outcomes, or suggest additional laboratory and observational research that might strengthen evidence for mechanisms underlying causal pathways. In addition, Tier III analyses also may take advantage of efforts to bring together information and results from international cohort studies in the International Agricultural Cohort Consortium (AgriCOH) in which AHS is a member. AgriCOH is actively working to identify opportunities and approaches for pooling data across studies, and the availability of these other cohort data should aid in assessing reproducibility and replication of exposure-outcome relationships as EPA considers, evaluates and weighs the epidemiological data.

#### C.2.4. OPP's open literature searching strategies and evaluation of study quality

An important aspect of the systematic review approach is the thorough, systematic, and reproducible searching of the open epidemiological literature such that much of the literature that meets the established eligibility criteria can be located.<sup>36</sup> OPP uses specific databases as part of their literature search and has specific guidance on their conduct (for example, OPP's open literature search guidance for human health risk assessments<sup>37</sup>). Evaluation of all relevant literature, application of a standardised approach for grading the strength of evidence, and clear and consistent summative language will typically be important components (NRC, 2011). In addition, a high quality exposure assessment is particularly important for environmental and occupational epidemiology studies.

A second important component of the above systematic review approach is the assessment of the validity of the findings from the identified studies. Generally speaking, the quality of epidemiological research, sufficiency of documentation of the study (study design and results), and relevance to risk assessment will be considered when evaluating epidemiology studies from the open literature for use in agency risk assessments. When considering individual study quality, various aspects of the design, conduct, analysis and interpretation of the epidemiology studies are important. These include (from US-EPA, 2016):

- clear articulation of the hypothesis, or a clear articulation of the research objectives if the study is hypothesis-generating in nature;
- adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment;
- reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population);
- appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias;
- adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed.

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価を行い、これを動物毒性学や MOA/AOP 情報とより正式に統合しようとするものである。このような Tier III 評価は、 疫学的文献のシステマティックレビューの形をとり、毒性及び有害な転帰の経路の評価と併せて実施される。

AHS 由来の農薬化学物質については、Tier III 解析では、他の質の高い疫学調査の評価結果を取り入れ、より多 様な情報源を再調査するために「エビデンスの重み付け」をより多く取り入れることが理想的である。これらの調査の結 果は、AHS の結果との再現性と一貫性を評価するために使用される。多くのケースにおける初期の AHS の結果は、 特定の健康影響を示した少数の参加者、または参加者を追跡した年数が比較的少ない参加者に基づいている。AHS コホートの高齢化に伴い、AHS からのいくつかの化学物質の 2 回目の評価の発表は、さらなる追跡調査の年数と、ば く露と健康影響の間の正負の関連を解釈するためのより強固な根拠となると期待されるより多くの症例数に基づいて行 われることになるであろう。さらに、AHS では、疫学研究の結果の解釈を助けるために、かなりの量の生化学的、遺伝 的マーカー及び分子データの生成が増加している。このような結果は、AHS の結果をさらに明確にしたり、ばく露と健 康影響を結びつける生物学的基盤のエビデンスを提供したり、あるいは因果関係の経路の基礎となるメカニズムのエビ デンスを強化する可能性のある追加の実験研究や観察研究を示唆したりする可能性がある。さらに Tier III 解析では、 AHS がメンバーである国際農業コホートコンソーシアム(AgriCOH)の国際的なコホート研究からの情報と結果をまと める努力を利用することもできる。AgriCOH は、研究間のデータを蓄積するための機会と方法を特定するために積極 的に取り組んでおり、これらの他のコホートデータの利用可能性は、EPA が疫学的データを検討、評価、重み付けする 際に、ばく露ー健康影響の関係の再現性と反復を評価するのに役立つはずである。

#### C.2.4. OPP の公表文献検索戦略及び研究の質の評価

システマティックレビューアプローチの重要な側面は、確立された適格基準を満たす多くの文献をみつけることがで きるように、公表されている疫学的文献を徹底的に、体系的に、再現可能に検索することである<sup>35</sup>。OPP は文献検索の 一部として特定のデータベースを使用しており、その実施に関する特定のガイダンスがある(例えば、ヒト健康リスク評価 に関する OPP の公表文献検索ガイダンス<sup>36</sup>)。すべての関連文献の評価、エビデンスの強固さを評価するための標準 化されたアプローチの適用、明確で一貫性のある総括的表現は、一般的に重要な要素となる(NRC、2011 年)。さらに、 質の高いばく露評価は、環境疫学研究や職域疫学研究において特に重要である。

上記のシステマティックレビューのアプローチの第二の重要な要素は、同定された研究から得られた結果の妥当性 の評価である。一般的に言えば、疫学研究の質、研究の文書化の妥当性(研究の計画と結果)、リスク評価との関連性 は、政府機関のリスク評価に使用するために公表されている文献から疫学研究を評価する際に考慮される。個々の研 究の質を検討する際には、疫学研究の計画、実施、解析、解釈の様々な側面が重要である。これらには以下が含まれ る(US-EPA、2016年より)。

- 1) 仮説を明確に明示することで、たとえその研究が本質的に仮説生成的なものであったとしても、その仮説を明確に 示されていること。
- 2)健康影響の関連する臨界期、リスク評価対象集団の関係あるばく露範囲、試験から得られる用量/ばく露反応の 傾向の入手可能性など、ばく露評価の資質の中で、適切なばく露評価が十分であること。
- 3)合理的に有効で信頼性の高い結果の確認(研究集団における健康影響の有無を正しく識別されていること)。
   4)対象集団を代表するサンプル集団となり、系統的な偏りがない適切な組み入れ基準と除外基準。
- 5) 観察されたリスク推定値における複数の農薬ばく露、または混合物ばく露の役割の評価または考察を含む、潜在 的な交絡変数の適切な評価及び解析。

<sup>&</sup>lt;sup>36</sup> Some advocate looking at the grey or unpublished literature to lessen potential issues associated with publication bias.
<sup>37</sup> See https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open and specifically p. 10 of the document 'Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment' dated 28.8.2012 at https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf for Special Notes on Epidemiologic Data.

<sup>35</sup> 出版バイアスに関連する潜在的な問題を軽減するために、はっきりしない文献や未発表の文献を見ることを提唱する者もいる。

<sup>36</sup> 疫学的データに関する特別な注意事項については、

<sup>&</sup>lt;u>https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-identifying-selecting-and-evaluating-open</u>及び2012年 8月 28日付けの文書「Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment」の10ページを参照のこと。



- 6) overall characterisation of potential systematic biases in the study including errors in the selection of participation and in the collection of information, including performance of sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented;
- adequate statistical power for the exposure-outcome assessment, or evaluation of the impact of statistical power of the study if under-powered to observed effects, and appropriate discussion and/or presentation of power estimates; and
- 8) use of appropriate statistical modelling techniques, given the study design and the nature of the outcomes under study.

#### References

- Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730–741.
- Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36, 781–792.
- Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C, 2008. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Critical Reviews in Toxicology, 38, 87–96.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295–300.
- Meek, ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J and Vickers C, 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. Journal of Applied Toxicology, 34, 595–606.
- Meek, ME, Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of Applied Toxicology, 34, 1–18.
- NAS (National Academy of Sciences), 2007. Toxicity Testing on the 21st Century: A Vision and a Strategy. Board on Environmental Studies and Toxicology. Available online: https://www.nap.edu/catalog/11970/toxicity-testingin-the-21st-century-a-vision-and-a
- NAS (National Academy of Sciences), 2009. Science and decisions: advancing Risk Assessment. Board on Environmental Studies and Toxicology. Available online: http://dels.nas.edu/Report/Science-Decisions-Advanc ing-Risk-Assessment/12209
- NAS (National Academy of Sciences), 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Board on Environmental Studies and Toxicology. Available online: https:// www.nap.edu/download/13142
- Simon TW, Simons SS, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Crisp PF, McMullin TS, McQueen CA and Rowlands JC, 2014. The use of mode of action information in risk assessment: Quantitative key events/dose response framework for modelling the dose-response for key events. Critical Reviews in Toxicology, 44 (Suppl 3), 17–43.
- US-EPA (Environmental Protection Agency), 2010a. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment. Presented to FIFRA Scientific Advisory Panel on February 2-4 2010a. January 7. Available online: https://www.regulations.gov/document?D=EPA-HQ-OPP-2009-0851-0004
- US-EPA (Environmental Protection Agency), 2010b. Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. MEMORANDUM dated 22 April, 2010b. SAP Minutes No. 2010-03. Available online: https://www.re gulations.gov/document?D=EPA-HQ-OPP-2009-0851-0059
- US-EPA (Environmental Protection Agency), 2012. Office of the Science Advisor. Risk Assessment Forum. Draft Framework for Human Health Risk Assessment to Inform Decision Making. July 12, 2012.
- US-EPA (Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic and Incident Data in Risk Assessments for Pesticides. December 28, 2016. Available online: https://www3.epa.gov/pesticides/EPA-HO-OPP-2008-0316-DRAFT-0075.pdf

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- 6)参加者の選択や情報収集における誤りを含む、研究における潜在的な系統的な偏りの全体的な特性。これには、 提示されたリスク推定値に対する系統的誤差の潜在的な影響を調査するための感度分析の実施を含む。
- 7)ばく露・健康影響評価のための適切な統計的検出力、または観察された影響に対して検出力が不足している場合の研究の統計的検出力の影響の評価及び検出力推定値の適切な考察及び/または提示。
- 8)研究デザインと対象となる結果の性質を考慮した適切な統計的モデル化技術の使用。

#### 参考文献

- Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730–741.
- Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36, 781–792.
- Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J and Vickers C, 2008. IPCS framework for analyzing the relevance of a noncancer mode f action for humans. Critical Reviews in Toxicology, 38, 87–96.
- Hill AB, 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine, 58, 295–300.
- Meek, ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J and Vickers C, 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. Journal of Applied Toxicology, 34, 595–606. Meek, ME, Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance
- Meek, ME, Palermo CM, Bachman AN, North CM and Lewis RJ, 2014. Mode of action human relevance (species concordance) framework: evolution of the Bradford Hill considerations and comparative analysis of weight of evidence. Journal of Applied Toxicology, 34, 1–18. NAS (National Academy of Sciences), 2007. Toxicity Testing on the 21st Century: A Vision and a Strategy.
- NAS (National Academy of Sciences), 2007. Toxicity Testing on the 21st Century: A Vision and a Strategy. Board on Environmental Studies and Toxicology. Available online: https://www.nap.edu/catalog/11970/ toxicity-testing: in-the-21st-century-a-vision-and-a
- NAS (National Academy of Sciences), 2009. Science and decisions: advancing Risk Assessment. Board on Environmental Studies and Toxicology. Available online: http://dels.nas.edu/Report/Science-Decisions-Advance ing-Risk-Assessment/12209
- NAS (National Academy of Sciences), 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Board on Environmental Studies and Toxicology. Available online: https:// www.nap.edu/download/13142
- Simon TW, Simons SS, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Crisp PF, McMullin TS, McQueen CA and Rowlands JC, 2014. The use of mode of action information in risk assessment: Quantitative key events/dose response framework for modelling the dose-response for key events. Critical Reviews in Toxicology, 44 (Suppl 3), 17–43.
- US-EPA (Environmental Protection Agency), 2010a. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment. Presented to FIFRA Scientific Advisory Panel on February 2-4 2010a. January 7. Available online: https://www.regulations.gov/document?D= EPA-HQ-OPP-2009-0851-0004
- US-EPA (Environmental Protection Agency), 2010b. Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. MEMORANDUM dated 22 April, 2010b. SAP Minutes No. 2010-03. Available online: https://www.re gulations.gov/document?D=EPA-HQ-OPP-2009-0851-0059
- US-EPA (Environmental Protection Agency), 2012. Office of the Science Advisor. Risk Assessment Forum. Draft Framework for Human Health Risk Assessment to Inform Decision Making, July 12, 2012.
- US-EPA Environmental Protection Agency), 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic and Incident ata in Risk Assessments for Pesticides. December 28, 2016. Available online: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

#### Annex D – Effect size magnification/inflation

As described in the main text of this document, a potential source of bias may result if a study has low power. This lesser known type of bias is known 'effect size magnification'. While it is as widely known that, generally small, low-powered studies can result in false negatives since the study power is inadequate to reliably detect a meaningful effect size, it is less well known that these studies can result in inflation of effect sizes if those estimated effects are required to pass a statistical threshold (e.g. the common p < 0.05 threshold used for statistical significance) to be judged important, relevant, or 'discovered'. This effect – variously known as effect size magnification, the 'winners curse', truth inflation, or effect size inflation – is a phenomenon by which a 'discovered' association (i.e. one that has passed a given threshold of statistical significance to be judged meaningful) from a study with suboptimal power to make that discovery will produce an observed effect size that is artificially and systematically inflated.

Such truth inflation manifests itself as (systematic) bias away from the null in studies that achieve statistical significance in instances where studies are underpowered (Reinhart, 2015). This is because low-powered (and thus generally smaller) studies are more likely to have widely varying results and thus be more likely to be affected by random variation among individuals than larger ones. More specifically, the degree of effect size magnification that may be observed in any study depends, in part, on how widely varying the results of a study is expected to be and this depends on the power of the study; low powered studies tend to produce greater degrees of effect size magnification in results that are found to be statistically significant (or pass other threshold criteria) than higher powered studies.

As an example of this 'effect size magnification' concept and why it may come about, it is useful to imagine a trial run thousands of times with variable sample sizes. In this case, there will be a broad distribution of observed effect sizes. While the observed medians of these estimated effect sizes are expected to be close to the true effect size, the smaller trials will necessarily systematically produce a wider variation in observed effect sizes than larger trials. However, in low powered studies, only a small proportion of observed effects will pass any given (high) statistical threshold of significance and these will be only the ones with the greatest of effect sizes. Thus, when these generally smaller, low powered studies with greater random variation do indeed find a significance-triggered association as a result of passing a given statistical threshold, they are more likely to overestimate the size of that effect. What this means is that research findings of low-powered and statistically significant studies are biased in favour of finding inflated effects. As summarised by Gelman and Carlin (2014): 'when researchers use small *[underpowered]*<sup>38</sup> samples and noisy measurements to study small effects..., a significant result is often surprisingly likely to be in the wrong direction and to greatly overestimate an effect'. In general, it can be shown that low background (or control or natural) rates, low effect sizes of interest, and smaller sample sizes in the study end to produce lower power in the study and this leads to a greater tendency towards and magnitude of (any) inflated effect sizes.

It is important to note that the effect size inflation phenomenon is a general principle applicable to discovery science in general and is not a specific affliction or malady of epidemiology (Ioannidis, 2005; Lehrer, 2010; Button, 2013; Button et al., 2013; Gelman and Carlin, 2014; Reinhart, 2015). It is often seen in studies in pharmacology, in gene studies, in psychological studies, and in much of the most-often cited medical literature. When researchers have limited ability to increase the sample size such as in most epidemiological studies, effect size magnification is not a function or fault of the research or research design, but rather a function of how that the results of that research are interpreted by the user community. Thus, unlike other possible biases such as selection or information bias in epidemiology studies, the bias is not intrinsic to the study or its design, but rather characteristic of how that study is interpreted.

In order to determine (and quantify) the potential degree of effect size magnification for any given study that produces a statistically significant result, the reviewer must perform various power calculations. More specifically, when the association between a chemical exposure and a disease is found to be statistically significant, a power analysis can be done to determine the degree to which the statistically significant effect size estimate (e.g. odds ratio, relative risk or rate ratio) may be artificially inflated.

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#### 付属書 D-影響量の拡大/膨張

この文書の本文で説明されているように、研究の検出力が低い場合には、バイアスの潜在的な原因が生じる可能性 がある。このあまり知られていないタイプのバイアスは、「影響量の規模」として知られています。一般的に、小規模で検 出力の低い研究では、研究の検出力が意味のある影響量を確実に検出するには不十分であるため、偽陰性が生じる 可能性があることは広く知られているが、推定された影響が重要、関連性がある、または「発見された」と判断されるため に、統計的閾値(例えば、統計的有意性に使用される一般的な p<0.05 の閾値)を通過する必要がある場合、これらの 研究が影響量の膨張をもたらす可能性があることはあまり知られていない。この影響は、影響量の拡大、「勝者の呪い」、 真実の膨張、または影響量の膨張として様々に知られているが、これは、「発見された」関連性(すなわち、意味がある と判断されるために統計的有意性の所定の閾値を通過した関連性)が、その発見を行うために最適ではない検出力を 持つ研究から得られる現象であり、その結果、意図的かつ体系的に膨張した影響量が生じることになる。

このような真偽の不明確化は、検出力の低い研究で統計的有意差を達成する研究では、帰無値から離れる(系統的 な)バイアスとして現れる(Reinhart、2015年)。これは、検出力の低い(したがって一般的には小さい)研究は、結果が 大きく変動する可能性が高く、大規模な研究よりも個人の間のランダムな変動の影響を受けやすいからである。より具 体的には、どのような研究でも観察されるかもしれない影響量の拡大の変化の程度は、研究の結果がどの程度広く変 化すると予想されるかに部分的に依存しており、これは研究の検出力に依存する;検出力の低い研究は影響量の拡大 の程度を大きくする傾向があり、その結果検出力の高い研究よりも統計的有意性(または他の閾値基準をパスする)を 見出す。

この「影響量の拡大」の概念とその理由の例として、可変のサンプルサイズで何千回も試験を実施した場合を想像す ると便利である。この場合、観察された影響量の広い分布がある。これらの推定影響量の観察された中央値は、真の影 響量に近いと予想されるが、小規模な試験では、大規模な試験に比べて、観察された影響量のばらつきが必然的に大 きくなる。しかし、低検出力の研究では、観察された影響のうち、統計的に有意な(高い)関値を通過するのはごく一部 であり、これらの影響は最大の影響量を持つものだけである。このように、一般的に小規模で、ランダム変動が大きい低 検出力の研究では、与えられた統計的関値を通過した結果、実際に有意性起因の関連を発見した場合、その影響の 大きさを過大評価する可能性が高くなる。これが意味するのは、低検出力で統計的に有意な研究の結果は、膨張効果 となるように偏っているということである。Gelman 及び Carlin(2014 年)が要約しているように、「研究者が小さな影響 を研究するために小さな[検出力不足]<sup>37</sup>サンプルとノイズの多い測定を使用した場合、有意な結果はしばしば驚くほど 間違った方向に行き、影響を大幅に過大評価する可能性が高い」のである。一般的に、バックグラウンド(または対照ま たは無処置)率が低い、対象となる影響量が小さい、研究中のサンプルサイズが小さいと、研究の検出力が低下し、そ の結果、(あらゆる)膨張した影響量の傾向と規模が大きくなることが示されている。

影響量の膨張現象は、発見科学全般に適用される一般的な原則であり、疫学の特殊な現象や弊害ではないことに 注意することが重要である(Ioannidis, 2005 年; Lehrer, 2010 年; Button, 2013 年; Button ら、2013 年; Gelman 及び Carlin, 2014 年; Reinhart, 2015 年)。これは、薬理学の研究、遺伝子研究、心理学の研究、そして最もよく引 用される医学文献の多くでよくみられる。ほとんどの疫学研究のように、研究者がサンプルサイズを増加させる能力が限 られている場合、影響効果量の拡大は、研究や研究デザインの機能や欠陥ではなく、むしろ、その研究の結果がユー ザーコミュニティによってどのように解釈されるかという機能である。したがって、疫学研究における選択や情報バイアス のような他のバイアスの可能性とは異なり、バイアスは研究やその計画に内在するものではなく、むしろその研究がどの ように解釈されるかに特徴がある。

統計的に有意な結果をもたらす研究について、影響量の規模の潜在的な程度を決定(定量化)するために、査読者 は様々な検出力の計算を実行しなければならない。より具体的には、化学物質ばく露と疾患との間の関連が統計的に 有意であることが判明した場合、検出力解析は、統計的に有意な影響量の推定値(例えば、オッズ比、相対リスクまた は率比)がどの程度人工的に膨張しているかを決定するために行われる。

<sup>&</sup>lt;sup>38</sup> [italics added]

<sup>37 [</sup>斜体を付けた]

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In order to perform the requisite power calculation, the reviewer must know or obtain four values:

- 1) the number of subjects in non-exposed group;
- 2) the number of subjects in the exposed group;
- the number of <u>individuals with the disease of interest (or cases)</u> in the <u>non-exposed</u> group; and
- a target value of interest to detect a difference of a given (predetermined) size in a comparison of two groups (e.g. exposed vs. not exposed)

The first three listed values are provided in or must be obtained from the publication while the target value of interest (typically an OR or RR in epidemiology studies) is selected by the risk managers (and is ultimately a policy decision).<sup>39</sup> This Annex examines this effect size inflation phenomenon in a quantitative way using simulations. The annex uses two example published studies and simulations of hundreds of trials to evaluate the degree to which effect size magnification may play a role in producing biased effect sizes (such as odds ratios, rate ratios or relative risks) due to low power.

The first example uses data from Agricultural Health Study prospective cohort publication examining diazinon exposure and lung cancer and illustrates the effect size magnification issue for a calculated RR. The second example uses ever-never data from a case–control study studying malathion exposure and NHL and illustrates the effect size magnification concept from the point of view of an estimated OR.

#### An Example Illustrating Effect Size Magnification and Relative Risk (Jones et al. (2015))

The power associated with a comparison between those that are not exposed to diazinon to those that are exposed at the highest tertile (T) can be computed from the information provided in the AHS study publication 'Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study - an updated analysis' by Jones et al. (2015) for lung cancer. The number of subjects at each exposure level was provided in the article (non-exposed group: N = 17710, and T(ertile)1, T2 and T3 were categorised based on exposure distribution; specifically: N of each tertile = (2,350 + 2,770)/3 = 1,710 from the publication's Table 1 where: (a) the value of 2,350 represents the number in the lowest exposed *level* and (b) the value of 2,770 represents the number of the two highest exposed levels when the exposed subjects were dichotomously categorised. Since we have (i) the number of subjects in the reference non-exposed group = 17,710; (ii) the number of subjects in each of the exposed groups (tertiles) = 1710; and (iii) the number of diseased individuals (lung cancer) in the reference non-exposed group = 199 (from Table 3 of the cited publication), we can calculate the power of the comparisons between T1 vs non-exposed, T2 vs non-exposed and T3 vs non-exposed that were presented in the article, given the assumption that any true Rate Ratio = 1.2, 1.5, or 2.0, etc.

Here, we are interested in evaluating the power associated with the estimated background rate of 199/17710 (= 0.011237), and, as a form of sensitivity analysis, one half of this background rate (or 0.005617), and twice this rate (0.022473) for detecting (admittedly arbitrary) relative rates of (possible regulatory interest of) 1.2, 1.5, 2.0 and 3.0 among the subjects in each tertile of the diazinon exposed individuals. This analysis was performed using Stata statistical software and is shown below in both tabular and graphical format for true Rate Ratios of 1.2, 1.5, 2.0 and 3.0 for

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必要な検出力計算を行うために	、査読者は以下の4	つの値を知っているか、	または得なければならない。
1) 非ばく 露 起の 被 験 者 数 .			

2)ばく露群の被験者数。

3)非ばく露群の対象疾患を持つ個人の数(または症例数)及び

4)2 つのグループ(例えば、ばく露群 vs.非ばく露群)の比較において、所定の(事前決定の)量の差を検出するための対象となる目標値。

最初の3 つの値は文献に記載されているか、文献から入手しなければならないが、対象となる目標値(一般的に疫 学研究ではORまたはRR)はリスク管理者によって選択される(最終的には政策決定である)<sup>38</sup>。本付属書では、シミュ レーションを用いて、この影響量の膨張現象を定量的に検討する。本付属書では、2 つの公表された研究例と数百件 の試験のシミュレーションを用いて、検出力が低いために偏った影響量(オッズ比、率比、相対リスクなど)を生み出す のに影響量の拡大がどの程度の役割を果たしているかを評価している。

最初の例では、ダイアジノンばく露と肺がんを調査した Agricultural Health Study の前向きコホート出版物からの データを使用し、計算された RR の影響量の偏りの問題を説明している。2番目の例は、マラチオンばく露とNHLを研 究した症例対照研究からの ever-never データを使用して、推定 OR の観点から影響量の拡大の概念を説明する。

#### 影響量の拡大の説明と相対リスクを説明する例(Jones ら、2015 年)

ダイアジノンにばく露されていないものと、最も高い三分位(T)でばく露されているものとの間の比較に関連した検出 力は、肺がんに対する Jones ら(2015 年)の AHS 研究発表「Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study - an updated analysis」で提供された情報から計算することができる。各ばく露量での被験者数は文献中に記載されてい た(非ばく露群。N=17710 及び T(ertile) 1, T2 及び T3 は、ばく露分布に基づいて分類された;具体的には、各三分 位の N=(2,350 + 2,770)/3=1,710 文献の表 1 から。(a) 2,350 の値は最も低いばく露量を表し、(b) 2,770 の値は、 ばく露された被験者を二分法で分類したときの、2 つの最も高いばく露量を表している。(i)参照非ばく露群の被験者数 =17,710; (ii) ばく露群(三分位)のそれぞれの被験者数=1710; (iii)参照非ばく露群の罹患者数(肺がん)=199 人 (引用文献の表 3 より)とすると、真の率比=1.2、1.5、または 2.0 と仮定すると、文献に示された T1 対非ばく露群、T2 対非ばく露群、T3 対非ばく露群の比較の検出力を計算することができる。

ここで、我々は、199/17710(=0.011237)の推定バックグラウンド率及び感度分析の形態として、このバックグラウン ド率の1/2(または0.005617)及びダイアジノンにばく露された個人の各三分位の被験者の間で1.2、1.5、2.0及び3.0 の(可能な規制関係の)相対率を検出するためのこの率の2倍(0.022473)に関連する検出力を評価することに注目し ている。この解析はStata統計ソフトウェアを使用して行われ、1.2、1.5、2.0及び3.0の真の率比について、199人の 罹患者/17,710人のバックグラウンド率に対して1/2x・、1x・(太字/網掛けで以下に示す)及び

<sup>&</sup>lt;sup>39</sup> This target value is an effect size of interest, often expressed as either a relative risk (for cohort studies) or an odds rate (for case control studies). That is, the target value is generally an OR or RR of a given magnitude that the risk manager desires to detect with a given degree of confidence. The higher the OR or RR, the greater the magnitude of the estimated association between exposure and the health outcome. While there are not strict guidelines about what constitutes a 'weak' association vs a 'strong' one - and it undoubtedly can be very context-dependent - values less than or equal to about 1 (or sometimes  $\leq$  1.2) are considered to be 'null' or 'essentially null' (this ignores the possibility of a protective effect which in some contexts – for example, vaccination efficacy - may be appropriate to consider). Values less than 2 or 3 are often considered by some as 'weak'. Values greater than 2 (or 3) and up to about 5 might be considered 'moderate', and values greater than 5 are considered by some to be 'large'. Monson (1990) describes as a guide to the strength of association a rate ratio of 1.0-1.2 as 'None', of from 1.2 to 1.5 as 'Weak', of from 1.5 to 3.0 as 'Moderate', and of 3.0-10.0 as 'Strong'. Other authors use Cohen's criteria to describe ORs of 1.5 as 'small' and 5 as 'large', with 3.5 as 'medium' in epidemiology (Cohen and Chen, 2010). Others describe 1.5 as 'small', 2.5 as 'medium' or 'moderate', 4 as 'large' or 'strong' and 10 as 'very large' or 'very strong' (Rosenthal, 1996) Taube (1995) discusses some of the limitations of environmental epidemiology in detecting weak associations (also see invited commentary illustrating counter-arguments in Wynder (1997). It should be recognized that none of the demarcation lines are 'hard' and there can be legitimate disagreements about where these are drawn and how these are considered and interpreted. Regardless, these can be very much context-dependent and the above demarcations should not be regarded as in any way official or definitive.

<sup>&</sup>lt;sup>38</sup> この目標値は対象となる効果量であり、相対リスク(コホート研究の場合)またはオッズ率(確例対照研究の場合)のいずれかで表されるとが多い、 すなわち、目標値は一般的に、リスク管理者が一定の確信度で検出したいと考えている一定の大きさのOR または RF である。OR または RF が 高ければあいほど、ばく驚と健康影響との間の推定関連性の規模が大きくなる。何が「弱い」関連性と「強い」関連性を構成するかについての厳密 なガイドラインはないが、約1以下(時には 1.2 以下)の値は「帰無」または「本質的に帰無」と考えられる(これは、いくつかの背景(例えば、ワクチ ン接種の有効性など)では考慮することが適切であるかもしれない保護効果の可能性を差視している)。2 または 3 未満の値は、しばしば「弱い」と 考えられていてる。2 (または 3) より大きく約5 までの値は「中等度」と考えられ、5 より大きい値は「大」であると考えられていて、 のmoson(1990)は、 関連性の強さの目安として、1.0~1.2 を「か」、5 を「中」と表現している)、2 または 3 未満の値は、しばしば「弱い」と 考えられていてる。2 (または 3) より大きく約5 までの値は「中等度」と考えられ、5 より大き、3 いであると考えられていて。 (なわれの方面では 5 いた、5 まで)、5 まで「明」と表見している(Cohen and Chen、2010)また、1.5 を「小」、5 まで」」またば「中 程度」、4 を「大」またば「強てに大きい」または「非常に強い」と表現する人もいる(Rosenthal, 1996年)、 Tabuら(P5年)は、弱い関 連性を検出する上での環境疫学の限界について議論している(Wynder(1997年)の反論を示す招待解説も参照のこと)。これらの境界線はどれ も「軽しい」ものではなく、どこに線が引かれ、どのように解釈されるかについては、正当な意見の相違があり得ることを認識す べきである。それにもかかわらず、これらの境界線は背景に大きく依存するものであり、上記のような境界線は、いかなる意味でも、公式なものでも、 次定的なためのでもあると考えるべきではない。

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1/2x-, 1x- (shown below in bold/shaded) and 2x- the (observed) background rate of 199 diseased individuals/17,710 persons<sup>40</sup>:

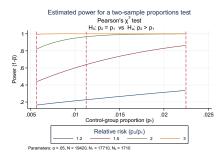
Results of p	ower analysis for	a one-sided, two-	Results of power analysis for a one-sided, two-sample proportions test ( $\alpha = 0.05$ ) <sup>(a)</sup>									
N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Relative risk	Power							
17,710	1,710	0.00562	0.00674	1.2	0.1634							
17,710	1,710	0.00562	0.00843	1.5	0.4353							
17,710	1,710	0.00562	0.01124	2.0	0.8182							
17,710	1,710	0.00562	0.01685	3.0	0.9935							
17,710	1,710	0.01124	0.01348	1.2	0.2259							
17,710	1,710	0.01124	0.01685	1.5	0.6379							
17,710	1,710	0.01124	0.02247	2.0	0.9652							
17,710	1,710	0.01124	0.03371	3.0	1							
17,710	1,710	0.02247	0.02697	1.2	0.3353							
17,710	1,710	0.02247	0.03371	1.5	0.8632							
17,710	1,710	0.02247	0.04495	2.0	0.9991							
17,710	1,710	0.02247	0.06742	3.0	1							

State code used to generate the above power calculation results: power two proportions ('= 0.5 \* 199/ 17710'= 199/17710'= 2 \* 199/17710), test(chi2) RR(1.2 1.5 2.0 3.0) nl(17710) n2 (1710) one-sided table(N1:'N control''N2:'N exposed''p1:'proportion control''p2:''proportion exposed''R:''relative risk'' power:''power'').

(a): One-sided test  $\alpha = 0.05$  Ho: p2 = p1 vs Ha: p2 > p1; N<sub>controls</sub> = 17,710, N<sub>exposed</sub> = 1,710; Number of Iterations = 1,000 (data sets).

(b): Representing 1/2×-, 1x- and 2x- the observed background rate of lung cancer of 199/17710 in Jones et al. (2015). Highlighted/bolded region in table above represents power associated with this 1x observed background rate of lung cancer in cited study.

These values can be graphed as shown below<sup>41</sup>:



Graph showing estimated power for a (one-sided) two-sample proportions test evaluating power as a function of control-group proportion at true RRs of 1.2-, 1.5-, 2.0- and 3.0. Dashed red vertical lines represent control group proportions at 1/2x of that observed, 1x of that observed and 2x of that observed and illustrate sensitivity of the power to these background rate assumptions.

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2x-(観察された)を表形式及びグラフ形式の両方で以下に示す39。

N <sub>control</sub>	$N_{exposed}$	Proportion control <sup>(b)</sup>	Proportion exposed	Relative risk	Power
17,710	1,710	0.00562	0.00674	1.2	0.1634
17,710	1,710	0.00562	0.00843	1.5	0.4353
17,710	1,710	0.00562	0.01124	2.0	0.8182
17,710	1,710	0.00562	0.01685	3.0	0.9935
17,710	1,710	0.01124	0.01348	1.2	0.2259
17,710	1,710	0.01124	0.01685	1.5	0.6379
17,710	1,710	0.01124	0.02247	2.0	0.9652
17,710	1,710	0.01124	0.03371	3.0	1
17,710	1,710	0.02247	0.02697	1.2	0.3353
17,710	1,710	0.02247	0.03371	1.5	0.8632
17,710	1,710	0.02247	0.04495	2.0	0.9991
17,710	1,710	0.02247	0.06742	3.0	1

上記の検出力計算結果を生成するために使用される Stata コード:検出力2比例('=0.5\*199/17710'=199/17710'=2\*199/17710), test(chi2) RR (1.2 1.5 2.0 3.0) n1(17710) n2

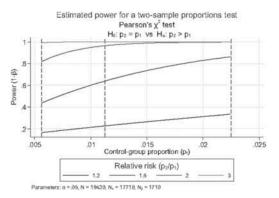
(1710)片側表(N1:"N コントロール" N2:"N エクスボージャー" p1:"割合コントロール" p2:"割合エクスボージャー" RR:"相対リスク" power:"検出力')。

(a):片側検定 a=0.05 Ho:p2=p1 vs Ha:p2>p1; Ncontrols=17,710、Nexposed=1,710;反復回数=1,000 回 (データセット)。

(b): Jones ら(2015)の 199/17710 の肺がんの観察されたバックグラウンド率を 1/2 倍-、1 倍-、2 倍-を表す。

上表の強調表示/太字の領域は、引用研究における肺がんのこの 1x 観測されたバックグラウンド率に関連した検出力を表している。

これらの値は以下のようにグラフ化することができる40。



<sup>&</sup>lt;sup>30</sup> 1.2、1.5、2.0、3.0の RRは、リスク管理者や意思決定者が関心を持ちそうな一連の相対リスクに関連する検出力検出力を示すために、やや态意的に選択されたものである。RR または OR=2.0 と 3.0 の値は、弱い影響量の大きさと強い影響量の間の境界線であると考えられている。RR 値 1.2 は、一部の人が「帰無に近い、または本質的に帰無」と考えるものであり、RR 値 1.5 はこれらの間の中間値である。疫学的証拠がばく露と健康影響との間の関係を示唆しているかどうかを判断する際に、リスク管理者は、許容可能な統計力(一般的には 80-90%と考えられている)を持つ強固な研究から得られた「本質的には帰無」RR1.2 を、関連性を見いだせなかったことを示サト分なエビデンスであり、事実上、ばく露と健康影響との間に観察可能な関連性がないという結論を支持するエビデンスを提供していると判断されることがあるかもしれない。

<sup>&</sup>lt;sup>40</sup> The RRs of 1.2, 1.5, 2.0 and 3.0 were selected somewhat arbitrarily to illustrate the power associated with a series of relative risks that might be of interest to the risk manager/decision-maker. The values of RR or OR = 2.0 and 3.0 are considered by some to be a demarcation between weaker effect sizes and stronger effect sizes. The RR value of 1.2 is what some consider 'near to or essentially null', and the RR of 1.5 is an intermediate value between these. In determining whether the epidemiological evidence suggests a relationship between an exposure and a health outcome, a risk manager might consider the 'essentially null' RR of 1.2 from a robust study with acceptable statistical power (generally considered 80-90%) as sufficient evidence for failing to find an association and, in effect, may provide supporting evidence for a conclusion of no observable association between the exposure and the outcome.

<sup>&</sup>lt;sup>41</sup> State code for generating the above graph: power twoproportions (' = 0.5 \* 199/17710'(0.0001) '= 2 \* 199/17710'), test(chi2) rrisk(1.2 1.5 2.0 3.0) n1(17710) n2(1710) graph (recast(line) xline('= 0.5 \* 199/17710' '=199/17710' '= 2 \* 199/17710', lpattern (dash)) legend(rows(l)size(small)) ylabel(0.2(0.2)1.0)) one sided.

<sup>&</sup>lt;sup>40</sup> 上のグラフを生成するための Stata コード:乗2 比例(' = 0.5 \* 199/17710'(0.0001) '= 2 \* 199/17710'), test(chi2) rrisk(1.2 1.5 2.0 3.0) n1(17710) 0n 1(17710) n2(1710) グラフ(recast(line) xline('= 0.5 \* 199/17710" '= 199/17710" '= 2 \* 199/17710', lpattern(dash)) legend(rows(1)size(small)) ylabel(0.20.21).0)/F(周/ダラフである。

As can be seen in the above table and graph, this study had a power of about 23% at 1x the background rate (control-group proportion, equal to 199 diseased individuals/17,710 subjects = 0.011237) to detect a RR of 1.2. To detect an RR of 1.5, there is about 64% power. If the true background rate were in reality twice the observed background rate  $(2 \times 0.011237 = 0.022473)$ , we would have about 86% power to be able to detect a RR of 1.5 and essentially 100% power to detect an RR of 2.0.42

Given the above. SAS was used to simulate the degree to which there may be effect size magnification (aka effect size inflation) given true relative risks of 1.2, 1.5, 2.0 and 3.0. The table below illustrates the power analysis for diazinon and lung cancer which shows the extent of the effect size magnification from the simulation results. The analysis presented in the table below parallels that done by Ioannidis (2008) and presented in his Table 2 for a set of hypothetical results passing the threshold of formal statistical significance to illustrate the effect size magnification concept.

SAS simulation results illustrating effect size magnification given true odds ratios of 1.2, 1.5, 2.0 and 3.0<sup>(a)</sup>

True values				Distribution of observed significant RRs			
Proportion of diseased individuals in control	RR	N analysed data sets	Power <sup>(b)</sup>	N	10th percentile	Median (% inflation)	90th percentile
0.005617	1.2	1,000	0.16	157	1.6	1.7 (42)	2.0
(1/2 $\times$ background)	1.5	1,000	0.40	401	1.6	1.8 (20)	2.3
	2	1,000	0.82	823	1.7	2.1 (5)	2.8
	3	1,000	1	997	2.3	3.0 (0)	3.9
0.011237	1.2	1,000	0.22	224	1.4	1.6 (33)	1.8
(1 $\times$ background)	1.5	1,000	0.63	627	1.4	1.6 (7)	2.0
	2	1,000	0.98	977	1.6	2.0 (0)	2.5
	3	1,000	1	1,000	2.5	3.0 (0)	3.6
0.022473	1.2	1,000	0.33	331	1.3	1.4 (17)	1.6
(2 $\times$ background)	1.5	1,000	0.87	871	1.3	1.5 (0)	1.8
	2	1,000	1	1,000	1.7	2.0 (0)	2.3
	3	1,000	1	1,000	2.6	3.0 (0)	3.4

Poisson regression model was used to compare the rate of (relative risks) between the groups. The EXACT Test was used in the analysis of some data sets when the generalised Hessian matrix is not positive definite (due to a zero cases in one of the groups). (a): One-sided test,  $\alpha = 0.05$ , N Controls = 17,710, N diazinon Exposed = 1,710, Number of iterations = 1,000 (data sets).

(b): The power resulting from this simulation may be close but not precisely match the power calculated from built-in procedures in statistical software such as SAS (PROC POWER) or Stata (power two-proportion). This may be due to the number of data sets simulated being of insufficient size. However, 1.000 iterations is sufficient to adequately estimate the power and to illustrate the degree of effect size magnification given a statistically significant result (here,  $\alpha \leq 0.05$ ).

Note that – given a statistically significant result at p < 0.05 – the percent effect size inflation at the median of the statistically significant results varies from 0% to 42% depending on both the rate of lung cancer among individuals not exposed to diazinon (i.e. proportion of diseased individuals in the non-exposed group) and the true relative risk (ranging from 1.2 to 3.0). For example, if the true RR of a tertile of exposed vs non-exposed were 1.2, where the non-exposed group has a rate of lung cancer of 0.011237 (bolded row in the above table), half of the observed statistically significant RRs would be above the median of 1.6 and half would be below 1.6; this represents a median inflation of 33% over the true RR of 1.2 used in the simulation.

For the background rate found in the Jones et al. (2015) study (0.011237), a true RR of 1.2 that was found to be statistically significant would instead were the study to be repeated be observed to vary from 1.4 (at the 10th percentile) to 1.8 (at the 90th percentile) with the aforementioned median of 1.6. When the true RR is 2 or 3, the power is greater than 80% (as seen in the above table) and the median of observed RR is close to the true RR and the range of observed RRs are narrow. As the true RR increases to 3, the study's power increases such that the effect size inflation disappears and the median from the simulations indeed reflects the true RR.

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1.2-、1.5-、2.0-及び 3.0 の真の RR における制御群比率の関数としての検出力を評価する(片側)2 標本比率検定 の推定検出力を示すグラフ。検定では、1.2-1.5-2.0、2.0-3.0の真の RR でのコントロールグループ比率の関数として の検出力を評価している。

トの表とグラフからわかろように、本研究では、バックグラウンド率(対照比率、199人の罹患者/17.710人=0.011237) の1倍で約23%の検出力でRR1.2を検出することができた。約64%の検出力が1.5のRRを検出する。真のバック グラウンド率が実際には観測されたバックグラウンド率の2倍(290.011237=0.022473)であれば、RR1.5を検出でき る検出力は約86%、RR2.0を検出できる検出力は実質的に100%となる41。

上記を考えると、真の相対リスクを1.2、1.5、2.0、3.0とした場合に、どの程度の影響量の拡大(別名、影響量の膨張) があるかをシミュレーションするために SAS が使用された。下の表は、ダイアジノンと肺がんの検出力解析を示しており、 シミュレーション結果から影響量の拡大の程度を示している。下の表に示された解析は、Ioannidis(2008年)によって 行われたものと類似していて、彼の表2に示された、影響量の拡大の概念を説明するための正式な統計的有意性の閾 値を通過した仮説的な結果のセットである。

真値		解析した		観測された有意な RR の分布				
対照における罹患者		データセ	検出力 <sup>(b)</sup>		観測され7	こ有息な КК の分布		
の割合	RR	ット数 (N)	<b>换出力</b>	N	10 <sup>th</sup> percentile	Median (% inflation)	90 <sup>th</sup> percentile	
0.005617	1.2	1,000	0.16	157	1.6	1.7 (42)	2.0	
(1/2 9 background)	1.5	1,000	0.4	401	1.6	1.8 (20)	2.3	
	2	1,000	0.82	823	1.7	2.1 (5)	2.8	
	3	1,000	1	997	2.3	3.0 (0)	3.9	
0.011237	1.2	1,000	0.22	224	1.4	1.6 (33)	1.8	
(19 background)	1.5	1,000	0.63	627	1.4	1.6 (7)	2.0	
	2	1,000	0.98	977	1.6	2.0 (0)	2.5	
	3	1,000	1	1,000	2.5	3.0 (0)	3.6	
0.022473	1.2	1,000	0.33	331	1.3	1.4 (17)	1.6	
(2 9 background)	1.5	1,000	0.87	871	1.3	1.5 (0)	1.8	
	2	1,000	1	1,000	1.7	2.0 (0)	2.3	
	3	1,000	1	1,000	2.6	3.0 (0)	3.4	

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ボアソン回帰モデルが、グループ間の(相対リスク)率を比較するために使用された、EXACT 検定は、一般化ヘシアン行列が正のデ フィニートでない場合(グループの1つでゼロのケースがあるため)、いくつかのデータセットの解析に使用された。 (a): 片側検定, a=0.05, N コントロール=17.710, N ダイアジノンばく露者=1.710, 反復回数=1.000回(データセット)。 (b): このシミュレーションから得られた検出力は、SAS (PROC POWER)や Stata (power two-proportion)のような統計ソフトの組み 込まれた手順から計算された検出力に近いかもしれないが、正確には一致しないかもしれない。これは、シミュレーションされたデ ータセットの数が十分でないためかもしれない。しかし、1,000回の反復は、検出力を適切に推定し、統計的に有意な結果(ここで は、≤0.05)を与えられた影響量の拡大の程度を説明するのに十分である。

p<0.05 で統計的に有意な結果が得られた場合、統計的に有意な結果の中央値での影響量の拡大の変化率は、ダ イアジノンにばく露されていない個人の肺がんの割合(すなわち、非ばく露グループの罹患者の割合)と真の相対リスク (1.2 から 3.0 までの範囲)の両方に応じて 0%から 42%まで変化することに注意する。例えば、ばく露対非ばく露の三 分位の真のRRが1.2であった場合、非ばく露グループの肺がんの割合は0.011237(上記の表の太字の行)で、観察 された統計的に有意な RR の半分は 1.6 の中央値を超えており、半分は 1.6 以下になるだろう:これは、シミュレーショ ンで使用される 1.2 の真の RR の上に 33%の膨張があることを表している。

Jones ら(2015 年)の研究(0.011237)でみつかったバックグラウンド率については、統計的に有意であることが判明

<sup>&</sup>lt;sup>42</sup> Said another way, if the true (but unknown) background rate were actually twice the observed background rate, we could reasonably conclude (with 86% confidence) if no statistically significant relationship was found that the true OR did not exceed 1.5.

<sup>41</sup> 別の言い方をすると、真の(しかし未知の)バックグラウンド率が実際に観測されたバックグラウンド率の2倍であった場合、統計的に有意な関係 がみつからなかった場合、我々は合理的に(86%の信憑性で)真の OR が 1.5 を超えていないと結論付けることができる。 EFSA Journal 2017:15(10):5007

#### An Example Illustrating Effect Size Magnification and Odds Ratios in an Ever/Never Analysis (Waddell, et al. 2001)

Sometimes comparisons between exposed group vs non-exposed group are presented in an 'ever/never' comparison as opposed to a comparison based on some other categorisation or grouping such as terciles or quartiles. This exposure category-based analysis might be done because there are an insufficient number of cases to break the exposure categories into small (more homogenous) exposure classifications or groupings or because the measurements of exposure are not available or are less reliable (such as in case-control studies). In these situations, we similarly need (i) the total number of subjects in non-exposed group; (ii) the number of subjects in exposed group; (iii) the number of diseased individuals in the non-exposed group at some; (iv) given or preselected odds ratios.

To illustrate how a power and effect size magnification analysis might be done for a case-control study using ever-never exposure categorisations, a study investigating the association between malathion and NHL (Waddell et al., 2001) was selected. Here, we have (i) the number of subjects in the reference non-exposed group = 1,018 (from Table 1: non-farmers = 243 diseased individuals + 775 non-diseased individuals); (ii) the number of subjects in the exposed group = 238 (from Table 4: malathion exposed individuals = 91 exposed cases + 147 non-exposed controls); (iii) the number of diseased individuals in the reference non-exposed group = 243 (from Table 1: 243 diseased individuals in the non-farmer or non-exposed group), we can similarly calculate the power of the comparisons between the ever vs never exposed, given the assumption that any true OR = 1.2, 1.5, 2.0, etc.

As was described above for lung cancer and diazinon, we estimated a power of 30.5% to detect an OR of 1.2 at the study-estimated NHL proportion of 0.2387 among non-farmers (non-exposed), as illustrated in the table below:

Results of power analysis for a one-sided, two-sample proportions test ( $\alpha = 0.05$ ) <sup>(a)</sup>									
N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Odds Ratio	Power				
1,018	238	0.1194	0.1399	1.2	0.2279				
1,018	238	0.1194	0.1689	1.5	0.647				
1,018	238	0.1194	0.2133	2.0	0.9693				
1,018	238	0.1194	0.2891	3.0	1				
1,018	238	0.2387	0.2734	1.2	0.3047				
1,018	238	0.2387	0.3199	1.5	0.8149				
1,018	238	0.2387	0.3854	2.0	0.9971				
1,018	238	0.2387	0.4847	3.0	1				
1,018	238	0.4774	0.523	1.2	0.3522				
1,018	238	0.4774	0.5781	1.5	0.8779				
1,018	238	0.4774	0.6463	2.0	0.9992				
1,018	238	0.4774	0.7327	3.0	1				

Stata code used to generate the above results: power two-proportions ('= 0.5 \* 243/1018' '= 243/ 1018' '= 2 \* 243/1018'), test(chi2) OR (1.21.52.03.0) n1(1,018) n2(238) one-side table(N1: 'N control' N2: 'N exposed' p1: 'proportion control' p2' proportion exposed' OR: 'odds ratio' power: 'power').

(a). One-sided test  $\alpha = 0.05$  Ho: p2 = p1 vs Ha: p2 > p1;  $N_{controls} = 1,018$ ,  $N_{exposed} = 238$ , Number of iterations = 1,000 (data sets). (b): Representing  $1/2x_{-}$ ,  $1x_{-}$  and  $2x_{-}$  the observed background rate of lung cancer of 243/1018 in Waddell et al. (2001). Highlighted,

bolded region in table above represents power associated with this 1x observed background rate of NHL in cited study.

Such power relations for malathion and NHL are graphed below<sup>43</sup> – as was done in the above AHS prospective cohort study for diazinon and lung cancer – with the middle vertical dotted line in the graph showing power at the NHL proportion of 0.2387 among non-farmers/non-exposed and the lefthand and right-hand vertical dashed lines representing a form of sensitivity analysis at one-half and twice the NHL proportion among non-farmers/non-exposed, respectively.

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#### Epidemiological studies and pesticides

した真の RR の 1.2 は、前述の中央値 1.6 の代わりに 1.4(10%のパーセンタイルで)から 1.8(90%のパーセンタイル で)に変化することが観察される研究が繰り返されるだろう。真の RR が 2 または 3 のときには、検出力は 80%以上(上 記の表に見られるように)であり、観測された RR の中央値は真の RR に近く、観測された RR の範囲は狭い。真の RR が 3 になると、影響量の膨張がなくなり、シミュレーションの中央値が真の RR に回帰するように検出力が増加する。

エバー/ネバー解析における影響量の拡大とオッズ比を示す例(Waddellら、2001年)

ばく露群と非ばく露群の比較は、三分位や四分位などの他の分類やグループ分けに基づいた比較とは対照的に、 「これまでありと、決してない」の比較で示されることがある。このようなばく露カテゴリーベースの解析は、ばく露カテゴリ ーを小さな(より均質な)ばく露分類やグループに分けるには十分な数の症例数がないため、あるいはばく露の測定値 が利用できないか、あるいは信頼性が低いため(症例対照研究のような)に行われるかもしれない。これらの状況では、 (i)非ばく露群の被験者の総数、(ii)ばく露群の被験者の数、(iii)ばく露群と非ばく露群の間の比較の検出力を計算 するために、非ばく露群の罹患者の数、(iv)与えられた、または事前に選択されたオッズ比が同様に必要となる。

エバー/ネバー分類を用いた症例対照研究において、検出力と影響量の拡大の解析がどのように行われるかを説 明するために、マラチオンとNHLの関連性を調査した研究(Waddellら、2001)を選択した。ここでは、(i)基準非ばく 露群の被験者数=1,018人(表1より:非農家=243人の罹患者+775人の非罹患者)、(ii)ばく露群の被験者数=238 人(表4より:マラチオンばく露者=91人の非ばく露者+147人の非ばく露コントロール)、(iii)参照非ばく露群の罹患 者数=243人(表1より:非農家または非ばく露群の243人の罹患者)とすると、真の率比=1.2、1.5、または2.0と仮定 すると、「ばく露ありと、ばく露なし」の比較の検出力が計算できた。

肺がんとダイアジノンについて上述したように、非農家(非ばく露者)では、以下の表に示すように、研究で推定された NHL の割合が 0.2387 で 1.2 の OR を検出する検出力を 30.5%と推定した。

N <sub>control</sub>	N <sub>exposed</sub>	Proportion control <sup>(b)</sup>	Proportion exposed	Odds Ratio	Power
1,018	238	0.1194	0.1399	1.2	0.2279
1,018	238	0.1194	0.1689	1.5	0.647
1,018	238	0.1194	0.2133	2.0	0.9693
1,018	238	0.1194	0.2891	3.0	1
1,018	238	0.2387	0.2734	1.2	0.3047
1,018	238	0.2387	0.3199	1.5	0.8149
1,018	238	0.2387	0.3854	2.0	0.9971
1,018	238	0.2387	0.4847	3.0	1
1,018	238	0.4774	0.523	1.2	0.3522
1,018	238	0.4774	0.5781	1.5	0.8779
1,018	238	0.4774	0.6463	2.0	0.9992
1,018	238	0.4774	0.7327	3.0	1

上記の結果を生成するために使用される Stata コード: 2 乗比例('= 0.5 \* 243/1018' '= 243/1018' '= 2 \* 243/1018'), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1,018) n2(238) 片側

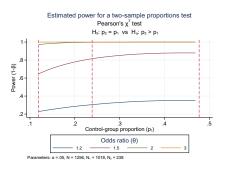
table(N1:"N コントロール" N2:"N ばく露" p1:"割合コントロール" p2"割合ばく露" OR:"オッズ比" power."検出力') (a): 片側検定 a = 0.05 Ho: p2 = p1 vs Ha: p2 > p1; Ncontrols = 1,018, Nexposed = 238、反復回数 = 1,000 回 (データセット)。 (b): Waddell ら (2001) の 243/1018 の肺がんの観察されたパックグラウンド率を 1/2 倍、1 倍、2 倍を表す。上表のハイライトされ 太字の領域は、引用された研究におけるこの 1 倍の NHL の観測されたパックグラウンド率に関連した検出力を表している。

このようなマラチオンとNHLの検出力関係は、上記のAHS前向きコホート研究(ダイアジノンと肺がん)と同様に以下のようにグラフ化されている42。グラフの中央縦点線は非農家/非ばく露群のNHL比が0.2387での検出力を示し、

<sup>42</sup> グラブを生成するための Stata コード・2 乗比例(= 0.5 \* 243/1018′(0.01) = 2 \* 243/1018′), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1018) 0) n1(1018) n2(238)graph(再キャスト(線) ×line(= 0.5 \* 243/1018′' = 243/1018′' = 2 \* 243/1018′, lpattern(dash) legend(rows(1)size(small)) y-label(0.2(0.21).0))<sup>4</sup>側。

<sup>&</sup>lt;sup>43</sup> Stata code for generating the graph: power two proportions ('=0.5\*243/1018'(0.01) '=2\*243/1018'), test(chi2) OR (1.2 1.5 2.0 3.0) n1(1018) n2(238)graph(recast (line) x-line('=0.5\*243/1018' '=243/1018' '=2\*243/1018', lpattern(dash)) legend(rows(1)size(small)) y-label(0.2(0.2)1.0)) one sided.





Graph showing estimated power for a (one-sided) two-sample proportions test evaluating power as a function of control-group proportion at true RRs of 1.2-, 1.5-, 2.0- and 3.0. Dashed red vertical lines represent control group proportions at 1/2x of that observed, 1x of that observed and 2x of that observed and illustrates the sensitivity of the power to these background rate assumptions.

At the study-estimated NHL proportion of 0.2387 among non-farmers/non-exposed, the power (one-sided) to detect ORs of 1.2, 1.5, 2.0 and 3.0 is shown to be 30.5%, 81.5%, 99.7% and > 99.9%, respectively. Note that Waddell et al. (2001) reported an OR of 1.6 with a 95% CI of 1.2–2.2, based on 91 NHL cases who used malathion and 243 cases that were among non-farmers who did not.

Given the above, SAS was used to simulate the degree to which effect size magnification may exist given *true* odds ratios of 1.2, 1.5, 2.0 and 3.0. Below is a SAS-generated table for the power analysis for malathion and NHL showing the magnitude of the effect size magnification from the SAS-based simulation results.

SAS simulation results illustrating effect size magnification given true odds ratios	of 1.2, 1.5, 2.0,
and 3.0 <sup>(a)</sup>	

True values				Dist	Distribution of observed significant ORs			
Proportion of diseased individuals in non-exposed group	OR	N analysed data sets	Power <sup>(b)</sup>	N	10th percentile	Median (% inflation)	90th percentile	
0.1194 (1/2 background)	1.2	1,000	0.22	220	1.4	1.5 (25)	1.8	
	1.5	1,000	0.66	661	1.5	1.7 (13)	2.0	
	2	1,000	0.97	972	1.6	2.0 (0)	2.5	
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7	
0.2387 (1 $\times$ background)	1.2	1,000	0.32	323	1.3	1.4 (17)	1.6	
	1.5	1,000	0.81	812	1.4	1.6 (7)	1.8	
	2	1,000	1.0	997	1.6	2.0 (0)	2.4	
	3	1,000	1.0	1,000	2.5	3.0 (0)	3.6	
0.4774 (2× background)	1.2	1,000	0.34	337	1.3	1.4 (17)	1.6	
	1.5	1,000	0.87	872	1.3	1.5 (0)	1.8	
	2	1,000	1.0	1,000	1.6	2.0 (0)	2.5	
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7	

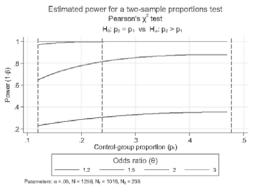
The logistic regression model was used to compute the odds ratios for the two groups. The EXACT Test was used in the analysis of some data sets when the maximum likelihood estimate did not exist (perhaps due to a zero cases in one of the groups). (a): One-side test,  $\alpha = 0.05$ , N non-exposed = 1,018, N malathino exposed = 238, N iterations = 1,000 (data sets).

(b): The power resulting from this simulation may be close but not match exactly with the power calculated from built-in procedures in statistical software such as SAS (PROC POWER) or Stata (power two-proportion). This may be due to number of data sets simulated being of insufficient size. However, 1,000 iterations are sufficient to adequately estimate the power and to illustrate the degree of effect size magnification given a statistically significant result (here, α ≤ 0.05).

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左端と右端の縦点線は非農家/非ばく露群のNHL比が1/2と2倍での感度分析の枠を示している。



1.2・、1.5・、2.0・及び 3.0 の真の RR におけるコントロール群比率の関数としての検出力を評価する(片側)2 標本比 率検定の推定検出力を示すグラフ。赤い破線の縦線は、観測された比率の 1/2 倍、観測された比率の 1 倍及び観測 された比率の 2 倍におけるコントロールグループの比率を表し、これらのバックグラウンドレートの仮定に対する検出力 の感度を示している。

非農業者/非ばく露者の NHL 割合を 0.2387 と推定した場合、OR 1.2、1.5、2.0、3.0 を検出する検出力(片側) は、それぞれ 30.5%、81.5%、99.7%、99.9%以上となる。なお、Waddellら(2001 年)は、マラチオンを使用した NHL 症例 91 例と使用しなかった非農家 243 例を対象に、OR は 1.6、95%CI は 1.2-2.2 と報告している。

以上のことから、真のオッズ比が 1.2、1.5、2.0、3.0 の場合に、影響量の拡大の差がどの程度存在するかをシミュレ ーションするために SAS が使用された。以下は、マラチオンと NHL の検出力解析のために SAS で作成された表で、 SAS ベースのシミュレーション結果から、影響の拡大のマグニフィケーションの大きさを示している。

真値			観察された有意な OR の分布				
非ばく露群における罹 患者の割合	OR	N 個の解析 データセット	Power <sup>(b)</sup>	N	10%台	中央値 (%inflation)	90%台
0.1194 (1/2 background)	1.2	1,000	0.22	220	1.4	1.5 (25)	1.8
	1.5	1,000	0.66	661	1.5	1.7 (13)	2.0
	2	1,000	0.97	972	1.6	2.0 (0)	2.5
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7
0.2387 (19 background)	1.2	1,000	0.32	323	1.3	1.4 (17)	1.6
	1.5	1,000	0.81	812	1.4	1.6 (7)	1.8
	2	1,000	1.0	997	1.6	2.0 (0)	2.4
	3	1,000	1.0	1,000	2.5	3.0 (0)	3.6
0.4774 (29 background)	1.2	1,000	0.34	337	1.3	1.4 (17)	1.6
	1.5	1,000	0.87	872	1.3	1.5 (0)	1.8
	2	1,000	1.0	1,000	1.6	2.0 (0)	2.5
	3	1,000	1.0	1,000	2.4	3.0 (0)	3.7

ロジスティック回帰モデルが、2 つのグループのオッズ比を計算するために使用された。最尤推定値が存在しない場合(おそらくいずれ かのグループの症例がゼロであったため)、EXACT 検定をいくつかのデータセットの解析に使用した。

(a):片側検定、a = 0.05、非ばく露群=1,018、マラチオンばく露群=238、反復 N=1,000 (データセット)。(b):このシミュレーション から得られた検出力は近いかもしれないが、組み込みの SAS (PROC POWER)や Stata (power 2-proportion)のような統計ソフトウェア のブロシージャを使用している。これは、シミュレーションされたデータセットの数が十分でないためかもしれない。しかし、1,000 回 の反復は、検出力を十分に推定し、統計的に有意な結果(ここでは、≤0.05)が得られた場合の影響量の拡大を説明するのに十分である

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Note that – given a statistically significant result at p < 0.05 – the median effect size varies from 1.4 to 3, depending on the NHL proportion in the non-exposed group, and the true odds ratio (ranging from 1.2 to 3.0). For example, if the true OR for a NHL proportion among non-farmers of 0.2387 was 1.2 (bolded row in the table), half of the *observed statistically significant* ORs would be above the median of 1.4 and half would be below. Further, most (90%) of the statistically significant ORs would be observed to be above 1.3, and a few (10%) would be observed even to be above 1.6.

In sum, then, the power of an epidemiological study is an important factor that should considered by regulators and others evaluating such studies. A study that is sufficiently powered will not only be more likely to detect a true effect of a given size if it is indeed present (the classic definition of power which relates to the issue of a Type II error or a false negative) but will also be less likely to magnify or exaggerate the effect if it is not there but (by chance) crosses a preselected threshold (such as the 0.05 level for statistical significance). If a study is suitably powered (say, 80% or more), the observed effect size is more likely to be a reflect a true effect size and any observed chance variation in this effect size will reflect a distribution symmetrically centred around the unknown true value. The take home message from these simulations and the original work by Ioannidis and extensions by Gelman and Carlin (2014) is that a study should be not only suitably powered to avoid a false negative (Type II error) but also suitably powered to avoid a magnification of the effect size for those effect sizes that are statistically significant (or pass some other threshold). Gelman and Carlin (2014) go further, stating that such 'retrospective design calculations may be more relevant for statistically significant findings than for nonsignificant findings. The interpretation of a statistically significant result can change drastically depending on the plausible size of the underlying effect'. Note that if a study is suitably powered, there is NO systematic risk inflation, but the effect estimates for underpowered studies that produce statistically significant effects are prone to what might be substantial risk inflation, the interpretation of which depends on realistic estimates of the true (underlying) effect.

Ideally, then, published literature studies should conduct and document power analyses. Short of that, published literature should provide adequate information for the reader to perform such power calculations (or, as Gelman and Carlin (2014) term them: (retrospective) design calculations). In the two examples provided above, the authors did provide sufficient information for the reader to calculate power and the potential for effect size magnification. This is not always the case. Sometimes information used for power calculations are only partially provided in the publications or provided information was structured in a way that does not permit such calculations.<sup>44,45</sup> For example, if authors use number of cases instead of level of exposure to determine tertiles or quartiles (which would be evidenced by a constant number of cases between groups) or if authors group multiple cancer outcomes together and use that number to determine tertiles, then the power (or design) calculations illustrated here are not possible since the required inputs are not able to be derived. Since the counts and data which are tabulated and reported are not necessarily standardised among authors and publications, one strong recommendation would be for publications to require reporting (even if in supplementary or online data) the necessary information to estimate power such that such evaluations can be done by both peer reviewers and interested readers.

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p<0.05 で統計的に有意な結果が得られた場合、影響量の中央値は、非ばく露群の NHL の割合と真のオッズ比 (1.2 から 3.0 まで)によって、1.4 から 3 まで変化することに注意する。例えば、非農家の NHL の割合が 0.2387 の場 合、真の OR が 1.2 (表中の太字の行)とすると、統計的に有意な OR の半分は中央値 1.4 を超え、半分は下回ってい ることになる。さらに、統計的に有意な OR の大部分 (90%)は 1.3 以上であることが観察され、少数 (10%)は 1.6 以上 であることさえ観察された。

まとめると、疫学研究の検出力は、規制当局や他の人がそのような研究を評価する際に考慮すべき重要な要素であ る。十分な検出力を持つ研究は、与えられた規模の真の効果が実際に存在する場合に検出する可能性が高くなるだ けでなく(検出力の古典的な定義は、II型エラーや偽陰性の問題に関連している)、影響が存在しないが(偶然にも) 事前に選択された閾値(統計的有意性の 0.05 レベルなど)を超えた場合に、影響を拡大したり誇張したりする可能性 が低くなるだろう。研究が適切な検出力を持っている場合(例えば、80%以上)、観察された影響量は真の影響量を再 現する可能性が高く、この影響量の観察された偶然の変動は、未知の真の値を中心に対称的に分布を再現する。これ らのシミュレーションと Ioannidis によるオリジナルの研究及び Gelman と Carlin(2014 年)による拡張研究から得ら れるメッセージは、研究は偽陰性(タイプ II エラー)を回避するために適切な検出力が必要であるだけでなく、統計的 に有意な(または他の閾値を通過した)影響量のために影響量の拡大を回避するために適切な検出力が必要であると いうことである。Gelman と Carlin(2014 年)はさらに進んで、そのような「後ろ向き計画の計算は、統計的に有意でな い影響量よりも統計的に有意な影響量に関連しているかもしれない。統計的に有意な結果の解釈は、基礎となる影響 のもっともらしい規模によって大きく変わる可能性がある」と述べている。研究が適切な検出力を持っていれば体系的な リスクの膨張は皆無であるが、統計的に有意な効果をもたらす検出力不足の研究の影響推定値は、実質的なリスク膨 張の可能性があり、その解釈は真の(基礎となる)影響の現実的な推定値に依存することに注意することである。

理想的には、公表されている文献研究は、検出力解析を実施し、文書化するべきである。それ以外にも、公表されて いる文献は、読者がこのような検出力計算(あるいは Gelman と Carlin(2014 年)が言うところの(後ろ向き)デザイン 計算)を行うのに十分な情報を提供すべきである。上記の2つの例では、著者は読者に検出力と影響量の拡大を計算 することができる十分な情報を提供していた。これは常にそうとは限らない。検出力の計算に使用された情報が文献に 部分的にしか提供されていなかったり、提供された情報がそのような計算ができない方法で構成されていたりすること がある<sup>43-44</sup>。例えば、著者が三分位または四分位を決定するためにばく露量の代わりに症例数を使用している場合(こ れはグループ間での症例数が一定であることから証明される)、または著者が複数のがん症例をまとめてグループ化し、 その数を使用して三分位を決定している場合、必要な入力が得られないため、ここに示されている検出力(またはデザ イン)の計算は不可能である。集積及び報告される数値及びデータは、著者及び文献の間で必ずしも標準化されてい るわけではないので、一つの強い推奨事項は、文献が(補足的またはオンラインデータであっても)検出力を推定する ために必要な情報を報告することを義務付けることであり、このような評価を査読者及び関心のある読者の両方ができ るようにすることであろう。

以上の解析から、影響量の膨張現象の潜在的な意味合いは、疫学研究を評価する上で重要な考慮事項であること が示唆されたが、この現象に関するいくつかの注意点を覚えておくことが重要であり、疫学研究の解釈にどのように考

<sup>&</sup>lt;sup>44</sup> For example, in the review of the association between malathion exposure vs aggressive prostate cancer presented in the publication 'Risk of Total and Aggressive Prostate Cancer and Pesticide Use in the Aggressive Prostate Cancer and Pesticide Use in the Aggressive Prostate Study' by Stella Koutros et al. (2012), the Panel was not able to calculate the power of the comparison between the malathion-exposed groups vs non-exposed group because critical information was not provided in the published article. From the publication and the supplemental document of the publication, we were able to easily find the number of <u>cases</u> in the non-exposed group (Table 2 in the main article), but the number of <u>subjects</u> in the non-exposed group or at <u>each</u> exposed level (i.e., quartile) appeared not to be available. We attempted to derive the number of <u>subjects</u> in the non-exposed group and number of <u>subjects</u> in each quartile from the information in Table 1 of the supplemental document of the article but were not able to do so since the information in Table 1 away that was not consistent with many other AHS publications in that the exposed subjects were categorized into groups based on the quartiles of number of cases.

<sup>&</sup>lt;sup>45</sup> Sometimes, information used for power calculations may have only been <u>partially</u> provided in the publications. For example, we calculated the powers associated with various thyroid cancer comparisons from the information provided in the AHS study publication in the AG cancer Incidence Among Pesticide Applicators in the Agricultural Health Study (1994-2007), by Laura Beane-Freeman et al. (2011). In this publication, the authors did not categorize the subjects into quartiles based on exposure but instead categorized or grouped the subjects based on the total number of all cancer cases combined. In this way, the number of sall cases of all types of cancer was the same between groups and thus both the number of subjects was not the same between groups. In this example, the publication provided (i) the reference Q11 N = 9,523, (ii) total subjects in Q2, Q3 and Q4: N = 26,834 (Table 1) and (iii) the number of thyroid cancer cases in the reference Q1 = 3 (Table 2). The exact number of subjects in each of the compared groups (Q2, Q3 or Q4) was, however, not available.

<sup>&</sup>lt;sup>45</sup> 例えば、Stella Koutros ら(2012 年)の出版物/Risk of Total and Aggressive Prostate Cancer and Pesticide Use in the Agricultural Health Study ごを基されたマラチオンは、2歳と良双性前立廠がんの関連性のレビューでは、発表された論文に重要な情報が提供されていなか ったため、バネルはマラチオンは、2歳能と非ばく2歳群しまは、2歳であった。文献及び文献の補足文書から、非ば、2歳群の症例表を容 易に把握することができたが(本文中の麦 2)、非ばく3歳群または各はく3歳量(四分位)の被験者数は入手できなかったようである。我々は、文献の 補足文書の表 1)の情報から非ばく3歳者能の被験者数及び各四分位の被験者数を導入したらしたが、表 1 の情報は、ばく3歳者を症例数の四分 位に基づいてグループに分類するという他の多くの AHS の出版物を一致しない方法で示されていたため、それを行うことができなかった。

<sup>44</sup> 検出力の計算に使用された情報は、文献では部分的にしか提供されていないことがある。例えば、我々は Laura Beane-Freeman ら(2011)に よる AHS 研究文紙「Atrazine and Cancer Incidence Among Pesticide Applicators in the Agricultural Health Study (1994-2007)」に記 載されている情報から、様々な甲状腺がんの比較に関連した検出力を計算した。この文献では、著者らは被験者をばく露に基づいて四分位に分 類するのではなく、その代わりに、すべてのがん症例を合わせた総症例数に基づいて被験者を分類またはグループ化した。このようにして、すべ てのタイプのがんの症例数は、分類されたグループ間で同じであり、したがって、対象となる任意の特定のがん(例えば、甲状腺、ここでは)の症例 数は、グループ間で同じではなく、被験者の数は、グループ間で同じではなかった。この例では、文献は、(i)参照 Q1:N=9,523、(ii) Q2、Q3 及 び Q4 の総被験者数を提供した。N=26,834 人(表 1) 及び(iii)参考 Q1 の甲状腺がん症例数=3 人(表 2)を提供した。しかし、比較詳(Q2,Q3 または Q4)のそれぞれの正確な披験者数は得られなかった。

While the above analysis suggests that potential implications of the effect size inflation phenomenon are important considerations in evaluating epidemiological studies, it is important to remember a number of caveats regarding the phenomenon and how its consideration should enter into any interpretation of epidemiological studies.

- First, while this phenomenon would tend to inflate effect sizes for underpowered studies for which the effect of interest passes a statistical (or other) threshold, there are other biases that may be present that bias estimates in the other direction, *towards* the null. This bias might be referred to as effect size *suppression*. Perhaps, the most well-known of these is non-differential misclassification bias discussed in the main body of the text. This can commonly (but not always) produce predictable biases towards the null, thereby systematically under-predicting the effect size. Recognising that this is not always true and there are potentially countervaling or counteracting factors like effect size magnification (at least for small underpowered studies) is an important step forward. Specifically, underpowered studies can result in biased estimates in a direction away from the null to a degree that that can potentially offset (and possibly more than offset) any biases towards the null that may result, for example, from non-differential misclassification bias. Regardless, what is of critical importance is to recognise that adequately powered studies are necessary to be able to have at least some minimal degree of confidence in the estimate of the effect size for a statistically significant result.
- Secondly and as stated in the main body of the text effect size magnification is linked to a focused effort on the part of the researcher (or regulators interpreting such a study) on identifying effects that pass a given threshold of significance (e.g. p < 0.05) or achieve a certain size (e.g. OR > 3) when that study is underpowered. This phenomenon, then, is of most concern when a 'pre-screening' for statistical significance (or effect size). To the extent that regulators, decision-makers and others avoid acting by focusing on only those associations that 'pass' some predetermined statistical threshold and then use that effect size to evaluate and judge the magnitude of the effect without acknowledging that it might be inflated if the study is underpowered, the phenomenon is of lesser concern. Note that effect size magnification is not a function or fault of the research or research design, **but rather a function of how that research is interpreted by the user community**.

Unfortunately, there is sometimes a tendency for attention to focus on effect sizes that are greater than a given size or that pass a certain statistical threshold and are as such 'discovered'. As recommended by Ioannidis with respect to how these 'discoveries' should be considered (Ioannidis, 2008):

'At the time of the first postulated discovery, we usually cannot tell whether an association exists at all, let alone judge its effect size. As a starting principle, one should be cautious about effect sizes. Uncertainty is not conveyed simply by CIs (no matter if these are 95%, 99% or 99.9%).

For a new proposed association, credibility and accuracy of the proposed effect varies depending on the case. One may ask the following questions: does the research community in the field adopt widely statistical significance or similar selection thresholds for claiming research findings? Did the discovery arise from a small study? Is there room for large flexibility in the analyses? Are we unprotected from selective reporting (e.g. was the protocol not fully available upfront?). Are there people or organisations interested in finding and promoting specific "positive" results? Finally, are the counteracting forces that would deflate effects minimal?'

 Thirdly, it should be remembered that the effect size inflation phenomenon is a general principle applicable to discovery science in general and is not a specific affliction or malady of epidemiology (Ioannidis, 2005; Lehrer, 2010; Button, 2013; Button et al., 2013; Reinhart, 2015). As indicated earlier, it is often seen in studies in pharmacology, in gene studies, in psychological studies, and in much of the most-often cited medical literature. Such truth inflation occurs in instances where studies are small and underpowered because such studies have widely varying results. It can be particularly problematic in instances where many researchers are performing similar studies and compete to publish 'new' or 'exciting' results (Reinhart, 2015).

#### **Summary and Conclusions**

Effect size magnification or 'truth inflation' is a phenomenon that can result in exaggerated estimates of odds ratios, relative risks or rate ratios in those instances in which these effect measures

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慮すべきであるかについても留意すべきである。

- -第一に、この現象は、対象となる影響が統計的(またはその他の)閾値を通過するような検出力不足の研究では影響量が膨張する傾向があるが、他にも推定値を帰無に向かって逆方向に偏らせるバイアスが存在する可能性がある。このバイアスは、影響量抑制と呼ばれることがある。おそらく、これらのバイアスの中で最もよく知られているのは、本文で議論されている非差異的誤分類バイアスである。これは、一般的に(常にではないが)帰無値に向かって予測可能なバイアスを生じさせ、それによって影響量を体系的に過小予測する。これが常に正しいとは限らず、(少なくとも小規模の低い検出力の研究については)影響量の拡大のような対抗要因や相殺要因が存在する可能性があることを認識することは、重要な前進である。特に、検出力不足の研究では、例えば、非差異的誤分類バイアスから生じるかもしれない帰無値へのバイアスを潜在的に相殺する(そして、多分相殺以上に)ことができる程度に、帰無値から離れた方向に偏った推定値をもたらすことがある。いずれにしても、重要なことは、統計的に有意な結果を得るための影響量の推定値に少なくともいくつかの最低限の一致度を持たせることができるようにするためには、十分な検出力のある研究が必要であるということを認識することである。
- -第二に、そして本文で述べられているように、影響量の拡大は、研究者(またはそのような研究を解釈する規制者)の側で、その研究が検出力不足の場合に、与えられた有意性の閾値(例:p<0.05)を通過するか、または一定の大きさ(例:OR>3)を達成する影響を特定することに焦点を当てた努力に関連している。この現象は、統計的有意性(または影響量)の「事前スクリーニング」を行う際に最も懸念される現象である。規制者や政策決定者などが、事前に決められた統計的閾値を「通過」した関連性のみに焦点を当てて行動することを避け、研究が検出力不足の場合に効果量の拡大を評価して判断するためにその影響量を使用する場合、この現象はあまり懸念されない。影響量の拡大の決定は、研究や研究デザインの機能や欠陥ではなく、むしろその研究がユーザーコミュニティによってどのように解釈されるかの機能であることに注意する。

残念なことに、与えられた量よりも大きい、またはある統計的閾値を通過して「発見された」影響量に注目が集まる 傾向が時々ある。これらの「発見」がどのように考慮されるべきかに関しては Ioannidis によって推奨されている (Ioannidis, 2008 年)。

「最初に仮定された発見の時点では、影響量を判断することはおろか、関連性が全く存在するかどうかもわからないのが普通である。最初の原則として、影響量については慎重にならなければならない。不確実性は、単に CI (95%、99%、99.9%であるかどうかは関係ありません)だけでは伝わらない。

新たに提案された関連性については、提案された影響の信頼性と正確性はケースによって異なる。ヒトは次のよう な質問をするだろう:この分野の研究コミュニティは、研究成果を主張するために、広く統計的な有意性や同様の選 択の閾値を採用しているのか?発見は小規模な研究から生まれたのか?分析に大きな変動の余地があるか?選択 的な報告から保護されていないか(例:プロトコールが前もって完全に利用可能ではなかったか)?特定の「ポジティ ブな」結果を発見し、促進することに興味を持っている人や組織はあるか?最後に、影響を相殺する力は最小限に 抑えられているか?

-第三に、影響量の膨張現象は、発見科学全般に適用可能な一般的な原則であり、疫学の特異的悩みや弊害ではないことを覚えておくべきである(Ioannidis、2005年;Lehrer、2010年;Button、2013年;Buttonら、2013年;Reinhart、2015年)。先に示したように、これは薬理学、遺伝子研究、心理学研究、そして最も頻繁に引用される医学文献の多くでしばしば見られる。このような真実性の膨張は、研究の規模が小さく、検出力が不足している場合に起こり、そのような研究では結果に大きなばらつきがあるからである。これは、多くの研究者が同様の研究を行っており、「新しい」または「刺激的な」結果を発表するために競争している場合には特に問題となる(Reinhart、2015年)。

### まとめと結論

影響量の拡大または「真実の膨張」とは、影響を「発見」するために統計的またはその他の閾値を満たす必要がある www.efsa.europa.eu/efsalourna EFSA.Journal 2017;15(10):5007

are derived from underpowered studies in which statistical or other thresholds need to be met in order for effects to be 'discovered'. The phenomenon is not specific to epidemiology or epidemiological studies, but rather to any science in which studies tend to be small and predetermined thresholds such as those relating to effect sizes or statistical significance are used to determine whether an effect exists. As such, it is important that users of epidemiological studies recognise this issue and its potential interpretational consequences. Specifically, any discovered associations from an underpowered study that are highlighted or focused upon on the basis of passing a statistical or other similar threshold are systematically biased away from the null. While we cannot know if any specific observed effect size from a specific study is biased away from the null as a result of being a 'discovered' association that passes a statistical threshold (just as we can't say that a specific study showing non-differential misclassification will necessarily be biased towards the null), we do know that that chance favours such a bias to some degree as illustrated by the explications presented and simulations performed here. Said another way: by choosing to focus on, report, or act upon effect sizes on the basis of those effect sizes passing a statistical or other threshold, a bias is introduced since it is inevitably more likely to select those associations that are helped by chance rather than hurt by it (Yarkoni, 2009). Again, this is an issue related to how studies are interpreted by users, not one that is intrinsic to the study design nor one that is related to good scientific principles or practices.

One (partial) solution to the above issue is for the reader to cautiously interpret effect sizes in epidemiological studies that pass a prestated threshold or are statistically significant if they arise from an underpowered study, recognising that the observed effect sizes can be systematically biased away from the null. Such an approach would require that either the authors report the power of the study or that the authors provide sufficient information for the reader to do so. Effects sizes from studies with powers substantially less than 80% should be interpreted with an appropriate degree of scepticism, recognising that these may be inflated - perhaps substantially so (particularly if the power is less than 50%). The potential degree of this inflation will depend on a number of issues including background rate of the health outcome of interest, the sample size of the study and the effect size of interest. More specifically, when (a) the smaller the background rate of the health outcome of interest is low, (b) the sample size of the study is small and (c) the effect size of interest is weak, then the power of the study (to detect that effect size) will be low and the tendency towards inflated effect sizes in statistically significant results will be high. Low power studies investigating small or weak effects in populations that have a low background rate of the health outcome of interest will tend towards the greatest degree of effect size inflation. As a result, the PPR Panel recommends that epidemiological publications either incorporate such calculations or include key information such that those calculations can be performed by the reader. Specifically:

When the association between a given pesticide exposure and a disease is found to be statistically significant, particularly in (presumed) low powered studies, data user should perform various power calculations (or a power analysis) to determine the degree to which the statistically significant effect size estimate (OR or RR) may be artificially inflated or magnified. This requires three values to be clearly reported by epidemiological studies: (i) the number of subjects in the non-exposed group (including diseased and non-diseased individuals); (ii) the number of subjects in the exposed group (including diseased and non-diseased individuals); and (iii) the number of diseased subjects in the non-exposed group. Risk managers can then select the target value of interest (typically an OR or RR) to detect a difference of a given (predetermined) effect size between the exposed and non-exposed subjects, and evaluate the degree to which effect size magnification could potentially explain the effect size that was estimated in the study of interest.

Since it appears that (i) many epidemiological studies are frequently underpowered; (ii) it is not common for authors to provide either power calculations or (sometimes) the information in publications required to do them, and (iii) the phenomenon of effect size magnification generally appears to be little recognised in the epidemiological field, the above PPR Panel recommendation will require effort on the part of researchers/grantees, publishers, and study sponsors to implement. While the above suggests that the current state of practice in this area may leave one pessimistic, an opinion piece on this topic by researcher Kate Button (Button, 2013) describing her work in Nature Reviews Neuroscience (Button et al., 2013) offered guarded reasons for optimism:

'Awareness of these issues is growing and acknowledging the problem is the first step to improving current practices and identifying solutions. Although issues of publication bias are difficult to solve overnight, researchers can improve the reliability of their research by adopting well-established (but

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検出力不足の研究から得られた効果測定値の場合に、オッズ比、相対リスク、または率比の推定値が誇張されることが ある現象である。この現象は、疫学や疫学研究に特有のものではなく、むしろ、影響が存在するかどうかを判断するた めに、影響量や統計的有意性に関連するような、研究の規模が小さく、事前に設定された閾値が使用される傾向にあ るあらゆる科学に見られる。このように、疫学研究の利用者がこの問題とその潜在的な解釈の結果を認識することが重 要である。特に、検出力不足の研究から発見された関連性は、統計的または他の同様の閾値を通過したことに基づい て強調されたり、注目されたりするが、それは帰無値から系統的にバイアスがかかっている。統計的閾値を通過した「発 見された」関連性の結果として、特定の研究で観測された影響量が帰無値から遠ざかるかどうかはわからないが(非差 異的な誤分類を示す特定の研究が必ずしも帰無値に向かって偏るとは言えないのと同じように)、ここで提示された説 明と実行されたシミュレーションによって説明されているように、偶然がそのような偏りをある程度有利にすることはわか っている。別の言い方をすると、統計的またはその他の閾値を通過した影響量に基づいて影響量に注目したり、報告し たり、行動したりすることを選択することで、バイアスが導入される(Yarkoni、2009 年)。繰り返しになるが、これは研究 がユーザーによってどのように解釈されるかに関連する問題であり、研究デザインに内在するものでもなければ、優れ た科学技術の原則や実践に関連するものでもない。

上記の問題に対する(部分的な)解決策の 1 つは、観察された影響量が帰無値から系統的に偏ってしまう可能性が あることを認識した上で、事前に定められた閾値を通過した疫学研究の影響量または検出力不足の研究から生じた場 合の統計的に有意な影響量を慎重に解釈することである。このようなアプローチでは、著者が研究の検出力を報告す るか、著者が読者に十分な情報を提供する必要がある。検出力が実質的に 80%未満の研究から得られた影響量は、 おそらく実質的に(特に検出力が 50%未満の場合)誇張する可能性があることを認識した上で、適切な程度の疑いを 持って解釈する必要がある。この誇張の潜在的な程度は、対象となる健康影響のバックグラウンド率、研究のサンプル サイズ、対象となる影響量など、多くの問題に依存する。より具体的には、(a)対象となる健康影響のバックグラウンド率 が低い場合、(b)研究のサンプルサイズが小さい場合、(c)対象となる影響量が弱い場合、研究の検出力(その影響量 を検出するための)は低く、統計的に有意な結果において影響量が誇張される傾向が高くなる。対象とする健康影響 のバックグラウンド率が低い集団で、小さな、または弱い影響を調査する低検出力研究では、影響量の誇張が最も大き くなる傾向がある。その結果、PPRパネルは、疫学的文献にこのような計算を組み込むか、または読者が計算を実行で きるような重要な情報を含めることを推奨している。具体的には以下の通り。

特定の農薬ばく露と疾病との間の関連が統計的に有意であることが判明した場合、特に(推定される)検出力の低い 研究では、データ利用者は、統計的に有意な影響量推定値(ORまたはRR)がどの程度人工的に拡大、または誇張さ れているかを判断するために、様々な検出力計算(または検出力解析)を実行すべきである。これは、疫学研究で明確 に報告される3つの値を必要とする。(i)非ばく露群の被験者数(罹患者と非罹患者を含む)、(ii)ばく露群の被験者数 (罹患者と非罹患者を含む)、(iii)非ばく露群の罹患者数である。リスク管理者は、次に、ばく露群と非ばく露群の間の 所定の(予め決められた)影響量の差を検出するために、対象となる目標値(典型的には、ORまたはRR)を選択でき、 影響量の規模が、対象となる研究で推定された影響量をどの程度説明できるかを評価することができる。

(i)多くの疫学研究はしばしば検出力不足であり、(ii)著者が検出力の計算や(場合によっては)計算に必要な情報 を文献の中で提供することは一般的ではなく、(iii)影響量の拡大の現象は一般的に疫学分野ではほとんど認識され ていないようであるため、上記の PPR パネルの勧告を実施するためには、研究者/助成機関、出版社、研究資金提 供者の側での努力が必要である。上記のように、この分野での実践の現状は悲観的になるかもしれないことを示唆して いるが、研究者である Kate Button(Button、2013 年)が Nature Reviews Neuroscience 誌に掲載したこのトピック に関するオピニオン・ピース(Button ら、2013 年)で、楽観について慎重な理由を提供した。

これらの問題に対する認識は高まっており、問題を認識することは、現在の実践を改善し、解決策を特定するための 第一歩である。出版バイアスの問題を一朝一夕に解決するのは難しいが、研究者は確立された(しかし、しばしば無視 される)科学技術の原則を採用することで、研究の信頼性を向上させることができる。また、研究者は、確立された(しか し無視されることが多い)科学技術の原則を採用することで、研究の有用性/信頼性を向上させることができる。

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1)研究の計画や結果の解釈において、統計的な検出力を考慮する。

often ignored) scientific principles; Also, researchers can improve the usefulness/reliability of their research by adopting well-established (but often ignored) scientific principles:

1) Consider statistical power in the design of our studies, and in the interpretation of our results;

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- 2) Increase the honesty with which we disclose our methods and results.
- 3) Make our study protocols, and analysis plans, and even our data, publically available; and
- 4) Work collaboratively to pool resources and increase our sample sizes and power to replicate findings."

Although the above set of recommendations and thoughts were set in the context of sample size and neurotoxicology, they have broad applicability to any discovery science, including epidemiology. In sum, while there is much room for improvement in the conduct and reporting of epidemiological studies for them to be useful to regulatory bodies in making public health-based choices, the issues are beginning to be better defined and recognised and - going forward - there is reason for optimism.

#### References

- Beane Freeman, LE, Rusiecki, JA, Hoppin, JA, Lubin, JH, Koutros, S, Andreotti, G, Hoar Zahm, S, Hines, CJ, Coble, JB, Barone Adesi, F, Sloan, J. Sandler, DP, Blair, A, and Alavanja, MCR. Atrazine and cancer incidence among pesticide applicators int eh agricultural health study (1994–2007). Environ Health Perspect, 119, 1253–1259.
- Button K, 2013. Unreliable neuroscience? Why power matters. The Guardian newspaper (UK). 10 April 2013 Available online: https://www.theguardian.com/science/sifting-the-evidence/2013/apr/10/unreliable-neuroscie nce-power-matters [Accessed 6 September 2017]
- Button K, Ioannidis JPA, Mokrysz C, Nosek BA, Flink J, Robinson ESJ and Munafo MR, 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nature Reviews Neuroscience, 14, 365-376.
- Cohen P and Chen S, 2010. How big is a big odds ratio: interpreting the magnitudes of odds ratios in epidemiological studies. Communications in Statistics: Simulation and Computation, 39, 860-864.
- Gelman A and Carlin J. 2014. Beyond power calculations: assessing type S (sign) and type M (magnitude) errors. Perspectives on Psychological Science, 9, 641-651.
- Ioannidis JP, 2005. Why most published research findings are false. PLoS Med, 2, e124.
- Ioannidis JP, 2008. Why most discovered true associations are inflated. Epidemiology, 19, 640-648.
- Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC and Beane Freeman LE. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. Occupational and Environmental Medicine, 72, 496-503.
- Koutros, S, Beane Freeman, LE, Lubin, JH, Heltshe, SL, Andreotti, G, Hughes-Barry, K, DelllaValle, CT, Hoppin, JA, Sandler, DP, Lynch, CF, Blair, A and Alavanja, MCR, 2013. Risk of total and aggressive prostate cancer and pesticide use in the agricultural health study. American Journal of Epidemiology, 177, 59-74.
- Lehrer J, 2010. The truth wears off: is there something wrong with the scientific method. New Yorker. 13 December, 2010. Available online: http://www.newvorker.com/magazine/2010/12/13/the-truth-wears-off [Accessed September 2017]
- Reinhart A, 2015. Statistics Done Wrong: the woefully complete guide. No Starch Press (San Francisco, CA).

Rosenthal JA, 1996. Qualitative descriptors of strength of association and effect size. Journal of Social Service Research, 21, 37-59.

Taubes G, 1995. Epidemiology faces its limits. Science, 269, 164-169.

- Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP and Blair A, 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control, 12, 509-517.
- Wynder EL, 1997. Epidemiology Faces its Limits Reply. Invited Commentary: Response to Science Article, "Epidemiology Faces Its Limits". American Journal of Epidemiology, 143, 747–749.
- Yarkoni T, 2009. Ioannidis on effect size inflation, with quest appearance by Bozo the Clown. 21 November 2009. Available online: http://www.talyarkoni.org/blog/2009/11/21/ioannidis-on-effect-size-inflation-with-guest-appea rance-by-bozo-the-clown/ [Accessed on 6 September 2017]

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2)研究方法と結果の開示には誠実さを高める。

3)研究計画書、解析計画、さらにはデータまでもが公表されるようにする。 4)供給源を共有し、サンプルサイズと再現力を向上させるために協力して作業する

上記の一連の推奨事項と考えは、サンプルサイズと神経毒性学の背景で設定されているが、疫学を含むあらゆる発 見科学に広く適用可能である。まとめると、疫学研究の実施と報告が公衆衛生に基づいた選択をする際に規制機関に

とって有用なものとなるためには、改善の余地が大いにあるが、問題点はより明確にされ、認識され始めており、今後も 楽観的に考えられる理由がある。

#### 参考文献

- Beane Freeman, LE, Rusiecki, JA, Hoppin, JA, Lubin, JH, Koutros, S, Andreotti, G, Hoar Zahm, S, Hines, CJ, Coble, JB, Barone Adesi, F, Sloan, J. Sandler, DP, Blair, A, and Alavanja, MCR. Atrazine and cancer incidence among pesticide applicators int eh agricultural health study (1994-2007). Environ Health Perspect, 119, 1253-1259.
- Button K, 2013. Unreliable neuroscience? Why power matters. The Guardian newspaper (UK). 10 April 2013 Available online: https://www.theguardian.com/science/sifting-the-evidence/2013/apr/10/unreliable -neuroscie nce-power-matters [Accessed 6 September 2017]
- Button K, Ioannidis JPA, Mokrysz C, Nosek BA, Flink J, Robinson ESJ and Munafo MR, 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nature Reviews Neuroscience, 14. 365-376.
- Cohen P and Chen S, 2010. How big is a big odds ratio: interpreting the magnitudes of odds ratios in epidemiological studies. Communications in Statistics: Simulation and Computation, 39, 860-864.
- Gelman A and Carlin J. 2014. Beyond power calculations: assessing type S (sign) and type M (magnitude) errors. Perspectives on Psychological Science, 9, 641-651.
- Ioannidis JP, 2005. Why most published research findings are false. PLoS Med. 2, e124.
- Ioannidis JP, 2008. Why most discovered true associations are inflated. Epidemiology, 19, 640-648.
- Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC and Beane Freeman LE. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. Occupational and Environmental Medicine, 72, 496-503.
- Koutros, S, Beane Freeman, LE, Lubin, JH, Heltshe, SL, Andreotti, G, Hughes Barry, K, DelllaValle, CT, Hoppin, JA, Sandler, DP, Lynch, CF, Blair, A and Alavanja, MCR, 2013. Risk of total and aggressive prostate cancer and pesticide use in the agricultural health study. American Journal of Epidemiology, 177. 59-74.
- Lehrer J, 2010. The truth wears off is there something wrong with the scientific method. New Yorker. 13 December, 2010, Available online: http://www.newvorker.com/magazine/2010/12/13/the-truth-wears-off [Accessed September 2017]
- Reinhart A, 2015. Statistics Done Wrong: the WOEfully complete guide. No Starch Press (San Francisco, CA).
- Rosenthal JA, 1996. Qualitative descriptors of strength of association and effect size. Journal of Social Service Research, 21, 37-59.
- Taubes G, 1995. Epidemiology faces its limits. Science, 269, 164–169. Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP and Blair A, 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control, 12, 509–517.
- Wynder EL, 1997. Epidemiology Faces its Limits Reply. Invited Commentary: Response to Science Article, 'Epidemiology Faces Its Limits'. American Journal of Epidemiology, 143, 747–749.
- Yarkoni T, 2009. Ioannidis on effect size inflation, with guest appearance by Bozo the Clown. 21 November 2009. Available online: http://www.talyarkoni.org/blog/2009/11/21/ioannidis-on-effect-size-inflationwith-guest-appea rance-by-bozo-the-clown/ [Accessed on 6 September 2017]

# **EXTERNAL SCIENTIFIC REPORT**

# Literature review on epidemiological studies linking exposure to pesticides and health effects<sup>1</sup>

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# ABSTRACT

We performed a systematic and extensive literature review of epidemiological studies examining the association between pesticide exposure and any health outcome published after 2006. We searched 43,259 citations and identified 603 published articles examining a very wide variety of outcomes and presenting over 6,000 analyses between pesticide exposure and health outcomes. We divided the different outcomes into 23 major disease categories. The largest proportion of studies pertains to cancer outcomes (N=164) and outcomes related to child health (N=84). The majority of studies were case-control studies and cross-sectional studies (N=222) and examined occupational exposure to pesticides (N=329). A wide and diverse range of pesticides was studied with studies using various definitions of pesticides; it is very hard to harmonise between studies this information. Despite the large volume of available data and the large number (>6,000) of analyses available, firm conclusions cannot be made for the majority of the outcomes studied. This observation is disappointing especially when one accounts for the large volume of research in the area. However, this observation is in line with previous studies on environmental epidemiology and in particular on pesticides which all acknowledge that such epidemiological studies suffer from many limitations and that the heterogeneity of data is such that does not allow firm conclusions to de made. We also performed updated metaanalysis for major outcomes and for those where a relevant meta-analysis published after 2006 was identified. This has only been possible for childhood leukaemia and for Parkinson's disease. For both these outcomes we found significant associations between pesticide exposure and disease in line with previous evidence.

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# **KEY WORDS**

Pesticides; epidemiological studies; pesticide exposure; health outcomes; mortality; case control studies; cohort studies

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#### **BACKGROUND AND TERMS OF REFERENCE**

Over the last years an abundance of epidemiological studies investigating possible associations of pesticide exposure with adverse health effects on humans have become available. In these studies exposure to pesticides e.g. via inhalation, ingestion, dermal contact or across the placenta has been established as being, or suggested to be, causative for instance for cancer in various organs and tissues, disturbed neurodevelopment of children, allergies, decreased fertility (male and female), birth defects and Parkinson's disease.

However, for many adverse health effects that are attributed to pesticide exposure contradictive or ambiguous studies also exist. Studies vary generally greatly in design (e.g. case control versus cohort studies), sample size and in many cases exposures are rather estimated or assumed than actually determined.

A comprehensive up-to-date literature collection and review covering relevant publications from 1<sup>st</sup> January 2006 to 31<sup>st</sup> March 2012 should be carried out in which also the quality of these studies is evaluated.

The objectives of the contract resulting from the present procurement procedure are as follows:

Objective 1: To collect and compile scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated.

Objective 2: To review and evaluate each collected study in regard to its qualitative aspects (e.g. the corner points of the investigations).

Objective 3: Provision of a database and a report of epidemiological studies.

This contract was awarded by EFSA to: The Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Grecce.

Contractor: The Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Grecce.

Contract title: Literature review on epidemiological studies linking exposure to pesticides and health effects.

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#### **INTRODUCTION AND OBJECTIVES**

This project aims to systematically collect, review and appraise epidemiological studies carried out to investigate possible links of pesticide exposure to health-related outcomes in order to improve understanding of already established or suggested associations with adverse effects in humans. The review focuses on all exposure types either through occupation or in general population with a particular focus on investigating sources of heterogeneity. In particular, we have collected scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated. The available evidence is under review and evaluation with regard to its qualitative aspects. Finally, a database of studies, which examine adverse health effect of pesticides, was compiled.

The final report is structured around health outcome categories and is linked to a *data extraction database*. In the methods we provide a detailed documentation of the search criteria and search strategy used for the literature review and the study selection process. This section also describes the analytical framework with the detailed documentation on the selected exposure and indicators of exposure and the surrogate and clinical outcomes examined. We present the results of the literature search with the full list of eligible studies and the contents of the *data extraction database*. We also present the results of the outcomes and pesticides examined and conclusions based on the literature review findings.

#### **BACKGROUND AND AIMS**

Pesticides have been widely used against pests that can damage crops such as insects, fungi, rodents, noxious, weeds, in order to prevent or reduce losses and improve product quality, for many years. Their use is very popular; in 2006 and 2007, the world used approximately 5.2 billion pounds of pesticides. However, despite their extensive use, and the associated benefits from pesticide use, there have been concerns on adverse effects in human health as these chemicals are designed to have adverse biological effects on target organisms. Indeed, there is evidence between pesticide use and adverse health outcomes such as cancers, neurodegenerative disease and birth defects; however, results so far have been inconsistent and firm conclusions cannot be drawn for several pesticides.

The aim of this review is to systematically collect, review and appraise epidemiological studies carried out to investigate possible links of pesticide exposure to health-related outcomes. This review includes all exposure types either through occupation or in the general population with a particular focus on investigating sources of heterogeneity. In particular, we have collected and compiled scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated. The available evidence has been reviewed and evaluated with regard to its qualitative aspects and data from each eligible study has been extracted. Finally, a database of studies, which examine adverse health effects of pesticides, has been compiled with the aim to facilitate the continuous update of results.

The aforementioned aims constitute a stimulating task due to the methodological challenges of environmental epidemiology and pesticide exposure in particular and the vast volume of the peer-reviewed literature.

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#### MATERIALS AND METHODS

#### 1. Search strategy

A comprehensive literature search was conducted of peer-reviewed original research pertaining to pesticide exposure and any health outcome. The search strategy was designed so as to identify observational epidemiologic studies published between 1<sup>st</sup> of January 2006 to 30<sup>th</sup> of September 2012 and examining the relationship between pesticide exposures during critical exposure time windows (preconception, pregnancy, childhood, adulthood) and any health-related outcome as discussed previously. The search strategy was developed to search primarily the MEDLINE (1950–to date), and EMBASE (Excerpta Medica Database; 1980 to-date) databases as well as TOXNET (Toxicology Data Network; U.S. National Library of Medicine 2012), OpenSigle (2012), and ProQuest Digital Dissertations and Theses (2012) as supplemental searches.

#### 2. Search algorithm for original studies in MEDLINE and EMBASE

This systematic review aimed to identify studies examining any clinical outcome or valid biomarker acting as surrogate for a clinical outcome that has been associated with exposure to pesticides. In order to achieve maximum sensitivity, we did not include any outcome-related search terms in the search algorithm that we developed. For the formation of the search algorithm, we concentrated on pesticides related terms, identified through the MEDLINEMESH terms and EMBASE classification trees on pesticides. In MEDLINE, the MESH terms of pesticides and pesticides (pharmacological action) were examined. Similarly, we examined the pesticide term in the EMBASE Emtree index. We have looked for pesticide categories (i.e. insecticides, herbicides, fungicide etc.) and for specific pesticide names as described in the literature or as pharmacological terms (e.g. DDT or Dichlorodiphenyltrichloroethane) in order to be comprehensive. We have also examined the search terms used in published systematic reviews on pesticide exposure during the past 10 years and looked for any additional terms.

Our first constructed algorithm was long including all aforementioned terms. We piloted different searches and shortened the search to improve the sensitivity of the algorithm with modest impact on the precision. All searches were limited to Humans and to publication date after 1<sup>st</sup> of January 2006.

The long list of pesticide names provided from the MESH database for pesticides pharmacological names only provided 2,270 citations on top of the pesticides related words search (pesticid\* OR pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields] OR "pesticides"[Pharmacological Action]) in MEDLINE. Examination of 200 from those 2,270 citations showed that these did not include epidemiological studies and referred to chemical studies on the substances and chemical formation of pesticides. We therefore adopted the search algorithm including the generic terms. The algorithm was constructed in EMBASE as the database provides a function to study MEDLINE and EMBASE simultaneously (see textbox below). The following algorithm was developed:

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# **Table 1:**Search algorithm for EMBASE and MEDLINE

pesticid\* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid\* OR 'fungicide'/exp OR herbicid\* OR 'herbicide'/exp OR insecticid\* OR 'insecticide'/exp OR molluscacid\* OR 'molluscacide'/exp OR molluscicid\* OR 'molluscicide'/exp OR rodenticid\* OR 'rodenticide'/exp OR carbamat\* OR 'carbamate'/exp OR pyrethroid\* OR 'pyrethroid'/exp OR 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp AND [humans]/lim AND [2006-2013]/py

The algorithm resulted in 43,259 citations in EMBASE and MEDLINE combined. Of those, 14,539 were unique to EMBASE. The algorithm includes all pesticides related terms and subcategories used either as emtree entries with the explode option and also as text words. The explode option ensures that when a term has any more specific, or narrower, index terms within the Emtree thesaurus, they are also automatically retrieved as part of the search. Terms such as organochlorine, glyphosate, paraquat and maneb were excluded as they are part of the pesticide tree of the explode option and are searched. Inclusion of these terms would lead to the same set of results. Figure 1 below shows examples of the indexing trees in EMBASE for some of our search terms.

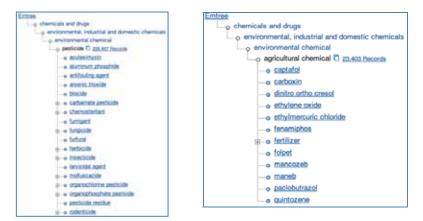


Figure 1: Examples of Emtree classification trees

#### **3.** Supplemental searches

The database of TOXNET, which lists databases on toxicology, hazardous chemicals, environmental health, and toxic releases, was also searched to identify any information missed from previous search in MEDLINE and EMBASE. We used only the Databases, which look for references in the biomedical literature (i.e. the Toxicology Literature Online (TOXLINE) and the Developmental Toxicology Literature (DART)). The remaining TOXNET databases provided summaries of Chemical, Toxicological, and Environmental Data per chemical substance and were not relevant to this search. For TOXLINE and DART, we used the generic terms "Pesticide OR Pesticides" as longer search algorithms with the inclusion of pesticides subcategories had only minor impact on the number of references identified. The searches were limited to publication dates after 2006, excluding references identified through MEDLINE. The function to identify chemical synonyms to the search term was enabled. Overall, 893 references were retrieved from TOXLINE and 34 from DART.

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We also looked into the System for Information on Grey Literature in Europe (OpenSigle), which includes 700.000 bibliographical references of grey literature (paper) produced in Europe. There were no bibliographical references on pesticides (search term pesticid\*) published after 2006.

We have also constructed a search algorithm to search the ProQuest Digital Dissertations and Theses database. We excluded from our search articles published in scholarly journals as those will have been identified through MEDLINE and EMBASE. We used the search term "pesticide\* AND health" and limited our search to specific subjects (environmental science OR public health OR environmental health OR epidemiology OR pesticides OR nutrition OR occupational health) and to publication dates between 2006 and 2012. This search strategy resulted in 1,713 results. Results were numerous when no subject limits were used (12,135) or when the term "health" was excluded from the initial algorithm (18,195).

Finally, the reference lists of all identified eligible studies and systematic reviews are scanned during data extraction for additional references.

### 4. Search for literature systematic reviews and meta-analysis

We also performed targeted searches for systematic reviews and meta-analysis in relation to specific outcomes. We restricted the search for reviews on those outcomes where more than 4 studies had been identified and we performed targeted searches in MEDLINE using the name of the outcome along with the keywords "systematic review OR meta-analysis" limited to the title or the abstract of the paper.

## 5. Structure of this report

This report is structured around health outcome categories and provides the results for each outcome group separately. A section on general conclusions is presented at the end. At the end of each section on outcomes and tables and figures are presented to allow ease of reading. Also, the ID numbers of each eligible article are referenced throughout the text. These correspond to the ID for each health outcome group in the *data extraction database* which has been provided as a separate file to this report. The ID is defined with an abbreviation for the specific health outcome and a study number.

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			N referen
Database	Search terms	Limits	ces
MEDLINE	pesticid* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'herbicide'/exp OR insecticid* OR 'insecticide'/exp OR molluscacid* OR 'molluscacide'/exp OR molluscacid* OR 'molluscicide'/exp OR rodenticid* OR 'rodenticide'/exp OR carbamat* OR 'carbamate'/exp OR pyrethroid* OR 'pyrethroid'/exp OR carbamat* OR 'pyrethroid'/exp OR 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp OR 'chlorinated hydrocarbon'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'fungicide'/exp OR molluscacid* OR 'herbicide'/exp OR molluscacid* OR 'nsecticide'/exp OR molluscacid* OR 'molluscacide'/exp OR carbamat* OR 'rodenticide'/exp OR pyrethroid* OR 'rodenticide'/exp OR carbamat* OR 'rodenticide'/exp OR carbamat* OR 'carbamate'/exp OR pyrethroid* OR 'pyrethroid'/exp OR carbamat* OR	Humans, Publication date: 2006- 2012 Humans, Publication date: 2006- 2012, no references identified	28,729
EMBASE	chemical'/exp	through MEDLINE Publication date: 2006-2012, no references identified through	14,530
TOXLINE	Pesticide OR Pesticides	MEDLINE Publication date: 2006-2012, no references identified through	893
DART OpenSigle ProQuest	Pesticide OR Pesticides Pesticide* Pesticide* AND health	MEDLINE Publication date: 2006-2012 Publication date: 2006-2012, Subjects (environmental science, public health, environmental health, epidemiology, pesticides, nutrition, occupational health), no articles published in scholarly journals	34 0 1,713 Total:

### Table 2: Summary of recourses searched, search terms and references identified

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#### 6. Selection of studies

All titles identified through the literature search of various databases were screened to identify studies, which evaluated the association between pesticides and health outcomes including any surrogate outcome. All abstracts of the selected titles are then screened in duplicate to identify epidemiological studies linking pesticide exposure to any health outcome including surrogate outcome. Both primary studies and systematic reviews or meta-analyses are selected. Articles that potentially meet eligibility criteria at the abstract screening stage have been retrieved and the full text articles have been reviewed in duplicate for eligibility. The reason for rejection of all full text articles has been recorded.

# 6.1. Eligibility criteria for full text articles

We included observational studies assessing the association between pesticide exposure and healthrelated outcomes. We included cohort, cross-sectional and case- control studies. We included studies performed in humans published from 1<sup>st</sup> of January 2006 to 30<sup>th</sup> of September 2012. Animal studies and studies performed in human cells have been excluded. We had no language, population or geographical restrictions. To enhance totality of the evidence, all types of pesticides have been considered. Exposure to pesticides was defined as reported use of pesticides by the study participant or by government registry data (self administrated questionnaires, interviewer administrated questionnaires, job exposure matrix (JEM)), by residential status (proximity to pesticide exposure), by detecting biomarkers associated with pesticide exposure or by any other means as defined by each study. Eligible health-related outcomes were "major" clinical outcomes, such as neoplasias or Parkinson's disease, clinical surrogate outcomes such as neurocognitive scales, or laboratory surrogate outcomes with an established association with clinical outcomes, such as liver enzymes.

Narrative reviews, case-series and case-reports (studies without control populations) are excluded. We also excluded studies assessing the health-related effect of pesticide poisoning or accidental high-dose pesticide exposure. We have excluded studies with no availability of sufficient quantitative information reported in the article (e.g. effect estimates) so that effect sizes or measures of associations can be calculated. Whenever reports pertained to the same study at different follow-up periods and examining the same outcome, we retained the one with the longer follow-up to avoid data duplication. We also excluded studies that referred to fertilizers (exploded from the algorithm term "agricultural chemical") as well as studies referred to the adverse effects of substances used as therapy for various medical conditions such as warfarin for anticoagulation or agents used in the treatment of scabies. Solvents and other non-active ingredients in pesticides/herbicides were not considered eligible. We excluded studies that investigate the various effects of Agent Orange on chemical warfare veterans as they represent cases of very high dose exposures. Finally, studies which examined the association between exposure and biomarkers of exposure were also not considered eligible as they do not examine health outcomes. Finally, following consultation with EFSA, we excluded studies/analyses investigating exposure to pesticides: arsenic,  $\alpha$ ,  $\beta$ , hexachlorocyclohexane (HCH), lead, dioxins and dioxin-like compounds including polychlorinated biphenyls (PCBs) as these chemicals were not considered relevant for the current project.

Regarding systematic reviews and meta-analyses, we considered all systematic reviews and metaanalyses that systematically assessed the effect of pesticide exposure to health-related outcomes, regardless of the pesticide, exposure window and outcome assessed. We included all publications where a systematic approach was endorsed (systematic literature search, assessment of methodological characteristics of the included studies and, if a meta-analysis was performed, the use of standard analytical tools including the use of a weighted summary estimate and a formal appraisal of heterogeneity). Narrative reviews are excluded.

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### 6.2. Quality control measures

The pilot literature searches have all been performed in duplicate. In addition, the first 500 results of all title searches were performed in duplicate and results were compared between investigators, which displayed high levels of agreement. The kappa statistic for agreement was 0.78. Two independent research group members performed in duplicate the abstract screening, the full text screening and the data extraction. All discrepancies are resolved by consensus or by a third arbitrator.

### 6.3. Data extraction database

We have constructed and tested the *data extraction database* with data extraction items and quality assessment items that were implemented through the whole process. The data extraction database has been structured in 7 domains: Reference, Time period, Study characteristics, Exposure assessment, Outcomes, Statistical analysis and Quality assessment (separate database file). The first 6 domains pertain to information directly extracted from the full-texts of eligible studies and would be primarily used to select studies for quantitative synthesis and aid quantitative synthesis. Studies contribute one row in the database for each outcome examined and for each exposure examined. When studies present various definitions of exposure we select for data extraction the most comprehensive definition of exposure and subsequently the one with the largest sample size. However when studies include any type of quantitative information for different biomarkers used for the identification of the same chemical substance e.g. p,p'-DDT and p,p'-DDE for dichlorodiphenyltrichloroethane (DDT), they are all reported in separate rows. When studies present data in subgroups (e.g. males and females) we extract only their main analysis (whole group) unless the data is only presented in subgroups in which case multiple rows are presented. Analyses regarding different pesticide classes and different health outcomes are extracted individually. Appendix II explains all the items used in the *data extraction* database.

The data extraction form was validated through a robust and systematic procedure. Specifically, various versions of the form were validated after blinded loops of extracting information for studies randomly selected from the database. We opted for the maximum agreement while preserving the comprehensiveness of the database. Two investigators extract each item independently and discrepancies are resolved with discussion.

# 6.4. Quality appraisal

The last part of the *data extraction database*, concerns the methodological appraisal of each eligible paper. The areas that we have focused are the study design, the study population, the level of details in exposure definition and the methods of exposure measurement and the specificity of the measurement. These are crucial questions to be asked in exposure assessment epidemiology. We have also focused on the efforts undertaken to account for confounders through matching or multivariable models, blinded exposure assessment and well-defined and valid outcome assessment. We have also looked at whether the source of funding was acknowledged. The elements of the methodological appraisal were considered from the RTI item bank. The RTI item bank is a practical and validated item bank for evaluating the risk of bias and precision of observational studies of interventions or exposures included in systematic evidence reviews. The questions were adapted to reflect exposure assessment. Across the quality appraisal questions we consistently coloured the responses to qualitative assessment in green, orange and red with green representing low risk of bias and red high risk of bias. Table 3

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below explains which answers were considered low and high risk respectively. However, the quality appraisal questions should be interpreted with caution, as they are only suggestive of the risk of bias associated with each study. There may be studies which score high in this quality assessment and still have a high risk of bias and vice versa. A final column was constructed to grade the overall quality of the studies in low, intermediate and high. This classification was based on the answers to the methodological assessment questions as explained in Table 3.

Table 3:	Methodological	assessment of eligible studies

Question	High risk	Low risk
Study design (prospective, retrospective, mixed, cross-sectional)	Retrospective, mixed, NA	Prospective
Inclusion/exclusion criteria clearly stated (yes, partially, no)	No	Yes
Authors mention power calculations (yes, no)		Yes
Level of detail in describing exposure (high, medium, low)	Low	High
Robust measurement of exposure. (biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	No	Yes
Were measures of exposure specific? yes; based on broader, chemically- related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	No	Yes
Attempt to balance the allocation between the groups (e.g., through stratification, matching)	No	Yes
Adjustment performed for potential confounders (yes, some, no)	No	Yes
Assessors blinded to exposure status (for cohort studies)	No	Yes
Outcomes assessed using valid and reliable measures, implemented	N	-
consistently across all study participants?	No	Yes
Sample size	Low	Тор
Rough quality assessment	>6 answers high risk	>6 asnwers low risk

# 6.5. Quantitative synthesis

Quantitative synthesis of the results was only attempted when there were more than 4 studies per examined outcome and when there was no substantial heterogeneity among the published evidence. The presence and extent of heterogeneity was assessed by the  $I^2$  (ranging from 0% to 100%) (Ioannidis 2007). We have summarized the RR/OR estimates using fixed and random-effects models (Lau 1997). Fixed-effects models assume that there is a common underlying effect and the variability observed is attributed to chance alone; random effects models acknowledge that true between-study heterogeneity exists and take into account the presence of heterogeneity into their calculations. In the absence of heterogeneity, fixed-and random-effects models yield the same results. Publication bias was assessed using funnel plots and visual inspection of the results.

For each outcome with more than 5 eligible studies quantitative synthesis was attempted. We did not include data from the same cohort study; either presented in the same or in different publications in the same meta-analysis when the groups compared were not mutually exclusive. For each outcome with more than 4 studies, we also looked for previously published meta-analyses to compare results and to interpret our findings in the context of previous studies. Meta-analyses were found through a) systematic reviews and meta-analyses identified through our literature review and b) targeted searches in PUBMED to identify published meta-analyses for each outcome of interest. We attempted to update any previously published meta-analyses with our results when the meta-analysis a) included studies published by 2006 and b) when outcome and exposure definitions were comparable with the definitions used in this report. Finally, we also plotted funnel plots to visually inspect asymmetry when more than 10 studies were include in the meta-analysis.

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# RESULTS

## 7. Overall results

This section focuses on the literature review results including flowcharts with number of studies screened and deemed eligible as well as number of excluded studies and corresponding reasons. It also provides an overview of the studies identified and their main characteristics.

# 7.1. Selection process for individual studies

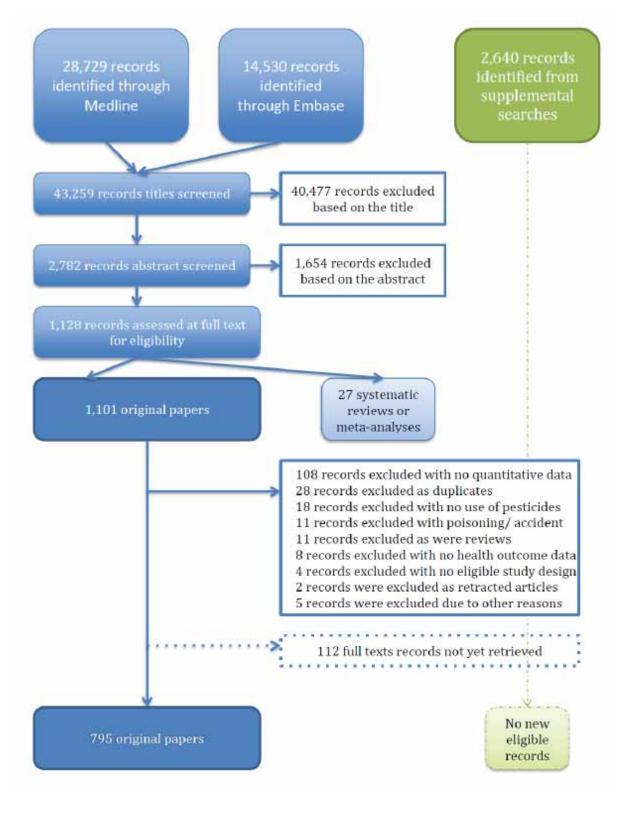
Of the 43,259 retrieved citations, 40,477 were excluded at the title screening level. Of the 2,782 remaining titles, a further 1,654 were excluded after the abstract screening. We thus deemed eligible, 1,128 citations to be scrutinized at the full-text level of which 1,101 were original research articles and 27 were systematic reviews or meta-analyses. Of the 1,101 original articles, 184 were excluded (Figure 2). For few (101) publications were the full text (or abstract for conference presentations) has not been found online, we sought the full text through letters to authors and investigations from our library. This has not been possible for 58 studies for which we extracted information from the abstract only.

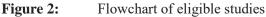
Main reasons for exclusion at the full-text level pertained to: no quantitative information/ data (these were mainly abstract presentations or comments/ editorials which did not present any quantitative information for the association between pesticides and health outcomes, n=108); duplicate records (n=28), no implied use of pesticides (n=18), studies on poisoning or accidental very high doses (n=11), reviews with no primary data (n=11), no data on health outcomes (n=8). Supplemental searches did not succeed to provide additional references as they resulted in a large number of policy documents, grant applications documents and studies already retrieved. Supplemental searches through reference lists of identified studies and especially the reference lists of identified systematic reviews will continue during data extraction and any new identified studies will be added to the current list of eligible studies. During full text screening and data abstraction a further 301 studies were excluded. The main reason for exclusion was no eligible pesticide, such as Polychlorinated Biphenyls (PCBs) (Figure 2). Overall, 602 individual publications were eligible for inclusion in the present review. These 602 publications correspond to 6,479 different analyses, which are also present in the *data extraction database*.

Any enquiries related to this output should be addressed to <u>pesticides.ppr@efsa.europa.eu</u>

Suggested citation: Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013:EN-497, 159 pp.

Available online: www.efsa.europa.eu/publications





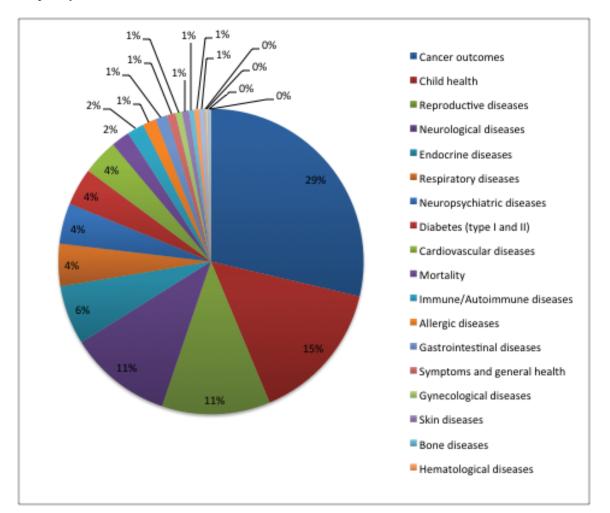
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#### 7.2. Evidence map tables and outcomes examined

We observed a great variety of assessed outcomes covering a wide range of pathophysiologies. "Hard" clinical outcomes as well as many surrogate outcomes are present in the database reflecting the different methodologies endorsed to approach the assessed clinical research questions. We divided the different outcomes into 23 major disease categories (Table 4 and Figure 3). The largest proportion of studies pertains to cancer outcomes (N=164) and outcomes related to child health (N=84). Table 4 corresponds to the Evidence map Table and shows the outcome mapping of the project describing all outcomes that have been associated with pesticide exposure between 2006 and 2012 and their frequency.



**Figure 3:** Major outcome categories and corresponding percentage of studies examining those outcomes among the eligible publications

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**Table 4:**Evidence map table including all major outcome categories examined by eligiblestudies.

Major outcome	N studies
Cancer outcomes	164
Child health	84
Reproductive diseases	64
Neurological diseases	61
Endocrine diseases	35
Mental and psychomotor development	32
Respiratory diseases	25
Neuropsychiatric diseases	15
Diabetes (type I and II)	22
Cardiovascular diseases	31
Hematological diseases	15
Mortality	11
Immune/Autoimmune diseases	10
Allergic diseases	8
Gastrointestinal diseases	7
Symptoms and general health	5
Gynecological diseases	4
Skin diseases	4
Bone diseases	3
Kidney diseases	3
Benign tumors	1
Dental diseases	1
Men health	1
Metabolic diseases	1

# 7.3. Characteristics of eligible studies

The eligible studies were published from 2006 to 2012. The observed distribution of the publication year of the eligible studies indicates an approximately equal distribution of studies throughout the past 5 years (Figure 4). Of note, we expected a considerable presence of the results of the various reports of the Agricultural Health Study (AHS), the largest to-date observational study performed in the field. Indeed, the AHS publications (n=42) represent a recognizable proportion of the included studies (7%). Another 22 studies come form the cross-sectional National Health and Nutrition Examination Survey (NHANES) cohorts.

The majority of studies were case-control studies (N=222) and cross-sectional studies (Figure 5) and examined occupational exposure to pesticides (N=329). Almost half of the studies (N=285) were based in America (Figure 6). The most frequent method of pesticide assessment was measurement of biomarker or use of self reported questionnaire (Figure 7). Approximately half (N=261) studies were classified as 'high' in the methodological assessment.

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A wide and diverse range of pesticides was studied with studies using various definitions of pesticides; it is very hard to harmonise between studies this information. We also anticipated a considerable proportion of the published literature to be focusing on pesticides no longer approved for use in the European Union and in most of the developed countries. We acknowledge that this research lies on the rational of pesticide long-term residuals, as well as of the continuing use of these pesticides in developing countries. For example, studies focusing solely on DDT and its metabolites constitute almost 10% of the eligible studies.

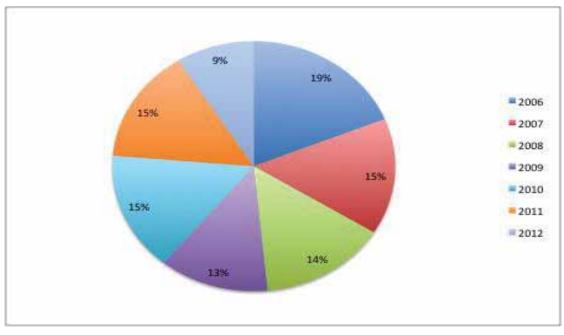
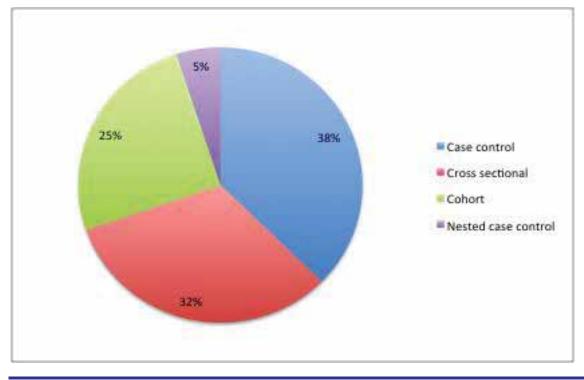


Figure 4: Percentage of eligible papers per publication year between 2006 and 2012



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# Figure 5: Epidemiological study designs of eligible publications

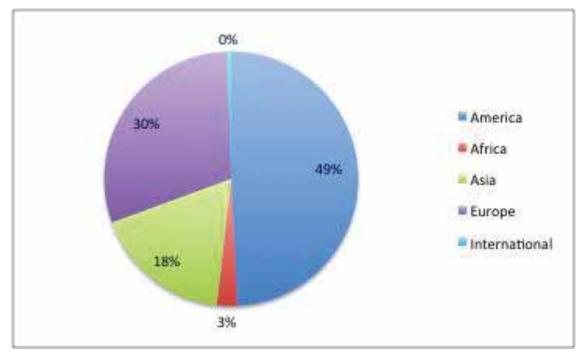
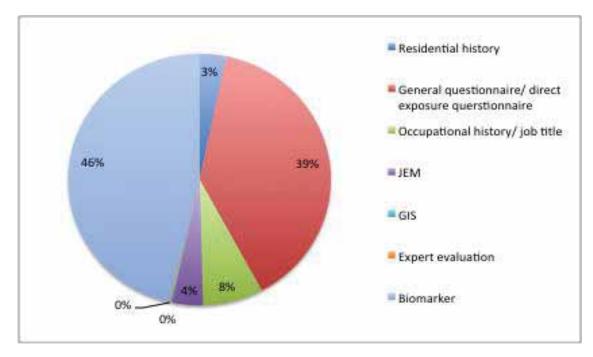
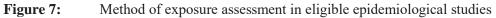


Figure 6: Location (continent) where eligible epidemiological studies were conducted





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## 7.4. Systematic literature review of systematic reviews and meta-analysis

Throughout our search strategy we also identified systematic reviews and meta-analyses. Overall, 21 different eligible reviews were identified published after 2006. The outcomes examined are shown in Table 5 below. Most reviews examined cancer related outcomes and some claimed positive associations between pesticides and examined outcome. The reviews are discussed in relevant outcome categories along with the individual studies.

Outcome	Natudios	Authors claim association	Author, Journal, Publication
Outcome	IN Studies	Authors claim association	year Sutadia NA at al. 2000
			Sutedja NA et al, 2009
	0	N	Kamel F et al, 2012
Amyotrophic lateral sclerosis	3	No	Malek et al, 2012
Cancers	11		
Breast cancer	1	No	Khanjani N et al, 2007
			Infante-Rivard C et al, 2007
Childhood cancer	2	Yes	Vinson F et al, 2011
			Wingle DT et al, 2009
			Turner et al, 2010
			Van Maele-Fabry G et al,
			2010
			Van Maele-Fabry G et al,
			2011
			Bailey HD et al, 2011
Childhood Leukaemia	6	Yes	Turner MC et al, 2011
Multiple cancers	1	Yes	Cooper et al, 2008
Prostate cancer	1	Yes	Budnik LT et al, 2012
Multiple health outcomes	1	Yes	Koureas M et al, 2012
•			Ismail AA et al, 2012
Neurobehavioral	2	No	Li AA et al, 2012
			Van der Mark M et al, 2012
			Van Maele Fabry G et al,
Parkinson disease	2	Yes	2012
Reproductive	1	No	Shirangi A, 2011
-			Snijder CA et al, 2012
Time to pregnancy	1	Yes	•

### Table 5: List of systematic reviews and meta-analyses identified in the literature review

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#### 8. Cancer Outcomes

Overall, 164 publications examined the effect of pesticide exposure on cancer outcomes, contributing more than 2000 separate analyses. As seen with other outcomes, the diversity of exposure definition is remarkable and poses special challenges to data synthesis. Only 36 out of the 164 were prospective cohort studies and other 13 were nested case-controls; the overwhelming majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. Also, out of the 49 prospective analyses, 30 (61%) were from the same prospective study, the Agricultural Health Study (AHS) and evidence beyond this prospective cohort is limited. This is an important observation as it emphasizes the fact that 60% of the evidence for prospective associations comes from a single population. The sample size of the analyses was often small; it ranged between 24 and 82,596 participants (median 301). In addition, 33 studies had information on biomarkers of exposure and only 7 assessed occupational exposures through job exposure matrix (JEM). Common limitations in studies included small sample sizes, self-reported exposure, potential for high false positive rates due to multiple testing (studies test multiple hypothesis without adjusting for multiple testing and therefore results are likely to be false positives), and retrospective design. A wide variety or pesticides were assessed, with many studies examining organochlorine insecticides.

The different cancer categories examined are presented in Table 6 along with the number of studies contributing to each outcome category and a recommendation for quantitative synthesis. Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for some cancer subgroups.

#### 8.1. Hematological neoplasms

#### 8.1.1. Leukemias

Overall, 26 studies (and 2 abstracts) examined associations between pesticide exposure and various forms of leukaemia. Fourteen out of these 26 studies were reports from the AHS with some overlapping results and examination of different pesticide groups. Only 2 studies, both on DDE (ID CAN 063, ID CAN 064) examined residential exposure and all the remaining studies examined occupation exposure to pesticides. Twelve out of 99 different analyses were statistically significant with effect sizes across all studies ranging between 6.1 and 0.2. Statistically significant results come from 7 different studies; with the exception of the AHS all were of modest to low quality. Table 7 shows summarised results across studies that reported information on the same pesticide class. The vast majority of results are non-significant and of small effect sizes. Figure 8 shows random effect meta-analyses keeping analyses with largest sample size form each study. The meta-analysis resulted in a non-significant pooled effect (OR 1.26, 95% CI 0.93, 1.71) and had modest heterogeneity. Previous meta-analyses on occupational exposure to pesticides and leukaemia were published in 2008 and 2007 (Merhi 2007, Van Maele-Fabry 2008). The overall summary effect estimates from previous meta-analyses suggested that there is a significantly positive, albeit weak, association between occupational exposure to pesticides and all hematopoietic cancers. But both reports acknowledged a wide range of limitations including the lack of sufficient data about exposure information and other risk factors for hematopoietic cancer and unclear definition of exposure and of leukemia type.

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# 8.1.2. Hodgkin lymphoma

Seven studies examined the associations between pesticide exposure and Hodgkin lymphoma. All studies assessed exposure through questionnaires, one studies had large sample size and all studies were retrospective. A wide range of pesticides classes was examined which did not allow any meaningful synthesis of the results. Twelve out of 75 separate analyses were statistically significant with effect sizes ranging from 8.4 to 0.4 across all analyses. We attempted random effects meta-analysis keeping only the Agricultural Health Study (AHS) analysis with the largest sample size. The result was not statistically significant and had high heterogeneity which can be attributed to the range of different pesticide classes examined by each study (Figure 9).

# 8.1.3. Other lymphomas

A very wide variety of definitions of lymphomas other than Hodgkin lymphoma were used in 44 studies of which 21 were reports from the Agricultural Health Study (AHS) and 2 from the BC (British Columbia) sawmill workers cohort study. Studies examined broad definitions of lymphomas and lymphoproliferative syndromes (ID CAN\_047, ID CAN\_049, ID CAN\_074) and other examined more specific definitions of follicular lymphoma, diffuse large cell lymphoma and peripheral T-cell lymphoma. Twenty-one studies provided effect sizes between pesticide exposure and broad definitions of Non-Hodgkin lymphomas. Five of those studies were prospective (ID CAN\_063, ID CAN\_064, ID CAN\_067, ID CAN\_118, ID CAN\_121) and seven examined associations with biomarkers of exposure (ID CAN\_056, ID CAN\_057, ID CAN\_064, ID CAN\_065, ID CAN\_067, ID CAN\_060, ID CAN\_052). However, the later analyses were all on organochlorine pesticides with only few significant results (6 analyses among a total of 35 analyses) without any firm evidence for associations. In the AHS, large and significant effect size was observed between butylate use and Non-Hodgkin lymphomas (RR 2.94, 95% 1.49–5.96, p=0.002; high vs. no exposure). However, again the AHS in the same publication has examined ten different outcomes and results need adjustment for multiple testing.

# 8.1.4. Multiple myeloma

Also, 11 studies examined associations between pesticides and multiple myeloma, myelodysplastic syndromes and monoclonal gammopathy of undetermined significance. These studies were generally heterogeneous and no quantitative synthesis was suggested. Overall, some analyses were statistically significant, but those were mainly from the French case control study (ID CAN\_049) which presented overall 147 separate analyses and results are prone to bias. The AHS also reported significant associations between permethrin, dieldrin, Carbon-tetrachloride/carbon disulfide mix and Chlorthalonil but again these were amongst 52 other analyses and require cautious interpretation. One study, reported very high significant effect size of 7.3 for myelodysplastic syndrome (ID CAN\_070) but the quality of the study was poor and adjustment of covariates very limited and results were not replicated by other studies on the same phenotype.

# 8.2. Prostate cancer

Overall, 39 studies (in 260 analyses) examined the effects of pesticide exposure on prostate cancer. One study was a conference abstract which provided little data on methodology to allow meaningful appraisal of its results (ID CAN\_107). Also, 25 of those 39 studies were studies from the AHS population with some overlapping results. For example, two studies (ID CAN\_022, ID CAN\_106) examined interactions between pesticide exposure and genetic variants in relation to prostate cancer. These AHS studies presented the same main effects for pesticide exposure; effects were largely null and, if anything, significant inverse effects were found e.g. for carbaryl, chlordane, metalachlor and

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others. The remaining AHS studies, examined associations between specific pesticides, again showing no statistically significant associations between any of the examined pesticides and prostate cancer with the exception of a weak significant effect between butylate exposure and prostate cancer. The remaining evidence stems from, rather small and of modest quality, retrospective studies. Most studies (ID CAN 103, ID CAN 101, ID CAN 100, ID CAN 094, ID CAN 143, ID CAN 142) examined the effects of organochlorines with largely small and non-significant results. Two studies (IDs CAN 099, ID CAN 095) showed high significant increased risk associated with pesticide exposure and prostate cancer but both studies were of low quality, had very broad definitions of exposure and results need cautious interpretation and do not match with those reported from well conducted large prospective studies (e.g. AHS). Notably, one population-based case-control study (ID CAN 104) in a highly exposure area found strong association of ambient exposure to methyl bromide with prostate cancer risk, but the study did not observe evidence for exposure-response. In summary, most evidence for prostate cancer risk in relation to pesticide exposure concerns the effect of organoclorines with studies showing weak non-significant effects. A meta-analysis (Maele-Fabry 2003) on occupational exposure to pesticides and prostate cancer was also identified published. The pooled effect estimate, based on 22 epidemiological studies, was 1.13 (95% CI 1.04 to 1.22) with substantial heterogeneity across studies. In addition, the studies reviewed contained insufficient qualitative and quantitative information on exposure in order to distinguish the influence of pesticides from other occupational, environmental, and lifestyle factors (Maele-Fabry 2003). Overall, there is no evidence supporting an association between pesticide exposure and prostate cancer.

### 8.3. Lung cancer

Thirty studies contributing 45 analyses examined associations between pesticide exposure and lung cancer; previously published meta-analysis was not identified. Again, 23 out of 30 published studies and 30 of the 45 analyses were analyses of the AHS. Amongst the 50 different analyses of the AHS, only one statistically significant result was observed. Three studies examined broad pesticides definition as their exposure (ID CAN\_080, ID CAN\_082, ID CAN\_083), one studied mosquito coil burns (ID CAN\_081), while the remaining studies examined a range of different pesticides with an emphasis on organochlorine insecticides. The diversity of pesticide categories and the repeated use of the same cohort population (AHS) in more than half of the studies does not allow for quantitative synthesis. Notably, the association between mosquito coil burn and lung cancer was statistically significant with large effect size (3.78 (1.55, 6.90); yes vs. no use) but the study is relative small, retrospective with limited examination of confounders and of overall modest quality. Two case-control studies (ID CAN\_082, ID CAN\_082) reported over a two-fold increased risk of lung cancer for occupational exposure to pesticides but individual pesticides were not examined. Another case-control study (ID CAN\_080) failed to replicate these observations between pesticide exposure and lung cancer mortality. Overall, the evidence on pesticide exposure and lung cancer is limited and inconclusive.

#### 8.4. Childhood cancer

#### 8.4.1. Childhood hematological neoplasms

Overall, 17 studies (and one abstract) which examined childhood hematological neoplasms in relation to pesticide exposure were identified. All 17 studies examined childhood leukemia and 4 of them also included other hematological neoplasms.

Previous meta-analysis on childhood leukemia concentrated on studies which assessed residential exposure to pesticides only. All studies that were included in the meta-analyses and were published after 2006 have been identified by our search which confirms that we identified all available evidence.

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Identified studies were generally small with the exception of two studies on national registries, the Northern Region Young Persons' Malignant Disease Registry (ID CAN 120) and the national registry-based case-control study ESCALE (Etude sur les cancers de l'enfant) (ID CAN 073). Results from these studies should be cautiously interpreted, despite their large sample size, due to the large number of hypothesis examined (high false positive rate); each study reported 42 and 64 separate analysis respectively. All were case control studies and vast majority examined residential exposures with few studies on occupation exposure identified. Although most studies assessed use of, or exposure to, pesticides or pesticide subgroups (insecticides, herbicides, fungicides), some studies also attempted to collect information on specific pesticides (ID CAN 031, ID CAN 032) and one study (ID CAN 032) assessed biomarker levels. There were few data regarding frequency or duration of pesticide use, with most studies reporting only "ever/never" use of/exposure to the pesticide of interest. Although confounding is difficult to assess because there are few established risk factors for childhood hematological neoplasms, most studies examined or adjusted for at least a range of sociodemographic and maternal characteristics. Almost all studies assessed pesticide exposure separately for preconception, pregnancy, and childhood time windows. One study of very low quality and incomplete statistical analyses results examined all exposure time windows and other 2 (ID CAN 073, ID CAN 044) examined preconception and pregnancy jointly.

Three studies were excluded from further quantitative analyses: study ID CAN\_040 was excluded due to lack of CIs; study ID CAN\_030 due to duplicate data from Northern California Childhood Leukemia Study (duplicate with ID CAN\_031), and study ID CAN\_037 due to a unique study population (Down syndrome cases only). We divided the quantitative synthesis of results by the time period (window of exposure).

### 8.4.1.1. Exposure during pregnancy

Seven studies had information for pesticide exposures during pregnancy. Eleven out of 86 analyses were statistically significant corresponding to 5 studies which all examined acute leukaemia as outcome of interest. Largest effect estimates were reported from the national registry-based casecontrol study ESCALE (Etude sur les cancers de l'enfant). Insecticide use during pregnancy was significantly associated with childhood acute leukemia (OR = 2.1; 95% CI, 1.7-2.5) and paternal household use of pesticides was also related to acute leukemia (OR = 1.5; 95% CI, 1.2–1.8) in this study. We performed a series of quantitative synthesis of results. We first selected analyses with the largest sample size within each published report and synthesized results (Figure 10). This analysis was associated with large heterogeneity ( $I^2 > 80\%$ ) as each study had different exposure assessment (type of pesticide and parental route of exposure) and variability in outcome assessment. The remaining metaanalysis in Figure 10 show synthesis or results based on pesticide class examined in an effort to harmonize results with the previously published meta-analysis (Turner 2010) on 'Residential Pesticides and Childhood Leukemia'. We performed quantitative synthesis of all studies on insecticides and pesticides identified in this systematic review and subsequently we updated the previously published meta-analysis keeping only studies assessing residential exposure. Overall, the results show modest heterogeneity across studies, which can be attributed to variability in pesticide exposure definition, outcome definition, definition of exposure time windows etc. However, the metaanalysis show a consistent increased risk of childhood leukemia associated with exposure to unspecified pesticides and insecticide (Summary OR=1.69; 96% CI=1.35, 2.11). Our updated metaanalysis resulted in more conservative results compared to the meta-analysis published in 2010 but still supported an association between exposure to pesticides during pregnancy and childhood leukaemia. Still the evidence merits careful interpretation as there were concerns around publication bias in the original meta-analysis, the studies are typically small and the exposure is measured through non-validated self-reported questionnaires that are prone to misclassification. Funnel plot shows relative symmetry around studies of small size. Further evidence from large studies, using valid

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biomarkers of past exposure are needed to confirm whether there is public health merit in reducing prenatal exposure to residential pesticides.

# 8.4.1.2. Preconception

Four studies examined preconception as the time window of exposure (ID CAN\_032, ID CAN\_043, ID CAN\_073, ID CAN\_120) but none reported statistically significant results.

## 8.4.1.3. Childhood

Seven studies with information on exposure during childhood were identified (ID CAN\_031, ID CAN\_032, ID CAN\_035, ID CAN\_036, ID CAN\_041, ID CAN\_043, ID CAN\_133). One study examined Endosulfan, which is no longer in use; the study was of very low quality and was not considered further. Meta-analysis of these studies is shown in Figure 14 below. Two analyses are presented A) one on identified studies from 2006 onwards based on the analysis of the largest sample size in each report (any pesticide) and B) an update on the 2010 meta-analysis on pesticide exposure during childhood and childhood leukemia. The meta-analysis on any pesticides had modest heterogeneity whereas the updated meta-analysis, which was restricted to residential exposure and insecticides/ unspecified pesticides only, displayed no heterogeneity in its results. The results of the updated meta-analysis are more conservative than the original meta-analysis but still very close to the pooled estimates reported in 2010 (Figure 14). Funnel plots indicated considerable symmetry around results. Overall, there is some evidence for association between childhood exposure to pesticide and childhood leukemia but this is weaker than exposure during pregnancy and requires more evidence from well-conducted large birth cohorts to draw firm conclusions.

# 8.4.2. Lymphomas

Evidence beyond leukaemia for childhood hematological neoplasms comes only from 3 studies, which reported many analyses (IDs CAN\_073, ID CAN\_120, ID CAN\_133) among which analyses for Non-Hodgkin and Hodgkin lymphomas. All analyses were not statistically significant and had weak effect estimates.

# 8.4.3. Other childhood cancers

Seven studies on other childhood cancers were identified. Four studies examined brain cancer (ID CAN\_006, ID CAN\_011, ID CAN\_089, ID CAN\_133), one childhood germ cell tumor (ID CAN\_114) and two examined a range of childhood cancers (ID CAN\_120, ID CAN\_133). Significant associations were only observed for brain cancers but again these pertain to only a small subset of many analyses and cannot be informative at this stage.

# 8.5. Colorectal cancer

Overall, 26 identified studies examined associations between pesticide exposure and colorectal cancer in 207 analyses. Separate analyses for colon cancer and rectum cancer were available in 24 and 11 studies respectively. A very large body of evidence comes from the AHS study, which examined all these 3 outcomes for associations with 194 out of the 207 identified analyses on colorectal cancer examining 50 different pesticides with no adjustments for multiple testing. Out of these 194 analyses, only 7 were statistically significantly positively associated with the outcome (Carbaryl, Aldicarb,

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Toxaphene, Pendimethalin, S-Ethyl dipropylthiocarbamate (EPTC), imazethapyr and Fonofos) but need to be interpreted with caution due to high false positive probability. Despite the fact that 27 published studies were identified, overall the evidence comes from only 7 different populations. This fact along with the range of different pesticides analysed by each of these studies in relation to colon cancer does not allow meaningful quantitative synthesis of the results. Table 8 shows the extent of publication of duplicate data from one cohort in multiple papers and shows good consistency of results. Previous meta-analyses on colorectal cancer and pesticide exposure have not been identified. Overall, the evidence for pesticides and colorectal cancer is very limited and current state of the literature does not support associations between pesticides and colorectal cancer.

#### 8.6. Skin cancer

Seventeen studies examined associations between melanoma and pesticide exposure. The majority of studies assessed organochlorine pesticides. Again, 14 out of 17 studies on melanoma were results from the AHS examining in each paper different pesticides categories and different definitions of exposure with some supplication of results present. Of the 26 different analyses of the AHS, 8 were statistically significant and all stemming from the same publication (ID CAN\_085) on dose response relationships for 50 agricultural pesticides with cutaneous melanoma. The study reported significant associations between cutaneous melanoma and maneb/mancozeb ( $\geq$  63 exposure days: OR = 2.4; 95% CI, 1.2–4.9; trend *p* = 0.006), parathion ( $\geq$  56 exposure days: OR = 2.4; 95% CI, 1.3–4.4; trend *p* = 0.003), and carbaryl ( $\geq$  56 exposure days: OR = 1.7; 95% CI, 1.1–2.5; trend *p* = 0.013) (155). Other studies did not report results on these pesticides to allow examination of replication of results. One case-control study showed increased statistically significant risk between indoor pesticide exposure and melanoma whereas in the same study outdoors pesticide exposure was not associated with melanoma (106). The remaining studies on organochlorines showed heterogeneous results with few statistically significant results (Hexachlorobenzene (HCB), mirex), which do not provide evidence for an association between these pesticides and melanoma.

#### 8.7. Breast cancer

Overall, 14 studies (and 3 abstracts) after 2006 examined the relationship between pesticide exposure and breast cancer. The vast majority of studies and analyses concentrate on organochlorine pesticide, which they are assessed through biomarker analyses. Two previous meta-analyses on breast cancer and DDT exposure have been published (Khanjani 2007, López-Cervantes 2004). Overall, previous meta-analyses did not show a significant association between any cyclodiene chemical and breast cancer except for heptachlor, but that was based on only two studies. Meta-analysis on identified studies in this systematic review on Dichlorodiphenyldichloroethylene (DDE) and breast cancer (5 studies) also shows no evidence for association. We have also performed a meta-analysis across all identified studies on breast cancer, selecting each time the analysis within each study with the largest sample size. Studies ID CAN 019 and ID CAN 023 were excluded from synthesis, as effect sizes and confidence interval to allow synthesis were not provided and study ID CAN 022 was excluded as it reported very tight confidence intervals which did not were assumed to be reported incorrectly. The synthesis here involves the pooled effect of many different pesticides definitions and biomarkers (DDE, lindane, and broad pesticide definition) and is difficult to be interpreted. The pooled effect shows a statistically significant increased risk of breast cancer (1.07 (0.87 to 1.31)) but this result need cautious interpretation. The meta-analysis combines very different categories of pesticides and is largely dominated by one study (ID CAN 022), which assessed pesticide exposure by self-reported residential pesticide use and is therefore of modest quality compared to the rest of the studies which assessed pesticides via biomarkers.

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## 8.8. Bladder cancer

Sixteen studies examining bladder cancer in relation to pesticide exposure were identified; however, 13 were studies from the same population the AHS as previously observed for other cancer outcomes. Among the 25 different analyses presented, only one provided statistically significant results for occupational exposure to imazethapyr in the AHS. However, due to multiple testing the results need cautious interpretation and based on the evidence reviewed in this report there is no suggestion for an association between pesticide exposure and bladder cancer.

# 8.9. Kidney cancer

Ten studies examined kidney cancer in relation to pesticide exposure; however, data from two populations only, the AHS and the BC (British Columbia) sawmill workers cohort study. Results form the BC sawmill workers cohort study (ID CAN\_129 and ID CAN\_125) were both on occupational exposure to pentachlorophenol and tetrachlorophenol but examining different approaches to statistical analyses. Results from the AHS were on different pesticide classes. Overall, no statistically significant results were observed and the limited number of contributing populations (n=2) does not allow further quantitative synthesis.

# 8.10. Pancreatic cancer

Seven studies examined pancreatic cancer in relation to pesticide exposure; 4 were reports from the AHS. The overwhelming majority of analyses considered organochlorine pesticides. In a small casecontrol study of modest quality significantly increased concentrations of hexachlorobenzene (HCB), sum of chlordanes and polybrominated diphenylethers (PBDEs) were found in the pancreatic cancer cases compared to healthy controls (ID CAN\_090). In the AHS, among 46 different analyses, significant associations were reported for Pendimethalin and S-Ethyl dipropylthiocarbamate (EPTC). Applicators in the top half of lifetime pendimethalin use had a 3.0-fold (95% CI 1.3–7.2, p-trend 5 0.01) risk compared with never users, and those in the top half of lifetime EPTC use had a 2.56-fold (95% CI 5 1.1–5.4, p-trend=0.01) risk compared with never users. Organochlorines were not associated with an excess risk of pancreatic cancer in the AHS. These findings suggest that herbicides may be associated with pancreatic cancer but require replication by future studies as they all come from a single population without adjustments for multiple testing.

# 8.11. Testicular cancer

Overall, 8 studies examined testicular cancer. Two studies also reported outcomes for seminoma cancer. All but one study assessed biomarker levels and concentrated on organochlorine pesticides with a range of different biomarkers assessed and studies showing a weak effect for an association with testicular cancer. However, information on more than 4 studies was available for p-p'DDE only and quantitative synthesis showed a non-significant effect and modest heterogeneity (Figure 20). Quantitative synthesis across any pesticide was not performed due to heterogeneity of biomarkers assessed in each study. Overall, there is no evidence to support an association between pesticide exposure and testicular cancer based on evidence reviewed herein.

# 8.12. Stomach cancer

Six studies examined association between pesticide exposure and stomach cancer. All studies examined occupational exposure to pesticides, a range of pesticide classes was studies; 2 studies had a prospective design but all had modest to small sample sizes. In agreement with previous meta-analysis

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on farmers (Saphir 1998), studies reported weak and mainly non-significant results. A nested casecontrol study (ID CAN 028) of gastric cancer embedded in the United Farm Workers of America (UFW) cohort reported significant associations: working in areas with high use of the phenoxyacetic acid herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was associated with gastric cancer (OR 1.85; 95% CI 1.05-3.25); use of the organochlorine insecticide chlordane was also associated with the disease (OR 2.96; 95% CI 1.48-5.94). Gastric cancer was associated with use of the acaricide propargite (OR 2.86; 95% CI 1.56-5.23). Nonetheless, the study is limited by a relatively small number of cases and controls, multiple testing and exposure misclassification, as assessment was ecological in nature. In the AHS, based on 15 exposed cases, stomach cancer risk increased monotonically with increasing methyl bromide use (RR = 3.13; 95 % CI, 1.25-7.80 for high use compared with no use; p for trend = 0.02). However, again the associations suffer from multiple testing as all other cancer subtypes have been associated with methyl bromide use in this study (ID CAN 147). Meta-analysis selecting the analysis with largest sample size is shown in Figure 21 but results require careful consideration. Despite a statistical significant pooled large effect size, this is dominated by two studies (ID CAN 125, ID CAN 147), which examine pentachlorophenol and methyl bromide; two compounds that are not approved in the European Union.

### 8.13. Liver cancer

Five studies (including 11 separate analyses) and one conference abstract examined associations between pesticide exposure and liver cancer. The majority of analyses examined exposure to organochlorine pesticides and all studies examined occupational exposure to pesticides. Both studies on DDT (IDs CAN\_076 and ID CAN\_079) reported statistically significant associations with liver cancer; the remaining analyses were non-statistically significant. These two studies largely dominate the meta-analysis on liver cancer, which shows a statistically significant pooled result largely driven by the DDT studies.

#### 8.14. Cancer subgroups with few studies

As illustrated in Table 6, for a large number of individual cancers only very few studies are available to allow synthesis of evidence for each cancer subgroup. Our systematic review did not identify any previously published meta-analyses on these cancer subtypes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these cancer subtypes were of small effect and not statistically significant with few exceptions concerning occupational exposure only. Given the large number of analyses within each study, these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these cancer subtypes.

There were also a large number of studies examining all cancers (composite cancer outcome) in relation to pesticide. Cancers represent a very heterogeneous group of disorders and simultaneous examination of all cancer subtypes may introduce bias in the associations. Overall, 30 analyses examining "all cancers" were identified and 28 of them were analyses of the same cohort, the AHS, not allowing further synthesis of the results. Only 4 results out of 31 were statistically significant were associated with poor quality of studies and therefore do not merit interpretation at this stage.

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# **Table 6:**Summary of eligible studies identified per cancer subgroup

Cancers	N studies	Meta-analysis recommended	Previous meta- analysis identified
Haematological neoplasms	88	Yes	Yes
Prostate cancer	39	No	Yes
Lung cancer	30	Yes	No
All cancers	30	No	No
Childhood cancer	45	Yes	Yes
Colorectal cancer	26	No	No
Skin cancer	17	Yes	No
Bladder cancer	16	Yes	No
Breast cancer	14	Yes	Yes
Kidney cancer	10	No	No
Pancreatic cancer	7	No	No
Testicular cancer	8	No	No
Lip, oral cavity and pharynx cancer	5	No	No
Stomach cancer	6	No	No
Liver cancer	5	No	No
Brain cancer	6	No	No
Bone cancer	5	No	No
Oesophageal cancer	5	No	No
Larynx cancer	3	No	No
Biliary tract cancer	2	No	No
Soft-tissue	2	No	No
Female reproductive system cancer	2	No	No
Other	9	No	No

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	Publication	Pesticide					95%	95%	Level of
Study ID	Date	Class	Pesticide Type	Outcome	Ν	OR	LCI	UCI	Adjustment
DDE									
CAN_064	2010	p,p'-DDE	Biomarker	Chronic Lymphocytic Leukemia	210	0.78	0.28	2.21	+++
CAN_063	2010	p,p'-DDE	Questionnaire	Chronic Lymphocytic Leukemia	148	0.62	0.29	1.3	++
CAN_056	2008	p,p'-DDE	Biomarker	Chronic Lymphocytic Leukemia	71	1	0.4	2.5	+
Insecticides									
CAN_072	2006	Insecticides	Questionnaire	All leukemias	1304	1	0.7	1.4	+
CAN_049	2009	Insecticides	Questionnaire	Chronic Lymphocytic Leukemia	37	0.8	0.3	2.1	+
CAN_024	2010	Insecticides	Questionnaire	Acute Myeloid Leukemia	158	1.52	0.16	2.04	+++
Herbicides									
CAN_072	2006	Herbicides	Questionnaire	All leukemias	1260	1.4	0.8	2.3	++
CAN_049	2009	Herbicides	Questionnaire	Chronic Lymphocytic Leukemia	39	0.5	0.2	1.3	+
CAN_024	2010	Herbicides	Questionnaire	Acute Myeloid Leukemia	45	1.83	0.99	3.38	+++
CAN_058	2008	Herbicides	Questionnaire	Chronic Lymphocytic Leukemia	523	1.15	0.76	1.74	++

**Table 7:**Summary results across eligible studies that reported information on the samepesticide class and risk of leukaemia (DDE: Dichlorodiphenyldichloroethylene)

**Table 8:**Examples of identified studies from the Agricultural Health Study (AHS) thatevaluated the same biomarkers of pesticide exposure in relation to colorectal cancer (DDVP:2,2-dichlorovinyl dimethyl phosphate)

				Sample	Effect	Lower	Upper	Adjust
Study ID	Pesticide	Outcome	Comparison	size	Estimate (OR)	95% CI	95% CI	ments
			Highest					
			tertile of					
	Dichlorvos/		exposure vs					
CAN_122	DDVP	Colon cancer	no	202	1.48	0.78	2.8	+
	Dichlorvos/							
CAN_024	DDVP	Colon cancer	Ever vs. never	56813	1.5	0.9	2.4	++
CAN_024	Fonofos	Colon cancer	Ever vs. never	56813	1.5	1	2.2	++
			Highest					
			tertile of					
			exposure vs					
CAN_119	Fonofos	Colon cancer	no	126	1.66	0.92	3.03	++
		Colorectal						
CAN_024	Malathion	cancer	Ever vs. never	56813	0.8	0.6	1.1	++
			Highest					
			tertile of					
		Colorectal	exposure vs					
CAN_121	Malathion	cancer	no	58	0.84	0.48	1.48	++
	L .	Rectum					_	
CAN_118	Toxaphene	cancer	Yes vs. no	75	2	1.1	3.5	+++
		Rectum						
CAN_024	Toxaphene	cancer	Ever vs. never	56813	2.1	1.2	3.6	++

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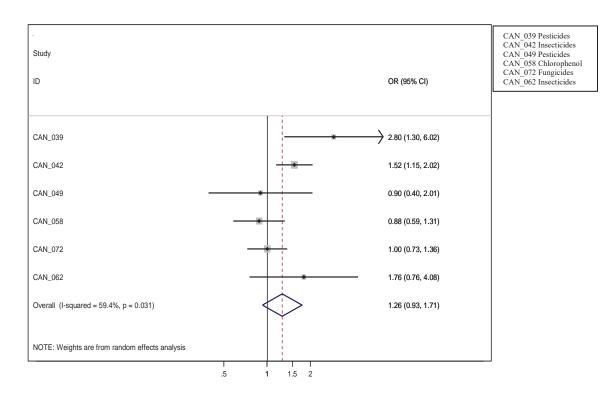
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**Table 9:**Studies on biomarkers of pesticide exposure and testicular cancer with morethan >2studiesperbiomarker(DDE:Dichlorodiphenyldichloroethylene;HCB:Hexachlorobenzene)

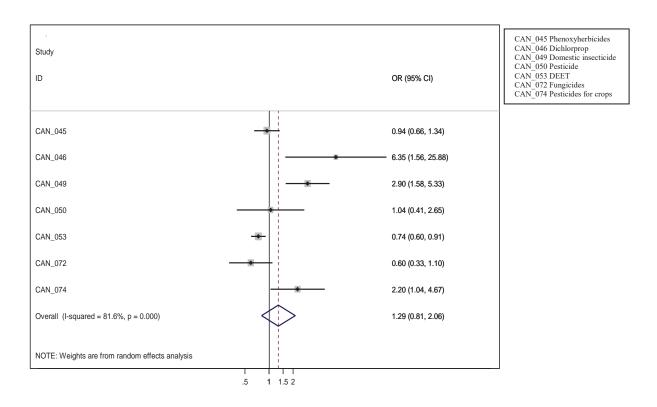
Study IDPesticidetypeComparison levelTotal NEffect estimate95% LCI95% UCIACAN_111DieldrinORhigh tertile vs low4180.790.441.41 +CAN_115DieldrinORhigh tertile vs low602.10.59.5 +CAN_113HCBORyes/no574.41.712 +CAN_115HCBORhigh tertile vs low702.90.515.2 +	+
CAN_115         Dieldrin         OR         high tertile vs low         60         2.1         0.5         9.5         +           CAN_113         HCB         OR         yes/no         57         4.4         1.7         12         +	
CAN_113         HCB         OR         yes/no         57         4.4         1.7         12 +	
CAN 115 HCB OR high tertile vs low 70 2.9 0.5 15.2 +	
CAN_111         Heptachlor epoxide         OR         high tertile vs low         407         0.67         0.35         1.29 +	+
CAN_115         Heptachlor epoxide         OR         high tertile vs low         68         2.4         0.6         9.1 +	
CAN_111         Mirex         OR         high tertile vs low         557         0.87         0.5         1.53         +	
CAN_112         Mirex         RR         high tertile vs low         1333         0.24         0.9         1.74 +	
CAN_115         Mirex         OR         high tertile vs low         66         1.2         0.4         3 +	
CAN_111         o,p-DDT         OR         high tertile vs low         514         1.3         0.67         2.53         +	+
CAN_115         o,p'-DDT         OR         high tertile vs low         71         1.4         0.4         4.5         +	
CAN_116         o,p'-DDT         Mean difference         unit increase         60         0.46         n/a         n	/a
CAN_111         p,p'-DDT         OR         high tertile vs low         533         1.17         0.68         2+	
CAN_112         p,p'-DDT         RR         high tertile vs low         1493         1.13         0.71         1.82 +	
CAN_115 p,p'-DDT OR high tertile vs low 63 2.1 0.6 7.2 +	
CAN_116 p,p'-DDT Mean difference unit increase 60 -1.2 n/a n/a n	/a
CAN_111         p,p'-DDE         OR         high tertile vs low         554         0.61         0.32         1.14 +           CAN_112         F         DDE         DD         bick tertile vs low         504         1.71         1.32         2.32	
CAN_112 p,p'-DDE RR high tertile vs low 884 1.71 1.23 2.38 +	
CAN_113         p,p'-DDE         OR         yes/no         44         1.3         0.5         3 +           CAN_115         p,p'-DDE         OR         high tertile vs low         65         2.2         0.7         6.5 +	
_ F/	
	/a
CAN_117         p,p'-DDE         OR         high tertile vs low         98         3.21         0.77         13.3         +	
CAN 111 Oxychlordane OR high tertile vs low 538 0.93 0.5 1.73 +	
CAN_112 Oxychlordane RR high tertile vs low 841 1.27 0.92 1.76 +	
CAN 115         Oxychlordane         OR         high tertile vs low         64         1.27         0.32         1.70           CAN 115         Oxychlordane         OR         high tertile vs low         68         3.2         0.6         16.8 +	TT
CAN 111 Total chlordanes OR high tertile vs low 562 0.93 0.51 1.68 +	+
CAN_112 Total chlordanes RR high tertile vs low 842 1.51 1.09 2.1+	
CAN 113 Sum of chlordanes OR yes/no 49 1.9 0.7 5+	
CAN_115 Total chlordanes OR high tertile vs low 70 2.3 0.6 7.2 +	
CAN_111 Trans -nonachlor OR high tertile vs low 564 0.89 0.49 1.61 +	+
CAN_112 Trans -nonachlor RR high tertile vs low 875 1.46 1.07 2 +	
CAN_115 Trans -nonachlor OR high tertile vs low 62 2.6 0.7 8.9 +	

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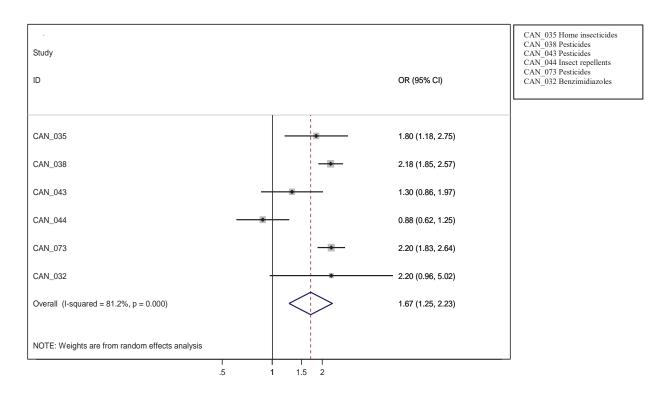
**Figure 8:** Random effects meta-analysis of the association between exposure to pesticides and Leukemia

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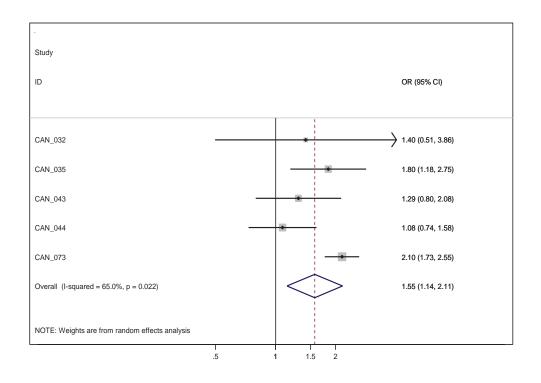
**Figure 9:** Random effects meta-analysis of the association between exposure to pesticides and Hodgkin Lymphoma

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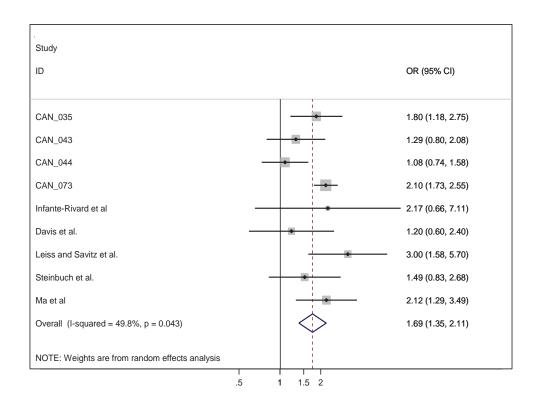
**Figure 10:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Any exposure to pesticide during pregnancy and childhood leukemia)

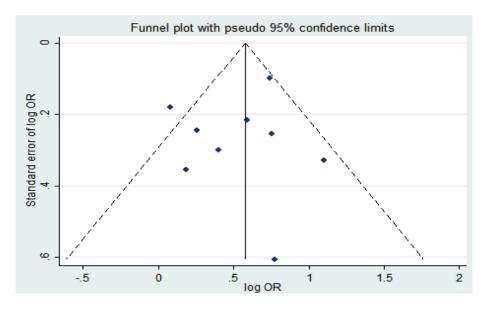
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**Figure 11:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Exposure to insecticides during pregnancy and childhood leukemia)

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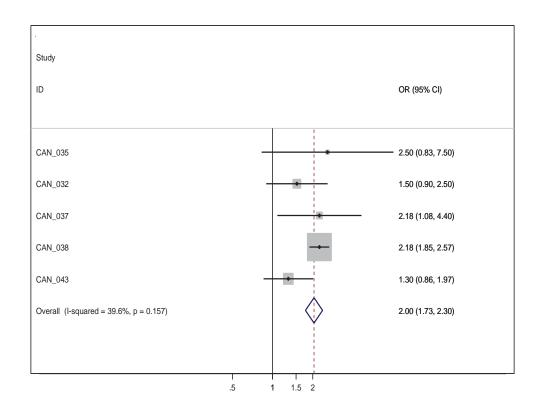




**Figure 12:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Residential exposure to insecticide during pregnancy and childhood leukemia) (update to meta-analysis 2010 using published effect sizes; Turner 2010) and associated funnel plot

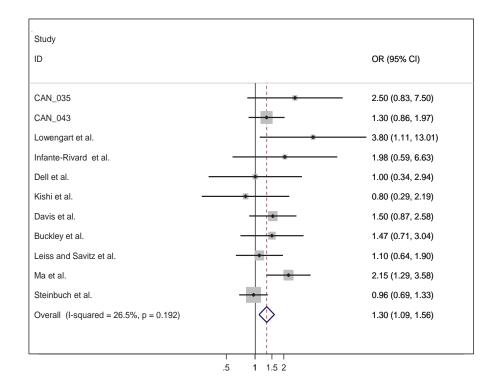
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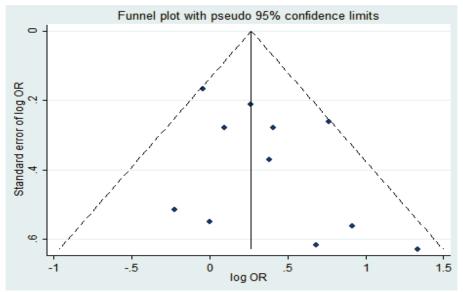
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**Figure 13:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Exposure to unspecified pesticides during pregnancy and childhood leukemia)

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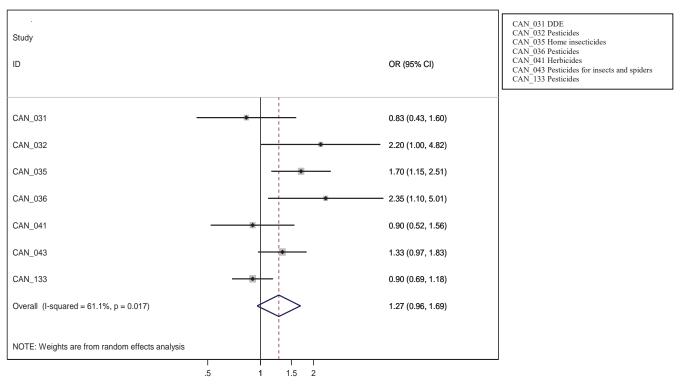


**Figure 14:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Residential exposure to unspecified pesticides during pregnancy and childhood leukemia (update to meta-analysis 2010, Turner 2010) and associated funnel plot)

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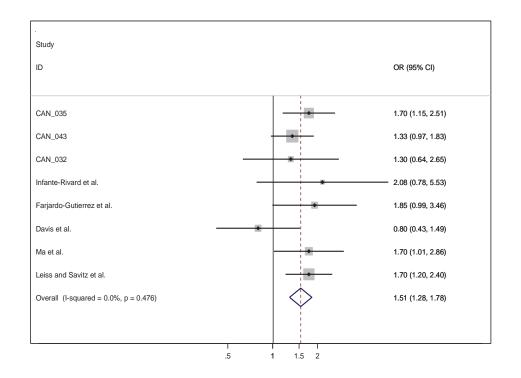
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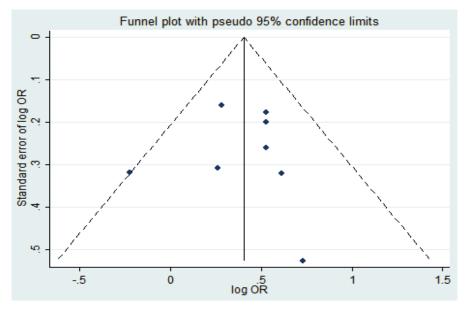
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**Figure 15:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Any exposure to pesticide during childhood and childhood leukemia)

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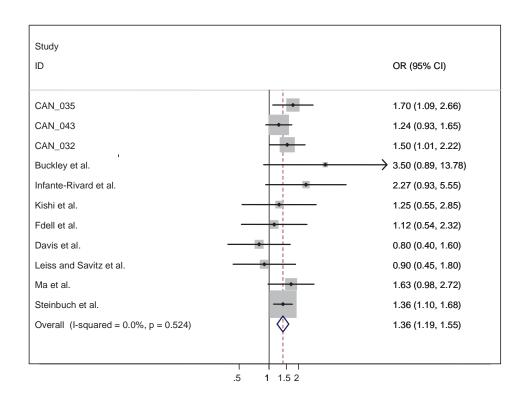


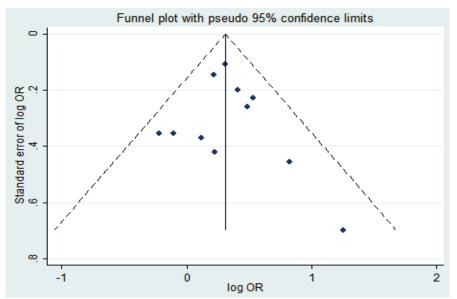


**Figure 16:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Residential exposure to insecticide during childhood and childhood leukemia (update to meta-analysis 2010 using published effect sizes, Turner 2010) and associated funnel plot)

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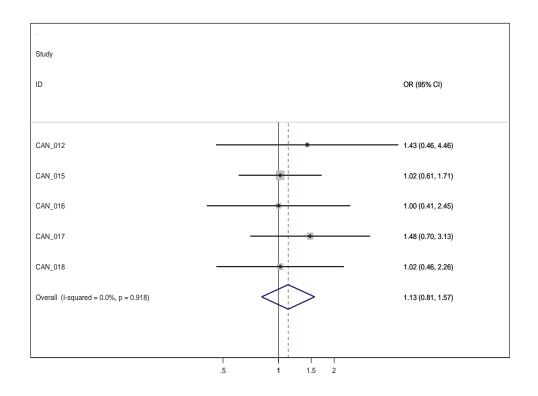




**Figure 17:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Residential exposure to unspecified pesticides during childhood and childhood leukemia (update to meta-analysis 2010 using published effect sizes, Turner 2010) and associated funnel plot)

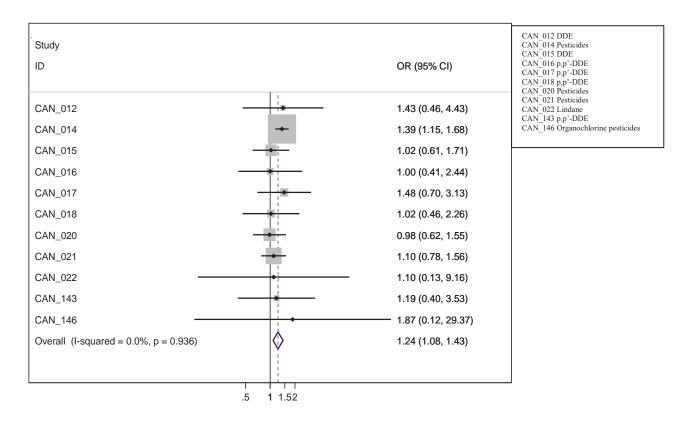
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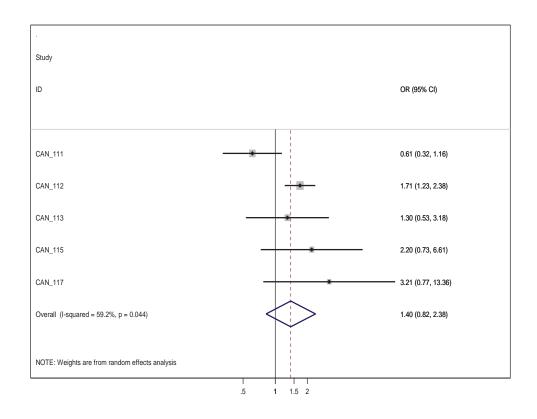
**Figure 18:** Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and breast cancer on studies that examined DDE exposure to pesticide with breast cancer

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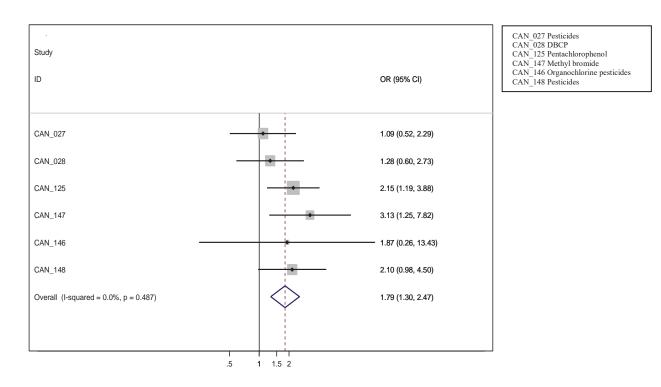
**Figure 19:** Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and breast cancer selecting analyses with the largest sample size within each study (pesticides assessed in each study are shown in on the right key).

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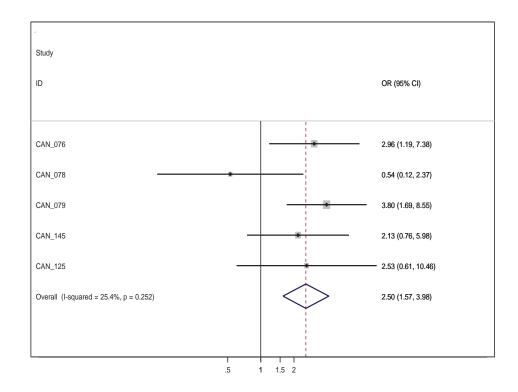
**Figure 20:** Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and testicular cancer

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**Figure 21:** Random effects meta-analysis for studies that examined any exposure to pesticide with stomach cancer selecting analyses with the largest sample size within each study

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**Figure 22:** Random effects meta-analysis for studies that examined any exposure to pesticide with liver cancer selecting analyses with the largest sample size within each study (pesticides assessed in each study are shown on the right key)

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## 9. Child health

Overall, 84 individual studies examined the effect of pesticide exposure on child health outcomes (median sample size: 267; IQR 119-811), contributing 821 separate analyses in the data extraction database. More than 120 health-related outcomes were assessed with a large proportion focusing on congenital malformations and developmental parameters including but not restricting to somatometrics (Table 10). As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 38 out of the 84 were prospective cohort studies and other 5 were nested case-controls; the majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 23 and 183,313 participants (median 267) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we observed no large clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS), while 26 studies assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was more prominent here (n=49, 58%) while 3 studies assessed occupational exposure through JEM. The different outcome categories examined are presented in Table 10 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis (Table 11). Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for urological malformations only.

## 9.1. Prematurity

Fifteen studies assessed the association between pesticide exposure during pregnancy and prematurity with a median sample size of 193 (IQR 87-469), contributing 54 separate extracted comparisons in the database. More than half of the studies were retrospective and in more than three-fourths of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (8 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 091) assessed a Dutch population of greenhouse workers and reported a decreased risk of preterm birth among male greenhouse workers (OR= 0.47; 95%CI= 0.35–0.65) while the observed increased risk in women was not statistically significant (OR= 1.14, 95%CI= 0.57–2.31). The remaining studies reported statistically non-significant results with effect estimates pointing towards a positive association. Moreover, no meta-analysis of published studies was identified. Based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and prematurity in general.

#### 9.2. Restricted fetal growth

Twelve studies assessed the association between pesticide exposure during pregnancy and restricted fetal growth and/or small for gestational age neonates with a median sample size of 422 (IQR 178-1,630), contributing 44 separate extracted comparisons in the database. Sixty percent of the studies were prospective, three assessed occupational exposure and in more than two-thirds of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (4 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a

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quantitative synthesis was not performed. The largest study (ID RPD 26) assessed in a retrospective cohort whether atrazine in drinking water is associated with increased prevalence of small-for-gestational age and preterm birth. The authors reported that atrazine in drinking water during the third trimester and the entire pregnancy was associated with a significant increase in the prevalence of SGA (Small for Gestational Age); atrazine in drinking water > 0.1 µg/L during the third trimester resulted in a 17–19% increase in the prevalence of SGA compared with the control group (< 0.1 µg/L). All the remaining studies reported statistically non-significant results without a consistent pattern regarding the effect direction of the effect magnitude. Moreover, no meta-analysis of published studies was identified. Based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and prematurity in general.

## **9.3.** Somatometrics (Body size metrics)

Numerous studies examined the association between pesticide exposure and growth.

# 9.3.1. Birth length / Height

Length at birth and height was assessed in 13 and 8 studies, respectively, contributing 78 separate comparisons in the database. In the vast majority of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently; nevertheless no single pesticide and related biomarker was assessed in more than 4 studies, thus a quantitative synthesis was not performed.

The largest prospective study (ID CH 073) assessing a North American population born before 1980, reported that only the highest prenatal concentrations of p,p'-DDE (>60 mg/l), as compared with the lowest (<15 mg/l), were statistically significantly associated with decreased height at age 7 years [adjusted coefficient (SE) -2.21 cm (0.67)]. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis was identified. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and birth length or height in general.

# 9.3.2. Body weight

Twenty-six studies assessed the association between pesticide exposure during pregnancy and birth weight, contributing 134 separate extracted comparisons in the database. Another 5 studies assessed the association between pesticide and ponderal index. In a large number of comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (11 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 014) was a Agricultural Health Study (AHS) publication and reported that first-trimester pesticide-related tasks were not associated with birth weight and that, after multiple analyses, ever use of the pesticide carbaryl was associated with decreased birth weight (-82 g, 95% CI = -132, -31). The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis of published studies was identified. We identified though a meta-analysis of individual participants data from European cohorts which reported that a  $1-\mu g/L$  increase in p,p'-

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DDE was associated with a 7-g decrease in birth weight (95% CI= -18, 4 g) (Govarts E 2012). Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and birth weight in general.

Twenty-six studies assessed the association between pesticide exposure and body weight at various time-points after birth, contributing 68 separate extracted comparisons in the database. In almost 85% of the assessed comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (10 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same outcome definition, the same time-point for the outcome assessment, the same pesticide, and the same comparison unit, thus a quantitative synthesis was not performed. The largest study (ID CH 074) assessing DDT exposure in a Mexican population of boys born in 2002 and 2003, reported that, overall, associations between prenatal DDE level and Body Mass Index (BMI) at any given age were not observed and that the predicted values showed that children with the highest exposure (DDE: 49.00 mg/g) compared to those least exposed (DDE: <3.01 mg/g) grew similarly and they had a BMI similar to the referent group. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis was identified. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and body weight in general.

# 9.3.3. Head circumference

Fourteen and three studies assessed the association between pesticide exposure during pregnancy and head circumference at birth and after birth, respectively, contributing 85 separate extracted comparisons in the database. In more than two-thirds of the comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed for birth head circumference, with DDT metabolites being assessed more frequently (7 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 026) was a Generation R study publication which explored associations between maternal occupational exposure to various chemicals and fetal growth in 4,680 pregnant women participating in this population-based prospective cohort study in the Netherlands (2002-2006). For fetal head circumference, only maternal occupational exposure to alkylphenolic compounds showed a statistically significant lower growth rate (-0.01752 SD per gestational week) compared with nonexposed mothers, adjusted for potential confounders. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis of published studies was identified. Given the large number of analyses the reported study results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and head circumference in general.

# 9.3.4. Congenital malformations

Five studies examined the association between pesticide exposure and congenital malformations in general. The largest study (ID CH 002) assessed a Canadian farm population, reported 146 potential associations, did not yield statistically significant results in the primary analysis and proposed that pre-

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conception exposure to cyanazine (OR = 4.99, 95% CI: 1.63-15.27) and dicamba (OR = 2.42, 95% CI: 1.06-5.53) were associated with increased risk of birth defects in male offspring. Nevertheless, given the number of the available comparisons and the self-reported nature of the exposure and outcomes in this study, the present findings should be considered with caution. The remaining four retrospective studies reported conflicting results (ID CH 043, occupational exposure (father), OR: 3.42, 95% CI: 1.97-5.92; ID CH 035, at least one parent exposed, OR = 1.3, 95%CI = 0.4 - 3.9; ID CH 008, HR for maternal urine metolachor, 95% 0.4-1.4). Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest association between pesticide exposure and congenital malformations in general.

# 9.3.5. Neural tube defects

Identified studies examined associations between pesticide exposure and neural tube defects (N=4 studies), including an encephaly and spina bifida and providing a very large number of reported analyses between different pesticides and neural tube defects, anencephaly and spina bifida with no adjustments for multiple testing (average 27 analyses per paper). Out of the 134 extracted analyses, 43 were statistically significantly positively associated with the outcome (of which 14 borderline significant) but need to be interpreted with caution due to high false positive probability. The range of different pesticides analysed by each of the 5 studies as well as the varying definitions of pesticide exposure do not allow for a meaningful quantitative synthesis of the results even using the "any pesticide" exposure definition since there is also considerable heterogeneity between studies regarding the exposure period as well as the parent analysed; three studies assessed maternal exposure, one study assessed paternal exposure and one study both. Previous meta-analyses on neural tube defects and pesticide exposure have not been identified. Overall, the evidence for pesticides and neural tube defects is limited and the current state of the most recent literature does not support a robust association. Of note, the largest study in the field (ID CH 044) investigated whether maternal residential proximity to applications of specific pesticides or physicochemical groups of pesticides during early gestation increases the risk of these malformations, included 731 cases and 940 controls and after reporting 107 different analyses for individual pesticides, pesticide physicochemical categories and any exposure, no exposure and multiple exposure definitions yielded 15 statistically significant results without correction for multiple testing and without a particular pattern with regards to a pesticide category or an additive effect.

#### 9.3.6. Urogenital malformations

Overall, 19 studies examined urogenital malformations, namely cryptorchidism (n=9) and hypospadias (n=9).

Cryptorchidism was assessed in nine mostly retrospective studies, of a median sample size of 199 (IQR 136-710). Four studies assessed DDT levels; hexachorobenzene (HCB) and chrordane were assessed in one study each, while general pesticide exposure was assessed in 2 studies. When we attempted to investigate the association between exposure to any pesticide and cryptorchidism across all assessed studies, the observed effect was not statistically significant (OR 1.19, 95% CI 0.96 – 1.49, I<sup>2</sup> 24%) (Figure 23). Moreover, when we assessed the potential association between DDT exposure and cryptorchism, we again observed a statistically non-significant association (OR 1.47, 95% CI 0.98- 2.2, I<sup>2</sup> 51%) (Figure 24). Given the large number of analyses, these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between any pesticide exposure and cryptorchidism.

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Hypospadias was assessed in 9 mostly retrospective studies, of a median sample size of 784 (IQR 200 - 861). Two studies assessed DDT levels, while general pesticide exposure was assessed in 6 studies. When we attempted to investigate the association between maternal exposure to any pesticide (during preconception and pregnancy) and hypospadias across all assessed studies, the observed effect was not statistically significant (OR 1.02, 95% CI 0.74 – 1.39,  $I^2$  72%) (Figure 25). When we included in the analysis the three studies that assessed a specific pesticide (DDT, n=2; chrordane, n=1), we again observed a statistically non-significant association (OR 1.00, 95% CI 0.84- 1.16, I<sup>2</sup> 66%) (Figure 26). Our systematic review retrieved one meta-analysis including original research published in English and indexed in PubMed from January 1966 through March 2008 (Rocheleau CM, 2009). Nine studies published before 2007 met all study inclusion criteria and the authors reported that elevated but marginally significant risks of hypospadias were associated with maternal occupational exposure (PRR of 1.36, CI=1.04-1.77), and paternal occupational exposure (PRR of 1.19, CI=1.00-1.41). Due to the different time-periods for the literature assessment and the resulting minimal overlap between our review and the published meta-analysis, we were able to synthesize the two efforts and again we retrieved a statistically non-significant result (OR 1.14, 95% CI 0.84 - 1.55, I<sup>2</sup> 73%). Thus, there is no recent evidence to suggest a robust, clinically significant association between any pesticide exposure and cryptorchidism.

#### 9.4. Child health outcomes with few studies

For all the assessed outcomes not included in Table 10, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a vast variety of captured information ranging from well-defined clinical entities yet with too few studies, such as gastroschisis, cardiac birth defects, diaphragmatic hernia, and esophageal atresia, as well as a large numbers of metrics pertaining to broad clinical entities but with a prominent lack of harmonization and standardization in the outcome definition. For example, outcomes related to neurodevelopment were assessed extensively; nevertheless the metrics used, ranging from IQ measurement to perceptual reasoning, deemed any further attempt towards a quantitative synthesis impossible. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

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## Table 10: Assessed outcomes in the field of child health as defined by eligible studies

Health outcome		
Abnormal urogenital distance	Body mass index (BMI) Z-score	Increased serum prolactin levels
Abnormal body mass index (BMI)	Body fat percentages (log transformed)	Increased serum total testosterone level
Abnormal bone age	Chordee	IQ
Abnormal breast size	Coarctation of the aorta	LH dysregulation
Abnormal change of body mass index	Congenital diaphragmatic hernia (CDH)	Low annual height velocity
Abnormal change of height	Congenital heart defects	Major congenital anomalies
Abnormal chest circumference	Congenital malformations	Male genital malformations
Abnormal gestational age	Cretinism	Maternal age
Abnormal head circumference-for-age	Crown-Heel Length	Maternal weight gain
Abnormal height	Cryptorchidism	Miscarriage or stillbirth
Abnormal hip circumference	Decreased inhibin B levels	Musculoskeletal defects
Abnormal length	Decreased serum FSH levels	Neural tube defects
Abnormal ovarian measurements	Decreased serum inhibin B levels	Obesity
Abnormal penis length (stretched)	Decreased serum SHBG levels	Oestradiol dysregulation
Abnormal penis width	Decreased testicular volume	Perceptual Reasoning
Abnormal serum DHT levels	Decreased testosterone levels	Performance IQ
Abnormal sitting height	Decresed serum LH levels	Ventricular septal defect
Abnormal standing height	Duration of lactation	Placental weight
Abnormal Tanner stage	Esophageal atresia	Placental weight
Abnormal upper arm circumference	Fetal death	Ponderal Index
Abnormal upper arm fold circumference	Fetal head circumference	Ponderal index
Abnormal uterine measurements	Fetal length	Precocious puberty
Abnormal waist circumference	Fetal weight	Preeclampsia
Abnormal weight	FGR	Premature breast development
Abnormal weight-for-length	Freedom from distractability	Premature oestradiol secretion
Affected breast development	FSH dysregulation	Premature puberty onset (pubic hair)
Anal position index	Gastroschisis	Prematurity
Androstendione dysregulation	Gestational age	Processing speed
Anencephaly	Gynecomastia	Rapid infant weight gain
Anti-mullerian hormone dysregulation	Head Circumference	SGA
APGAR 1-minute score	Hypospadias	SHC
APGAR 5-minute score	Idiopathic precocious puberty	Spina bifida
Atrioventricular septal defect	Increased FSH levels	Sum of four skin folds
Birth head circumference	Increased levels of SHBG	Testosterone dysregulation

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Pesticide epidemiology

Birth height	Increased ratio LH/testosterone	Tetralogy of Fallot	
Birth Weight	Increased serum AMH levels	Transposition of the great arteries	
Birth weight, adjusted for gestational age	Increased serum androstenedione levels	Verbal comprehension	
BMI	Increased serum DHEAS levels	Verbal IQ	
BMI at delivery	Increased serum free testosterone level	Working memory	
BMI before pregnancy			

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Outcome	N studies	Meta-analysis done	Previous meta-analysis result
Congenital malformations			
General	5	No	NA
Neural tube defects	4	No	NA
Urogenital malformations	19	Yes	<u>Hypospadias:</u> maternal occupational exposure (RR 1.36; 95% CI 1.04–1.77), and paternal occupational exposure (RR 1.19; 95% CI 1.00–1.41)
Development	40	No	NA
Growth			
Height/Birth length	21	No	NA
Weight	26	No	<u>Birth weight (individual</u> <u>participants' data meta-analysis</u> <u>of 12 European cohorts):</u> A 1- μg/L increase in p,p'-DDE was associated with a 7-g decrease in birth weight (95% CI: -18, 4 g).
Head circumference	17	No	<u>NA</u>
Sexual maturation	9	No	NA

# Table 11:Summary of studies identified per outcome subgroup with more than 4studies (NA: not available)

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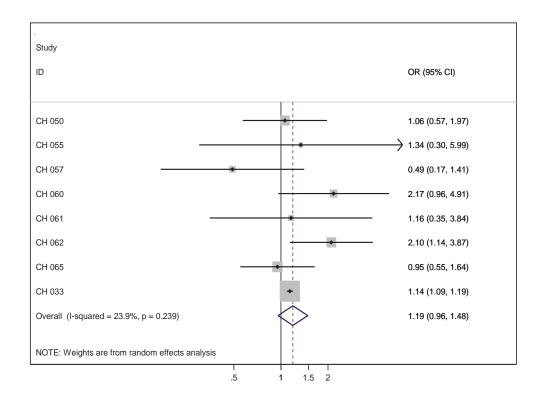
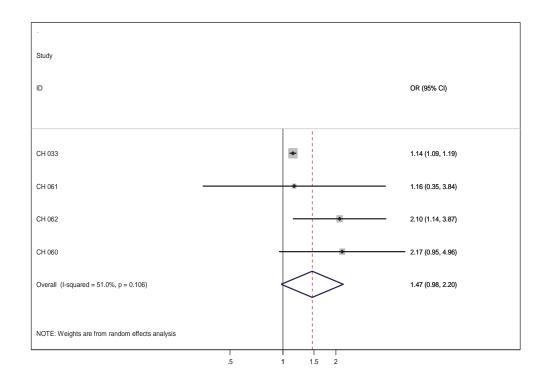


Figure 23: Random effects meta-analysis for studies with information on pesticide exposure and risk of cryptorchidism

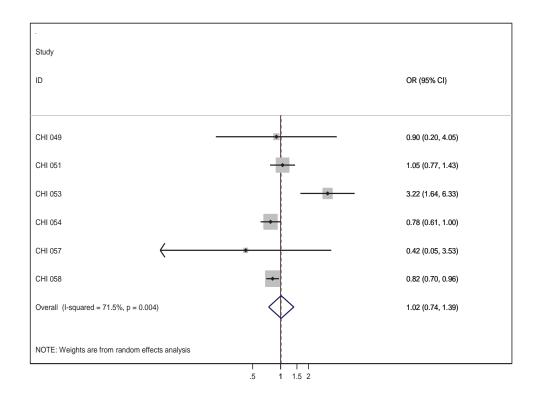
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**Figure 24**: Random effects meta-analysis for studies with information on DDT exposure and risk of cryptorchidism

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**Figure 25:** Random effects meta-analysis for studies with information on general pesticide exposure and risk of hypospadias

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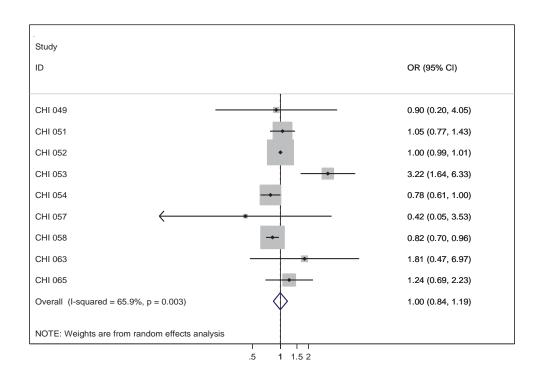


Figure 26: Random effects meta-analysis for studies with information on general pesticide exposure and risk of hypospadias, including studies on specific pesticides

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#### **10. Reproductive diseases**

Overall, 63 publications examined the effect of pesticide exposure on child health outcomes (median sample size: 299; IQR 111-544), contributing 578 separate analyses in the data extraction database. More than one third of the analyses (n=217, 38%) assess the sperm/semen quality, whereas other cluster of studies/analyses examine among others reproductive related hormones, infertily and spontaneous abortion As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 4 out of the 64 were prospective cohort studies whereas the vast majority of the studies were cross sectional (n=45, 70%). The sample size in the reported analyses was rather small; it ranged between 41 and 29,649 participants (median 161) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we observed a cluster of publications coming from INUENDO (INUit-ENDOcrine) research group (n=8), a project that has been established in three European countries together with a population of Inuits from Greenland and aims to enlighten the impact of Persistent Organic Pollutants (POPs) on human reproductive function. Almost 2/3 of the studies were conducted in Europe and America (n=22 and 20 respectively). Twenty-two studies assessed occupational exposures and, in addition, more than half of the studies had information on biomarkers of exposure (n=38, 59%), 3 studies assessed occupational exposure through Job Exposure Matrix (JEM), whereas 2 studies used both questionnaires and biomarkers. The different outcome categories examined are presented in Table 12 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data, statistical synthesis of the data (meta-analysis) was only performed for abortion.

## **10.1.** Impaired sperm parameters

Twenty-five studies (median 189: IQR 87-336) assessed the association of pesticides on sperm/semen quality using a variety of outcomes. The total analyses conducted for these outcomes are 217 and the sample size of the conducted analyses is small ranging from 41 to 763. The largest study is a European cross-sectional study from INDUENDO research group (ID RPD 009) and assess the impact of p,p'-DDE to sperm concentration, sperm motility and sperm morphology and showed that the sperm motility was negatively associated with p,p'-DDE across the four populations under study. Another large study from the same group (ID RPD 012) did not provide evidence that Persistent Organic Pollutants (POPs) may interfere with male reproductive function. Even though a large number analyses have been conducted no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit and analysis, thus a quantitative synthesis was not performed.

# **10.2.** Fecundability disorders

Eight studies including 30 different analyses assess the effect of pesticides on low fecundability. The sample sizes are rather small ranging from 41 to 2,365 participants. Different effect sizes and analyses are used for the assessment of potential associations therefore the synthesis of the results through meta-analysis is not feasible. The largest study (ID RPD 038) that examined pesticide exposure of female greenhouse farm workers reported a reduced fecundability (OR=0.68, 95% CI=0.49-0.94). However the second largest study in the field (ID RPD 034) on female greenhouse farm workers did not shown a significant association (OR=1.11, 95%CI=0.96-1.29). Fourteen additional analyses did not report significant findings; therefore the evidence is contradictory in the field.

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#### **10.3.** Spontaneous abortion

Ten studies of spontaneous abortion focused on occupational exposure. We were able to synthesize data from six studies that provided an effect estimate and a metric of its variation. The summary OR was 1.52 (95%: 1.09-2.13) using random effects models and large heterogeneity was observed ( $I^2=63\%$ ) (Figure 27). However, the largest cross-sectional study on this outcome conducted by the INUENDO research group (ID RPD 003) did not shown any statistical effect (OR=1) between p,p'-DDE and abortion. One more study compared full-time vs. part time farming and did not report a significant association (p-value=0.99). Three other studies did not provide adequate information for their inclusion in the meta-analysis.

#### **10.4.** Reproductive hormones

Nineteen studies (median sample size 257: IQR 97-322) contributing with 250 analyses for various reproductive hormones were identified in this systematic review. The studies were comparable to the other large group of impaired sperm parameters sample size-wise; their range was from 62 to 887. The largest study is a European cross-sectional study that assess the effect of hexachlorobenzene on the levels of testosterone and estradiol. Hormonal status of 14- to 15- year-old male adolescents was studies in relation to internal exposure to pollutants. The study shows that the exposure is associated with substantial differences in hormone concentrations. Different patterns were observed in study conducted by the INUENDO research group where the overall analysis between DDE and reproductive related hormones did not reveal any significant results. However in center-specific analysis, gonadotropin levels and sex-hormone-binding globulin seem to be affected by exposure on p,p'-DDE supporting substantial variations between different populations. The large variety of outcomes and pesticides assessed did not allow for any quantitative synthesis of the data.

#### **10.5.** Reproductive outcomes with few studies

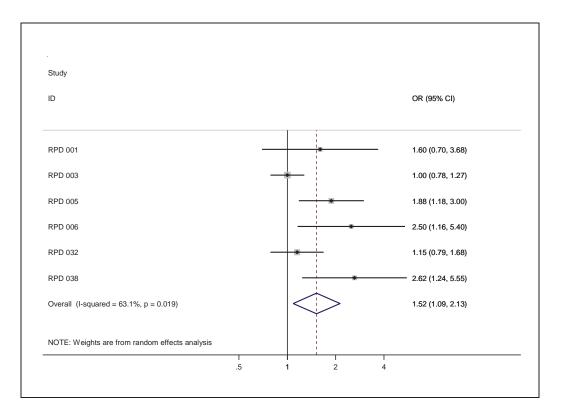
For all the assessed outcomes not included in Table 12, assessment of menstrual cycles cannot allow synthesis of the available evidence. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Results on different menstrual outcomes showed that it is unlikely that exposure to DDE is a main cause of menstrual disturbances.

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**Table 12:**Summary of studies identified per outcome subgroup with more than 4 studies (NA:not available)

Outcome	N studies	Meta-analysis done	Previous meta-analysis result
Impaired sperm parameters	25	No	NA
Fecundability disorders	8	No	NA
Abortion	10	Yes	NA
Reproductive hormones	19	No	NA



**Figure 27:** Random effects meta-analysis for studies with information on pesticide exposure and risk of abortion

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#### 11. Neurological diseases

Overall, 60 publications examined the effect of pesticide exposure on neurological outcomes (median sample size: 390; IQR 246-781), contributing 573 separate analyses in the data extraction database. More than thirty health-related outcomes were assessed with the largest proportion focusing on Parkinson's disease with 32 studies (Table 13). As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 8 out of the 60 were prospective cohort studies and other 2 were nested case-controls; the majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 46 and 143,325 participants (median 390) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we also observed large clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS), while 43 studies assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was far less prominent here (n=7, 12%). The different outcome categories examined are presented in Table 13; due to the small number of studies identified per assessed outcome, statistical synthesis of the data (meta-analysis) was only performed for Parkinson's disease and amyotrophic lateral sclerosis.

# 11.1. Parkinson's disease

Thirty-two studies assessed the association between pesticide exposure and Parkinson's disease with a median sample size of 399 (IQR 286-711), contributing 266 separate extracted comparisons in the database. Eighty percent of the retrieved studies assessed occupational exposures, only 10% were prospective and the exposure was assessed through a biomarker in a small number of studies (10%). A large variety of individual pesticides were assessed with the following pesticides being assessed more frequently: general pesticide (28 studies), as well as DDT (5 studies), paraquat (9 studies).

We initially assessed the association between general pesticide use and Parkinson's disease. The observed effect indicated a statistically significant association with the presence of considerable heterogeneity (random-effects OR 1.58, 95% CI 1.35 - 2.85, I<sup>2</sup> 61%) (Figure 28). With the exception of four studies where specific pesticides were assessed (e.g. paraquat), all the other studies assessed mainly occupational general pesticide use in mainly a retrospective fashion via a questionnaire. The results of the meta-analysis are in accordance with the largest studies on that research question.

We then proceeded to assess the association between DDT exposure and Parkinson's disease. The observed effect indicated a non-statistically significant association without the presence of heterogeneity (random-effects OR=1.01, 95% CI=0.78–1.30,  $I^2=0\%$ ) (Figure 29). Finally, we assessed the association between paraquat exposure and Parkinson's disease. The observed effect indicated a statistically significant association with the presence of moderate heterogeneity (random-effects OR=1.32, 95% CI=1.10–1.60,  $I^2=34\%$ ) (Figure 30). The results of the meta-analysis are in accordance with the largest studies on these research questions.

Our literature search yielded 7 systematic reviews and/or meta-analyses on the association between pesticide exposure and Parkinson's disease published from 2000 to 2013 (Pezzoli 2013, Van-Maele Fabry 2012, van der Mark 2012, Dick 2006, Priyadarshi 2001, Priyadarshi 2000, Allen 2013). Despite the considerable time interval between the oldest and most recent research synthesis effort and the different methodologies endorsed (prospective studies only assessed, methodological assessment of

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the included studies, etc.), the results are consistent across the meta-analyses and are also consistent with the present effort spanning from 2006 (Table 14).

## **11.2.** Amyotrophic lateral sclerosis (ALS)

Seven studies assessed the association between pesticide exposure and amyotrophic lateral sclerosis with a median sample size of 356 (IQR 201-1156), contributing 11 separate extracted comparisons in the database. All the retrieved studies assessed occupational exposures, while 4 also assessed residential exposure. Only one study was prospective and the exposure was assessed through a questionnaire in most of the studies (n=6).

We assessed the association between general pesticide use and ALS. The observed effect indicated a statistically significant association with the presence of small heterogeneity (fixed-effects OR=1.58, 95% CI=1.31 – 1.90, I<sup>2</sup> 10%) (Figure 31) and the results of the meta-analysis are in accordance with the largest studies on that research question.

Our literature search yielded 2 systematic reviews and/or meta-analyses on the association between pesticide exposure and ALS published in 2012 (Kamel 2012, Malek 2012). Regarding these efforts, the results are consistent with our findings and the authors' report of evidence on an association of exposure to pesticides and risk of ALS in male cases compared to controls (OR=1.88, 95% CI: 1.36-2.61), although the chemical or class of pesticide was not specified by the majority of studies.

## **11.3.** Neurological outcomes with few studies

With the exception of Parkinson's disease and amyotrophic lateral sclerosis, for all the remaining neurological outcomes, too few studies are available after 2006 to allow synthesis of evidence for each outcome alone; these outcomes comprise a vast variety of captured information ranging from well-defined clinical entities yet with too few studies, such as hearing loss or diabetic neuropathy, as well as a large number of metrics pertaining to neurological endophenotypes but with a prominent lack of harmonization and standardization in the outcome definition. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

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Health outcome		
Abnormal alternating hand movements	Alzheimer's disease	Narcolepsy with cataplexy
Abnormal ankle reflex	Amyotrophic lateral sclerosis	Neurological symptoms
Abnormal distal motor amplitude	Cryptogenic polyneuropathy	Parkinson's disease
Abnormal distal motor latency	Decline in hand-grip strength	Parkinsonism
abnormal facial expression	Delayed memory impairment	Peripheral neuropathy
abnormal nerve conduction velocity	Dementia	Progressive supranuclear palsy
Abnormal postural tremor	Essential tremor	Restless legs syndrome
Abnormal posture	Gait disorder	Romberg sign
Abnormal short F-wave latency	Hearing loss	Sporadic Motor Neuron Disease
Abnormal toe proprioception	Multiple System Atrophy	Subclinical neuropathy
Abnormal toe vibration perception	Narcolepsy (with and without cataplexy)	Tandem gait abnormality

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ID	Year	Location	Study design	Exposure type	Exposure assessment	Comparison unit	Adjustment	Sample size
DDT								
NRD 027	2007	America	Cohort	Occupational	Direct exposure questionnaire	ever/never	yes	8899
NRD 025	2011	America	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	808
NRD 032	2010	America	Case-control	Occupational	Direct exposure questionnaire	ever vs. never	yes	578
NRD 33	2010	Europe	Nested case- control	Environmental	Biomarker	per IQR increase	yes	292
NRD 019	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	no	184
Paraqua	t							
NRD 019	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	no	184
NRD 027	2007	America	Cohort	Occupational	Direct exposure questionnaire	ever/never	yes	7393
NRD 023	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709
NRD 030	2009	America	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	1030
NRD 037	2011	America	Nested case- control	Occupational	Direct exposure questionnaire	ever/never	yes	468
NRD 020	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709
NRD 022	2010	America	Case-control	Occupational	Direct exposure questionnaire	ever vs. never	yes	578

 Table 14:
 Characteristics of the studies assessing pesticide exposure and Parkinson's disease risk (n/a: not available)

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ID	Year	Location	Study design	Exposure type	Exposure assessment	Comparison unit	Adjustment	Sample size
NRD 038	2010	America	Case-control	Occupational	Occupational history	yes/no	yes	58
NRD 020	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709
Pesticio	des							
NRD 033	2010	Europe	Nested case- control	Environmental	Biomarker (HCB)	per IQR increase	yes	292
NRD 058	2010	Europe	Case-control	Mixed	Direct exposure questionnaire (insecticides)	yes/no	no	330
NRD 034	2010	Asia	Case-control	Occupational	Direct exposure questionnaire	ever/never	n/a	608
NRD 018	2008	Europe	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	233
NRD 017	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	yes	1666
NRD 014	2006	America	Cohort	Occupational	Direct exposure questionnaire	ever/never	yes	143325
NRD 029	2009	Europe	Case-control	Mixed	Direct exposure questionnaire and JEM	ever/never	no	388
NRD 036	2011	Europe	Cohort	Occupational	JEM	JEM class		
NRD 015	2007	Asia	Case-control	Occupational	Direct exposure questionnaire	yes/no	yes	308
NRD 020	2009	America	Case-control	Occupational	Occupational history	yes/no	yes	709
NRD 028	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	yes	615
NRD 023	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709

Pesticide epidemiology

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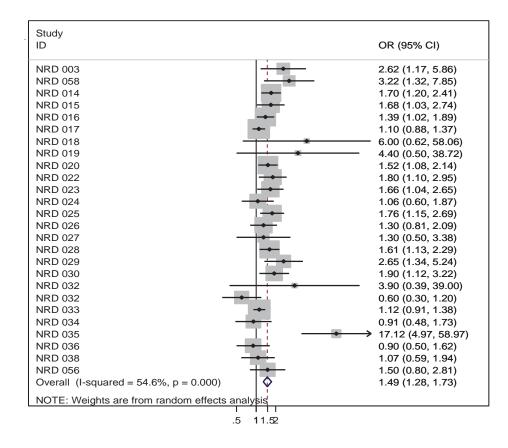
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ID	Year	Location	Study design	Exposure type	Exposure assessment	Comparison unit	Adjustment	Sample size
NRD 016	2007	Europe	Case-control	Mixed	Occupational history and direct exposure questionnaire	high vs. no exposure	yes	2756
NRD 024	2010	Europe	Case-control	Occupational	Occupational history	yes/no	no	387
NRD 025	2011	America	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	808
NRD 058	2006	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	no	278
NRD 027	2007	America	Cohort	Occupational	Direct exposure questionnaire	ever/never	yes	65183
NRD 035	2010	Asia	Case-control	Occupational	Occupational history	yes/no	no	525
NRD 026	2006	America	Case-control	Occupational	Direct exposure questionnaire	yes/no	yes	430
NRD 030	2009	America	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	1030
NRD 022	2009	Europe	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	781
NRD 019	2008	America	Case-control	Occupational	Direct exposure questionnaire	yes/no	no	184
NRD 032	2010	America	Case-control	Occupational	Direct exposure questionnaire	ever vs. never	yes	352
NRD 032	2010	America	Case-control	Occupational	Direct exposure questionnaire	ever vs. never	yes	578
NRD 003	2010	Europe	Case-control	n/a	n/a	yes/no	yes	264

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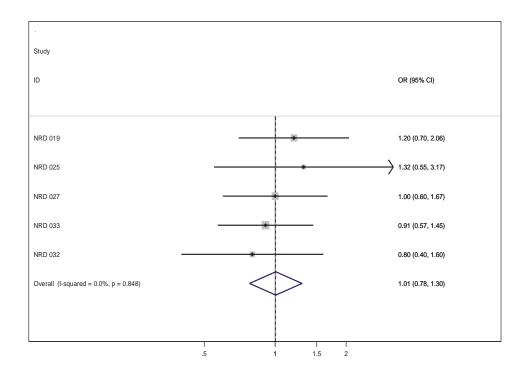
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**Figure 28:** Random effects meta-analysis for studies with information on any pesticide exposure and risk of Parkinson's disease (study with ID NRD 033, specifically assessed hexachlorobenzene)

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**Figure 29:** Fixed-effects meta-analysis for studies with information on exposure and risk of Parkinson's disease

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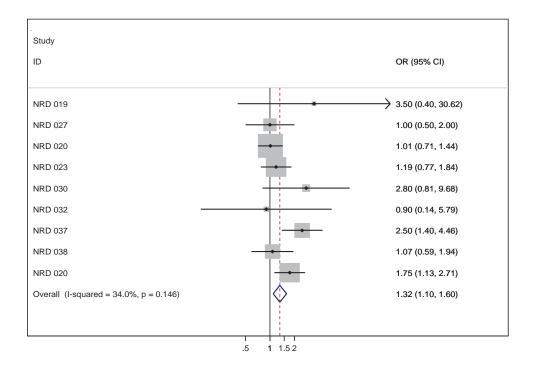
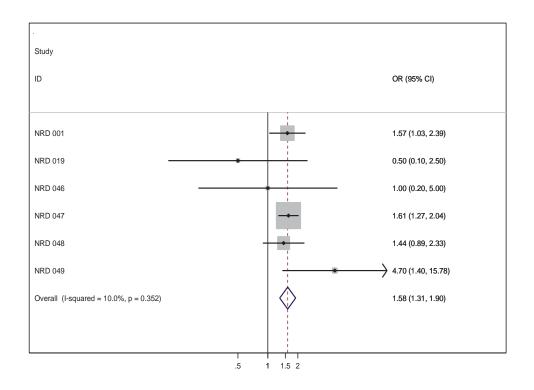


Figure 30: Fixed-effects meta-analysis for studies with information on paraquat exposure and risk of Parkinson's disease

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**Figure 31:** Fixed-effects meta-analysis for studies with information on general pesticide exposure and risk of amyotrophic lateral sclerosis

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#### 12. Endocrine diseases

Overall, 35 publications examined the effect of pesticide exposure on thyroid hormone dysregulation (median sample size: 226; IQR 130-453), contributing 343 separate analyses in the data extraction database. The main outcomes assessed were thyroxin (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) levels. Only 3 prospective cohort studies were conducted in the field; the majority of evidence comes from retrospective case-control or cross-sectional analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 27 and 16,529 participants (median 341). Here, we observed no large clusters of publications coming from large, well-known studies in the field, while the vast majority of the studies assessed environmental exposures (n=28, 80%). However, the presence of studies with information on biomarkers of exposure was more prominent here (n=29, 83%). Even though hypothyroidism, hyperthyroidism and other thyroid diseases contribute with more than 1/3 of the total analyses (n=123) the available evidence derives from Agricultural Health Study (AHS) which apparently is the largest in the field and examines the association between pesticide use and thyroid diseases in females. The study found an association between hypothyroidism and ever use of organochlorine insecticides (OR=1.2, 95% CI= 1.0-16) and fungicides (OR=1.4, 95% CI= 1.1-1.8). However, the results should be interpreted with caution due to borderline significance levels and absence of type-I error corrections due to multiple comparisons. Other studies in the field assessing several thyroid hormone levels are quite smaller and provide contradictory results. As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Due to heterogeneity of data and different analyses, effect sizes and metrics provided, statistical synthesis of the data (meta-analysis) was not performed.

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## **13.** Mental and psychomotor development outcomes

Overall, 32 publications examined the effect of pesticide exposure on mental and psychomotor development outcomes in pediatric populations (median sample size: 238, IQR 109-305), contributing 462 separate analyses in the *data extraction database*. Only one study was performed in a population of non-European (Asian) ancestry, while seventeen health-related outcomes were assessed with a large proportion focusing on attention-deficit hyperactivity disorder (ADHD, 6 studies, 102 analyses). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. A large majority of the studies (23 publications, 72%) referred to prospective cohort studies, while the sample size in the reported analyses was often small; it ranged between 25 and 7,440 participants with the largest study assessing retrospectively maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Here, we also observed clusters of publications coming from large, well-known studies in the field, such as the CHAMACOS (The Center for Health Assessment of Mothers and Children of Salinas) (5 publications), while 84% of the studies assessed environmental exposures. In addition, the presence of studies with information on biomarkers of exposure was prominent here (n=28, 88%). The different outcome categories examined are presented in Table 15 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, no statistical synthesis of the data (meta-analysis) was performed for any outcome.

## 13.1. Mental and psychomotor development outcomes with few studies

With the exception of mental and psychomotor development and Attention-deficit hyperactivity disorder (ADHD), for all the remaining assessed outcomes included in Table 15, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as autism, or pervasive developmental disorder, as well as a vast number of outcomes representing neurodevelopmental endo-phenotypes such as communication, fine and gross motor development or expressive language development. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the small number of studies and sample sizes, these results need cautious interpretation and, based on these outcomes.

# **13.2.** Attention-deficit hyperactivity disorder (ADHD)

Six studies assessed the association between pesticide exposure and ADHD with a sample size ranging from 278 to 2,539 participants, contributing 102 separate extracted comparisons in the database. Three studies were cohorts, all assessed environmental exposure and in all the exposure was assessed through a biomarker. General organophosphate exposure was assessed in three studies, DDT exposure in two studies, while trans-nonachlor, hexachlorobenzene, and 2,4,6-Trichlorophenol (TCP) were assessed in one study each. Thus, no single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. The largest study in the field is a National Health and Nutrition Examination Survey (NHANES) report (ID 17) used data from the 1999-2004 NHANES to evaluate the association between urinary trichlorophenols (TCPs) and parent-reported ADHD among 2546 children aged 6-15 years. The authors report that children with low levels (<3.58 mg/g) and high levels

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(>3.58 mg/g) of urinary 2,4,6-Trichlorophenol (TCP) had a higher risk of parent-reported ADHD compared to children with levels below the limit of detection (OR 1.54, 95% CI 0.97 to 2.43 and OR 1.77, 95% CI 1.18 to 2.66, respectively; p for trend=0.006) after adjusting for covariates.

Our systematic review did not identify any previously published meta-analyses on ADHD to allow for comparisons with previously published evidence (prior to 2006). Generally the results on ADHD were of small effect and not statistically significant with few exceptions. Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and ADHD.

# **13.3.** Neurodevelopment

Thirty-one studies assessed the association between pesticide exposure and aspects of neurodevelopment with a sample size ranging from 25 to 1,041 contributing 325 separate extracted comparisons in the database. Only one study assessed neurodevelopmental aspects in Asian children; all the rest pertained to populations of European ancestry. Seventy-four percent of the studies were cohort studies and, in 27 studies the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with the general category of organophosphate pesticides being assessed more frequently (Table 16). No single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. Actually, the assessment of neurodevelopment, as seen for cognitive function, is another typical example of a general outcome category where the multiplicity and complexity of the 35 tools and sub-tools used (Table 17) renders the attempt to systematically and quantitatively synthesize the results of the published literature fruitless.

The largest study in the field is a Collaborative Perinatal Project report (ID MPD 029) assessing inutero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. The authors report that although levels of DDT and DDE were relatively high in this population (median DDT concentration, 8.9 g/L; DDE, 24.5 g/L), neither were related to Mental or Psychomotor Development scores on the Bayley Scales nor to Full-Scale Intelligence Quotient at 7 years of age.

Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on neurodevelopmental outcomes were of small effect and not statistically significant with few exceptions. Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

#### **Table 15:** Summary of studies and mental and psychomotor development outcomes

Outcome group	N analyses
Attention Deficit Hyperactivity Disorder (ADHD)	102
Autism	2

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Intelligence Quotient (IQ)	13
Learning disability	4
Cognitive disorders	20
Mental and psychomotor development	318
Pervasive developmental disorder	3

 Table 16:
 Pesticides assessed in neurodevelopmental aspects

Pesticide assessed	N analyses
DDT	81
Chlordecone	5
Chlorpyrifos	8
Hexachlorobenzene (HCB)	5
Insecticides	6
Malathion	8
Mirex	13
Organochlorine pesticides	2
Organophosphate and carbamate pesticide	7
Organophosphate pesticides	115
Pesticides	80
Piperonyl butoxide	1

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Acuracy, impulse control Acuracy, impulse control Acuracy, impulse control Ages and Stages Questionnaire Behavioral Assessment and Research System (BARS) Bayley Psychomotor Development Index Scales for Infants Bayley Mental Development Index Scales for Infants Beery-Buktenica VMI developmental test Beenton Visual Retention Test (BVRT) Box test Brazelton neonatal behavioral assessment Brunet-Lezine scale of psychomotor development Children's Memory Scale combining the Picture Completion, Codin Continuous Performance Test (CPT) Digit Span Fagan test of Infant Intelligence (FTII) Finger Tapping Task Gesell Developmental Schedules Graham-Rosenblith test Griffiths Mental Developmental Scale Hit reaction time Large-pellet test Mullen Scales of Fairy Learning: AGS Ed Performance on Continuous Performance Test (CPT) Raven Test Santa Ana Form Board Score in Lincoln-Oseretsky Motor Small-pellet test Stanford-Binet Copying Test Teller visual Acuity Card (TAC) test Trail Making University of California Berkeley Preferential Looking Test Wisconsin Card Sorting Test	Outcome definition / Tool used			
Ages and Stages Questionnaire         Behavioral Assessment and Research System (BARS)         Bayley Psychomotor Development Index Scales for Infants         Bayley Mental Development Index Scales for Infants         Beery-Buktenica VMI developmental test         Benton Visual Retention Test (BVRT)         Box test         Brazelton neonatal behavioral assessment         Brunet-Lezine scale of psychomotor development         Children's Memory Scale         combining the Picture         Completion, Codin         Continuous Performance Test (CPT)         Digit Span         Fagan test of infant intelligence (FTII)         Finger Tapping Task         Gesell Developmental Schedules         Griffiths Mental Developmental Scale         Hit reaction time         Large-pellet test         Mullen Scales of Fairly Learning: AGS Ed         Performance on Continuous Performance Test (CPT)         Raven Test         Santa Ana Form Board         Score in Lincoln-Oseretsky Motor         Small-pellet test         Stanford-Binet Copying Test         Teller visual Acuity Card (TAC) test         Trail Making         University of California Berkeley Preferential Looking Test         Vechsler Intelligence Scale for children </td <td></td>				
Behavioral Assessment and Research System (BARS)         Bayley Psychomotor Development Index Scales for Infants         Bayley Mental Development Index Scales for Infants         Beery-Buktenica VMI developmental test         Benton Visual Retention Test (BVRT)         Box test         Brazelton neonatal behavioral assessment         Brunet-Lezine scale of psychomotor development         Children's Memory Scale         combining the Picture         Completion, Codin         Continuous Performance Test (CPT)         Digit Span         Fagan test of infant intelligence (FTII)         Finger Tapping Task         Gesell Developmental Schedules         Graham-Rosenblith test         Griffiths Mental Developmental Scale         Hit reaction time         Large-pellet test         Mullen Scales of Children's Abilities         Mullen Scales of Early Learning: AGS Ed         Performance on Continuous Performance Test (CPT)         Raven Test         Santa Ana Form Board         Score in Lincoln-Oseretsky Motor         Small-pellet test         Stanford-Binet Copying Test         Teller visual Acuity Card (TAC) test         Trail Making         University of California Berkeley Preferential Looking Test <tr< td=""><td></td></tr<>				
Bayley Psychomotor Development Index Scales for Infants         Bayley Mental Development Index Scales for Infants         Beery-Buktenica VMI developmental test         Benton Visual Retention Test (BVRT)         Box test         Brazelton neonatal behavioral assessment         Brunet-Lezine scale of psychomotor development         Children's Memory Scale         combining the Picture         Completion, Codin         Continuous Performance Test (CPT)         Digit Span         Fagan test of infant intelligence (FTII)         Finger Tapping Task         Gesell Developmental Schedules         Graham-Rosenblith test         Griffiths Mental Developmental Scale         Hit reaction time         Large-pellet test         Mullen Scales of Children's Abilities         Mullen Scales of Early Learning: AGS Ed         Performance on Continuous Performance Test (CPT)         Raven Test         Santa Ana Form Board         Score in Lincoln-Oseretsky Motor         Small-pellet test         Stanford-Binet Copying Test         Teller visual Acuity Card (TAC) test         Trail Making         University of California Berkeley Preferential Looking Test				
Bayley Mental Development Index Scales for Infants         Beery-Buktenica VMI developmental test         Benton Visual Retention Test (BVRT)         Box test         Brazelton neonatal behavioral assessment         Brunet-Lezine scale of psychomotor development         Children's Memory Scale         combining the Picture         Completion, Codin         Continuous Performance Test (CPT)         Digit Span         Fagan test of infant intelligence (FTII)         Finger Tapping Task         Gesell Developmental Schedules         Graham-Rosenblith test         Griffiths Mental Developmental Scale         Hit reaction time         Large-pellet test         Mullen Scales of Children's Abilities         Mullen Scales of Early Learning: AGS Ed         Performance on Continuous Performance Test (CPT)         Raven Test         Santa Ana Form Board         Score in Lincoln-Oseretsky Motor         Small-pellet test         Stanford-Binet Copying Test         Teller visual Acuity Card (TAC) test         Trail Making         University of California Berkeley Preferential Looking Test         Wechsler Intelligence Scale for children				
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University of California Berkeley Preferential Looking Test Wechsler Intelligence Scale for children	Teller visual Acuity Card (TAC) test			
Wechsler Intelligence Scale for children	Trail Making			
Wechsler Intelligence Scale for children	University of California Berkeley Preferential Looking Test			
Wisconsin Card Sorting Test				

 Table 17:
 Outcome definitions and tools used in the 31 studies assessing neurodevelopment

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## 14. Respiratory diseases

Overall, 29 publications examined the effect of pesticide exposure on respiratory outcomes (median sample size: 249, IQR 126-1728), contributing 399 separate analyses in the *data extraction database*. Sixty-seven percent came from Europe and America, while ten health-related outcomes were assessed with a large proportion focusing on asthma (N=9). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. Only 6 out of the 29 publications referred to prospective cohort studies and 12 were cross-sectional studies. The sample size in the reported analyses was often small; it ranged between 35 and 47,756 participants with the largest study being the Singapore Chinese Health Study. Here, we also observed large clusters of publications coming from large, well-known studies in the field, such as the AHS (6 publications), while 17 studies (68%) assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was less prominent here (N=8, 34%) while 1 study assessed occupational exposure through JEM. The different outcome categories examined are presented in Table 18 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for asthma.

# 14.1. **Respiratory outcomes with few studies**

With the exception of asthma, for all the remaining assessed outcomes included in Table 18, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as idiopathic pulmonary fibrosis, or sarcoidosis, as well as a numbers of biomarkers such as forced expiratory volume (FEV). Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the fact that most of the results come from the Agricultural Health Study (AHS), these results need cautious interpretation and, based on these data, there is no evidence to suggest a robust association between pesticide exposure and these outcomes.

# 14.2. Asthma

Nine studies assessed the association between pesticide exposure and asthma with a median sample size of 402 (IQR 127-724), contributing 196 separate extracted comparisons in the database. More than half of the studies were cross-sectional and in more than two-thirds of the studies, the exposure was assessed through a questionnaire. A large variety of individual pesticides were assessed with DDT, paraquat and chlorpyrifos being assessed more frequently. With the exception of DDT, chlorpyrifos and paraquat (Table 19), no other single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed.

When we attempted to investigate the association between exposure to DDT and asthma across the 5 available studies, the observed effect was statistically significant without indications of heterogeneity (OR 1.29, 95% CI 1.14 – 1.45,  $I^2$  0%) (Figure 32). We then attempted to investigate the association between exposure to paraquat and asthma across the 6 available studies and the observed effect was not statistically significant with indications of heterogeneity (OR=1.40, 95%CI=0.95–2.06,  $I^2$ =53%) (Figure 33). We finally attempted to investigate the association between exposure to chlorpyrifos and

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asthma across the 5 available studies and the observed effect was not statistically significant without indications of heterogeneity (OR= 1.03, 95% CI= 0.82-1.28, I<sup>2</sup>=0%) (Figure 34). We caution that the meta-analyses results are largely driven by the AHS; in the meta-analyses 4 entries belong to the AHS as the results were separately reported for men and women and for allergic and non-allergic asthma. We also acknowledge that the results of the meta-analyses are restricted to data published after 2006. We thus conclude that for DDT, but not for chlorpyrifos and paraquat, there is recent evidence to suggest a statistically significant, moderate association between exposure to this pesticides and asthma.

Table 18:	Summary	of str	tudies an	nd	outcomes	in	the	field	of	respiratory	medicine	(N/A:	not
available)													

Outcome Group	N studies	Meta-analysis performed	Previous published meta-analysis
Cough	2	No	N/A
Breathlessness	1	No	N/A
Cough/Phlegm	2	No	N/A
Volume that has been exhaled at the end of the first second of forced expiration (FEV <sub>1)</sub>	1	No	N/A
FEV <sub>1</sub> / Forced vital capacity (FVC)	2	No	N/A
Asthma	9	Yes	N/A
Chronic bronchitis	5	No	N/A
Hypersensitivity pneumonitis	2	No	N/A
Lower respiratory tract infection	2	No	N/A
Sarcoidosis	1	No	N/A
Wheeze	2	No	N/A

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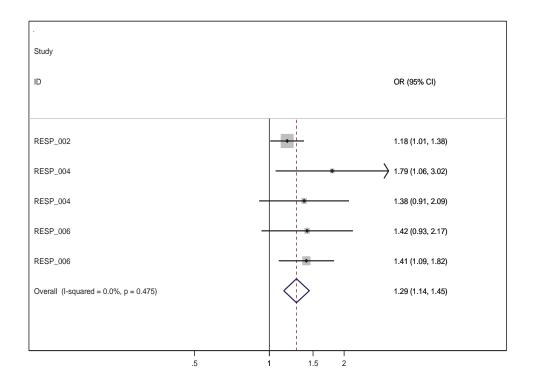
Pesticide epidemiology

ID	Year	Location	Study design	Exposure type	Exposure assessment	Comparison	Adjustment	Sample size
DDT								
RESP_002	2006	Europe	Cohort	Environmental	Biomarker	Yes/no	+++	402
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	936
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	946
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	4391
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	4468
Paraquat								
RESP_019	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	134
RESP_022	2012	Asia	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	125
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	292
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	294
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	3096
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	3108
Chlorpyrifo	S							
RESP_019	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	134
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	1017
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	1019
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	2174
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++	2199

# **Table 19:** Characteristics of the associations eligible for meta-analysis

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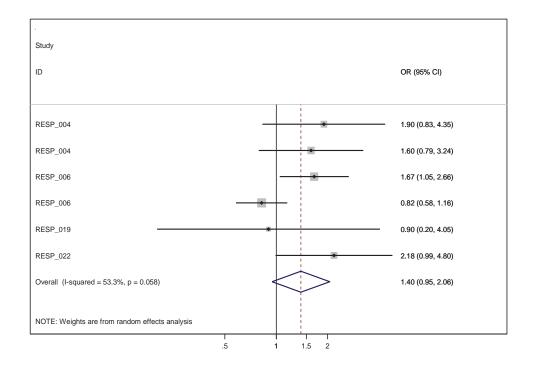
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**Figure 32**: Fixed-effects meta-analysis for studies with information on DDT exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

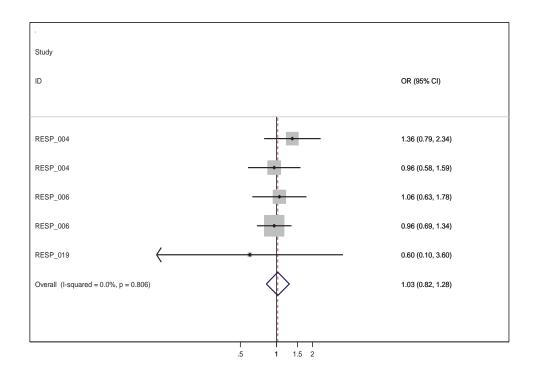
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**Figure 33**: Random-effects meta-analysis for studies with information on paraquat exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

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**Figure 34**: Fixed-effects meta-analysis for studies with information on chlorpyrifos exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

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### 15. Neuropsychiatric diseases

Overall, 15 publications examined the effect of pesticide exposure on neuropsychiatric outcomes in adult populations (median sample size: 596, IQR 158-12,263), contributing 358 separate analyses in the data extraction database. Three-quarters came from Europe and America, while 17 health-related outcomes were assessed with a large proportion focusing on cognitive function (9 studies, 246 analyses). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. Only 2 out of the 15 publications referred to prospective cohort studies and 60% of the publications were cross-sectional studies. The sample size in the reported analyses was often small; it ranged between 66 and 112,683 participants with the largest study being a retrospective American study. Here, we also observed clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS) (4 publications), while all but one study assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was far less prominent here (n=2, 13%). The different outcome categories examined are presented in Table 20, along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, no statistical synthesis of the data (meta-analysis) was performed for any outcome.

# **15.1.** Cognitive function

Nine studies assessed the association between pesticide exposure and cognitive function with a median sample size of 80 (IQR 141-205), contributing 246 separate extracted comparisons in the database. All but one of the studies were cross-sectional and, in seven studies the exposure was assessed through a questionnaire. A large variety of individual pesticides were assessed with the general category of organophosphate pesticides being assessed more frequently. No single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. Actually, the assessment of cognitive function is a typical example of a general outcome category where the multiplicity and complexity of the 62 tools and sub-tools used in the 15 available studies (Table 21) renders the attempt to systematically and quantitatively synthesize the results of the published literature fruitless.

The largest study in the field is an AHS report (ID NPD 014) assessing potential associations between long-term pesticide use and neurobehavioral function, with relevant tests administered to licensed pesticide applicators. The authors report that "test performance was associated with lifetime days of use of some pesticides". Ethoprop was significantly associated with reduced performance on a test of motor speed and visual scanning. Malathion was significantly associated with poor performance on a test of visual scanning and processing. Conversely, we observed significantly better test performance for five organophosphate pesticides. Specifically, chlorpyrifos, coumaphos, parathion, phorate, and tetrachlorvinphos were associated with better verbal learning and memory; coumaphos was associated with better performance on a test of motor speed and visual scanning; and parathion was associated with better performance on a test of sustained attention. Overall, we found no consistent evidence of an association between organophosphate pesticide use and adverse test performance among this older sample of pesticide applicators. Potential reasons for these mostly null results include a true absence of effect as well as possible selective participation by healthier applicators.

Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on neuropsychiatric outcomes were of small effect and not statistically significant with few exceptions.

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Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

### **15.2.** Neuropsychiatric outcomes with few studies

With the exception of cognitive function, for all the remaining assessed outcomes included in Table 20, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as depression, or obsessive-compulsive disorder, as well as a numbers of outcomes representing neuropsychiatric endo-phenotypes such as hostility or orientation disorders. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the fact that a number of the results come from the AHS, these results need cautious interpretation and, based on these data, there is no evidence to suggest a robust association between pesticide exposure and these outcomes.

Outcome group	N studies
Anxiety	3
Attention and calculation disorders	1
Cognitive function	9
Depression	4
Electroencephalographic (EEG) state	1
Hostility	1
Interpersonal sensitivity diosrder	1
Learning disability	1
Nausea	1
Neuropsychiatric symptoms	3
Obsessive-compulsive disorder	1
Orientation disorders	1
Paranoid ideation	1
Psychotisism	1
Rapid Eye Movement (REM) Sleep Behavior Disorders (RBD)	1
Somatization	1
Suicide commitment	3

 Table 20:
 Summary of studies and neuropsychiatric outcomes

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**Table 21**:Outcome definitions and tools used in the 15 studies assessing cognitive function(BARS: Behavioral Assessment and Research System, AVLT:Auditory Verbal Learning Test, BVFT:Benton Visual Form Discrimination Test CALCALP: California Computerised Assessment PackageManual, WAIS: Wechsler Adult Intelligence Scale, WMS: Wechsler Memory Scale)

Outcome definition / Tool used	
% Correct rejects (BARS)	Selective attention latency (BARS)
% Hits (BARS)	Selective attention trials (BARS)
Recall (AVLT)	Sequences A test performance (seconds)
Recognition (AVLT)	Sequences B test performance (seconds)
Total recall (AVLT)	Serial digit learning task (BARS)
Benton Visual Form Discrimination Test (BVFT)	Serial Digit Learning Test
Block design test	Simple Reaction Time Test (ms)
CALCAP choice test	Spatial span test
Continuous Performance Test Score (m/s)	Stroop test
Counting errors	Summary index (BARS)
Digit span backward task (BARS)	Symbol Digit Substitution Test (s)
Digit span forward task (BARS)	Symbol-digit latency task (BARS)
Digit-Symbol test score (seconds)	Symptom Checklist 90 revised (SCL-90-R)
False alarm latency (BARS)	Trails B test
Fine motor control test	Verbal fluency test
Finger tapping (preferred hand) (BARS)	WAIS-III picture arrangement test
Finger tapping , dominant hand (BARS)	WAIS-III arithmetic test
Finger tapping, (nonpreferred hand) (BARS)	WAIS-III comprehension test
Finger tapping, alternating hand (BARS)	WAIS-III digit span test
Graded naming test	WAIS-III digit symbol test
Grooved pegboard, dominant hand score	WAIS-III full scale IQ
Hit latency (BARS)	WAIS-III graded-naming test
Match-Sample (BARS)	WAIS-III similarities test
N100 latency (ms)	WAIS-III vocabulary test
N200 latency (ms)	WMS-III auditory delayed memory test
P200 latency (ms)	WMS-III auditory immediate memory test
P300 amplitude (μν), Cz	WMS-III auditory recognition test
P300 latency (ms)	WMS-III letter-number test
Progressive ratio (BARS)	WMS-III visual delayed memory test
Reaction time latency a (BARS)	WMS-III visual immediate test
Reaction time latency a (BARS)	Selective attention interstimulus interval (BARS)

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#### 16. Diabetes

Overall, 23 publications examined the effect of pesticide exposure on diabetes related outcomes (median sample size: 430; IQR 192-1721), contributing 125 separate analyses in the *data extraction* database. Four health-related outcomes were assessed with a large proportion focusing on type 1 diabetes (n=93, 74%) whereas 18 analyses focused on type 2 diabetes. The rest of the outcomes assessed was prediabetes (n=10), gestational diabetes (n=2) and other glucose and insulin related outcomes (n=2). Only one prospective cohort study was performed; the large majority was crosssectional designs (n=15), whereas 3 studies were case-controls and 4 studies used a nested casecontrols. The large majority of the studies was conducted in America (n=15, 65%) whereas 7 studies where Europeans and only one Asian. Here, we did not observe large clusters of publications coming from large, well-known studies in the field, such as the AHS. Only three study assessed occupational exposures the rest examined environmental exposures (n=19) or both (n=1). In addition, the presence of studies with information on biomarkers of exposure was limited to 9 studies, whereas 10 studies included information both on questionnaire and biomarkers. The different outcome categories examined are presented in Table 22 along with the number of studies contributing to each outcome category. For the pesticides accessed meta-analysis was feasible for DDE and DDT exposure and type 1 diabetes and DDE exposure and type 2 diabetes.

### 16.1. Type 1 diabetes

Thirtheen studies assessed the effect of pesticides on type 1 diabetes (median sample size: 309, IQR: 159-398) and a meta-analysis of ORs was feasible for DDE and DDT exposure. For DDE, 9 studies contributed a median sample size of 202, IQR=142-334. We were not able to include a prospective study that reported a (significant) Incidence Rate Ratio (IRR) of 7.1 and compared the highest vs. the lowest tertile of exposure with DDE. The computed summary OR was 1.90 (95% CI: 1.25-2.86) for the DDE exposure using random effects models. Moderate heterogeneity was observed (I<sup>2</sup>=49%). For DDT, 6 studies had available data for synthesis (median sample size: 577, IQR: 272-2163) providing a summary effect of 1.76 (95% CI: 1.20-2.59) with very large heterogeneity observed ((I<sup>2</sup>=76%)). Main source of heterogeneity is the different exposure levels used for the calculations of the effect estimates. Even though there is evidence from the random effects meta-analysis that an increased risk for type 1 diabetes exists, however the findings should be interpreted with caution due to the heterogeneity that was observed.

### 16.2. Type 2 diabetes

Four studies were eligible for the assessment of the DDE exposure and risk for type 2 diabetes (median sample size: 471, IQR=292-642). The summary OR derived from those studies was 1.30 (95% CI: 1.13-1.48). No heterogeneity was observed, however the summary results is driven by a case-control study that reported an effect size OR=1.30 (95% CI=1.11-1.52). Even though, there is evidence suggesting that DDE exposure is a risk factor for developing type 2 diabetes, this is based on small studies.

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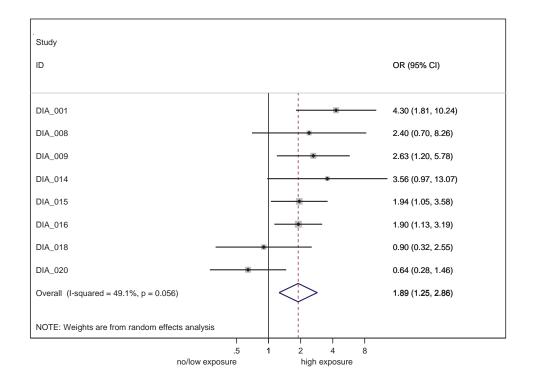
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 Table 22:
 Summary of studies identified per outcome subgroup with more than 4 studies (NA: not available)

Outcome	N studies	Meta-analysis done	Previous meta- analysis result
Type 1 diabetes	13	Yes	NA
Type 2 diabetes	6	Yes	NA
Gestational diabetes	2	No	NA
Insulin/ Glucose tolerance	2	No	NA

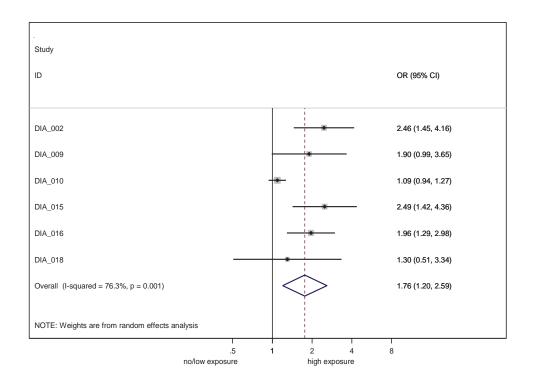
<sup>87</sup> 

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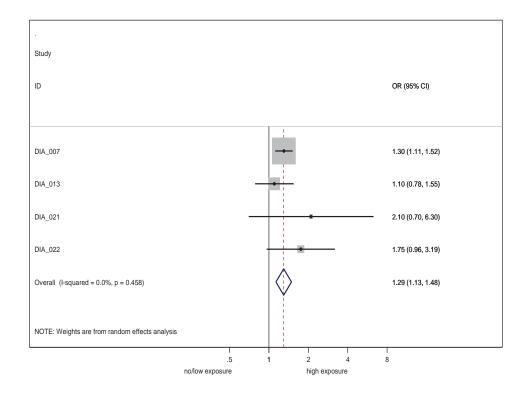
**Figure 35**: Summary odds ratio (OR) for the association between DDE exposure and type 1 diabetes

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**Figure 36**: Summary odds ratio (OR) for the association between DDT exposure and type 1 diabetes

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**Figure 37**: Summary odds ratio (OR) for the association between DDE exposure and type 2 diabetes

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### 17. Cardiovascular diseases

This section includes hard cardiovascular outcomes (myocardial infraction, stroke etc.), cardiovascular risk factors (lipids, blood pressure) and other cardiometabolic outcomes (metabolic syndrome and obesity). No previous meta-analysis has been identified for any of these traits. The evidence collected in this systematic review provides weak suggestions of associations in particular regarding cardiometabolic risk factors and organochlorines; however, other classes of pesticides were not studied and even results on organochlorines were limited and require prospective replication.

## 17.1. Hard cardiovascular outcomes

Five studies examined hard cardiovascular outcomes including myocardial infarction (ID CVD 005, ID CVD 006), peripheral arterial disease (PAD) (ID CVD 007), stroke (ID CVD 008), and composite cardiovascular disease (ID CVD 009). The Agricultural Health Study (AHS) contributed two prospective analyses (ID CVD 005, ID CVD 006) and National Health and Nutrition Examination Survey (NHANES) other two cross-sectional analyses (ID CVD 007, ID CVD 009). Studies on myocardial infarction (ID CVD 005, ID CVD 006) showed no evidence of an association between having used pesticides, individually or by class, and myocardial infarction mortality among men in the AHS. Similarly, among women of AHS, no overall association with pesticide use and myocardial infarction was seen. Six of 27 individual pesticides evaluated were significantly associated with nonfatal myocardial infarction among women (ID CVD 006), including chlorpyrifos, coumaphos, carbofuran, metalaxyl, pendimethalin, and trifluralin, which all had relatively high odds ratios (>1.7) but also high probability of false positive due to multiple testing.

Another prospective study (8) examined 21 persistent organic pollutants (POPs) in relation to stroke. After adjusting for known stroke risk factors, most polychlorinated biphenyls (PCBs) with 4, 5, or 6 chlorine atoms, p,p'-DDE, trans-nonachlor, and octachlorodibenzo-p-dioxin significantly predicted the risk of stroke. Nonetheless, results need replication from future studies. Peripheral arterial disease (PAD) and composite cardiovascular disease were studied in the cross-sectional NHANES cohort in relation to POPs. Compared with subjects without PAD, those with PAD had significantly higher concentrations of organochlorine pesticides but associations were not seen among non-obese participants. For composite cardiovascular disease, significant associations were observed for chlordane only. These findings need to be carefully interpreted because of the cross-sectional design and use of self-reported cardiovascular disease.

Overall, evidence for associations between pesticide exposure and cardiovascular outcomes is weak and mainly concentrated on organochlorine pesticides.

# 17.2. Cardiovascular risk factors

# 17.2.1. Blood pressure

Five studies examined associations between pesticides and blood pressure (ID CVD 002, ID CVD 003, ID CVD 004, ID CVD 010, ID CVD 011). All but one study (ID CVD 011) had cross-sectional

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designs. All effect sizes were very small and not suggestive of an association between pesticide exposure and blood pressure.

# 17.2.2. Metabolic syndrome components

Nine studies examined components of metabolic syndrome in relation to pesticide exposure including lipids levels, glucose and insulin levels. All but one study examined exposure to organochlorine pesticides and significant associations for some classes and lipid levels or glucose levels were observed. Highest quality evidence comes from the prospective Coronary Artery Risk Development in Young Adults (CARDIA) Study (ID CVD 016). In CARDIA, p,p'-DDE most consistently predicted higher triglycerides, and homeostasis model assessment value for insulin resistance (HOMA–IR) and lower High Density Lipoprotein (HDL)-cholesterol at year 20 after adjusting for various confounders. Oxychlordane, trans-nonachlor, and hexachlorobenzene also significantly predicted higher triglycerides. Finally, a case-control study in China, examined differences in glucose regulation in participants highly exposed to pyrethroids (occupational exposure). An indication of increased risk for abnormal glucose regulation was noted for exposure to pyrethroids (OR = 1.48, 95%CI = 1.24–1.77) (ID CVD 021). However, these results need external replication in other populations as the study is retrospective and residual confounding cannot be excluded.

# 17.2.3. Subclinical atherosclerosis

The population-based Prospective Investigation of the Vasculature in Uppsala Seniors examined in a cross-sectional study, whether POP levels were related to subclinical atherosclerosis. Circulating levels of PCBs were associated with atherosclerotic plaques and echogenicity of the intima-media complex independent of cardiovascular risk factors, but associations need to be confirmed in prospective studies.

# 17.3. Metabolic syndrome and obesity

Three studies (ID CVD 010, ID CVD 011) examined associations between organochlorine exposure and prevalence of metabolic syndrome. In National Health and Nutrition Examination Survey (NHANES) (ID CVD 010) significant association between organochlorine exposure and prevalence of Metabolic Syndrome was reported with ORs of 1.0, 1.5, 2.3 and 5.3 across organochlorine pesticide quartiles (p for trend <0.01). In the other case-control study (ID CVD 011) significant associations were noted for heptachlor only.

Overall, 12 cross-sectional studies examined associations between pesticide exposure and measures of body fatness or obesity. Also, 10 out of 12 studies examined associations between organochlorines and obesity or body fatness; evidence around other pesticide classes was scarce. Three studies (ID CVD 012, ID CVD 013, ID CVD 014) only presented correlation analysis with measures of body fatness. The remaining studies have shown some significant associations between waist circumference, Body Mass Index (BMI) and organochlorines (DDT and chlordane) but the evidence is limited to cross-sectional analysis and results are only suggestive of an association.

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# 18. Mortality

Overall, 11 publications examined the effect of pesticide exposure on mortality (median sample size: 1,986), contributing 318 separate analyses in the *data extraction database*. This section consists of a heterogeneous group of publications, which assessed associations between pesticides and all cause mortality of major mortality outcomes. Despite the fact that these studies were large, they were of modest quality and they are not very informative as they test a wide range of diseases simultaneously without corrections for multiple testing. The results do not show any apparent trend of pesticide exposure with overall mortality.

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### **19.** Immune/ Autoimmune diseases

Overall, 10 publications examined the effect of pesticide exposure on immune disorders (median sample size: 196, IQR 81-476), contributing 67 separate analyses in the *data extraction database*. Sixty studies were conducted in America, 3 in Europe and one study was Asian. Various health related outcomes including arthritis, osteoarthritis, rheumatoid arthritis and an extensive list of various antibodies, cytokines etc. as summarized in Table 23. Seven out of the 10 publications referred to prospective cohort studies whereas 2 studies were cross-sectional and only one was case-control. The sample size in the reported analyses was rather small; it ranged between 19 and 532 participants with the largest study being the Carolina Lupus Study. Half of the studies assess occupational exposures and information on biomarkers of exposure was available in 2 studies whereas 4 studies used both biomarkers and questionnaires. As seen with other outcomes, the diversity of the exposure definition and the outcomes assessed are extensive and poses special challenges to data synthesis. No single outcome was assessed in more than two studies therefore synthesis of the data was not feasible for the field of immune disorders.

Health outcome	
Antinuclear antibodies	Interleukin-4 (IL-4)
Arthritis	Interleukin-13 (IL-13)
Complement components C3, C4	Immunologic effects
Eosinophils	Leucocyte counts
Erythrocyte counts	Lymphocyte levels
Glycoproteins	Neutrophils
Hematocrit/Hemoglobin	Natural Killers (NK) cells
Interferon-γ (IFN-γ)	Osteoarthritis
Immunoglobulin 1 (lgG1)	Rheumatoid arthritis
Immunoglobulin 4 (IgG4)	Systematic Lupus
Immunoglobulin M (IgM)	

 Table 23:
 Health outcomes assessed in the field of immune disorders

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#### 20. Allergic diseases

Nine studies from eight different populations reported associations between pesticide exposure and allergic disorders. Seven studies examined occupational exposure whereas two studies examined environmental exposure. Eight studies were cross-sectional investigations and therefore conclusions are prone to reverse causality and other biases. In terms of outcomes examined, five studies examined self-reported allergic rhinitis, one examined self-reported asthma and the remaining 3 examined selfreported skin irritation, contact dermatitis, food allergy, hay fever and fragrance allergies. Statistically significant results were reported by four studies on allergic rhinitis (ID ALL 003, ID ALL 004, ID ALL 005, ID ALL 006). These studies reported significant association between various pesticide classes and allergic rhinitis. In particular, the Agricultural Health Study (AHS) reported significant association between allergic rhinitis and exposure to the herbicides 2,4-Dichlorophenoxyacetic acid (2,4-D) glyphosate and petroleum oil, the insecticide diazinon and the fungicide benomyl. However, the study has many limitations and results need cautious interpretation and require replication by future prospective studies. The study is limited by its ability to distinguish allergic from non-allergic symptoms of rhinitis and to establish temporality between exposure and symptoms due to its crosssectional design. One study with low overall quality reported high effect sizes (OR, 12.50; 95% CI, 2.00-78.05) for allergic rhinitis in greenhouse flower and ornamental plant growers with pesticide application by hand pump vs. without (ID ALL 006). Again, the study has low overall quality, concerns a heavily exposed population with definition of exposure related to the method of application rather than a chemical class. Overall, the evidence around allergic disorders and pesticide exposure is weak.

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### 21. Haematological diseases

## 21.1. Aplastic anaemia

Three studies examined associations between pesticide exposure and aplastic anaemia; a rare hematologic condition. All studies were case-control designs and had small sample sizes (range 9-310). Two studies reported significant associations with large effect sizes but it is difficult to draw firm conclusions due to the small number of studies available and the limitations of these studies (Table 24). The other case control study (ID APL\_002) did not report effect sizes but only the p value of association, which was non-significant. Further evidence is required to throw light into these suggestive results.

 Table 24:
 Summary of results between pesticide exposure and aplastic anemia in 2 case-control studies that reported effect sizes

Study ID	Pesticide assessed	Comparison	OR	Lower 95% Cl	Higher 95% Cl	N cases	N controls
APL_001	Organophosphates	yes/no	2.1	1.1	4.2	21	32
APL_001	DDT	yes/no	6.7	1.5	30	5	4
APL_001	Carbamates	yes/no	7.4	1.7	31	8	3
APL_001	Paraquat	yes/no	2.3	1	5.1	12	24
APL_001	Other occupational pesticides	yes/no	1	0.4	2.2	11	32
APL_001	Any household pesticides	yes/no	1.3	0.9	1.9	64	238
APL_001	Organophosphates	yes/no	2.1	1	4.4	17	26
APL_001	Paraquat	yes/no	1.9	0.7	4.9	7	20
APL_001	Other occupational pesticides	yes/no	1.1	0.4	2.7	9	24
APL_003	Agricultural use of pesticides	yes/no	2.2	1.1	4.7	12	23
APL_003	Home use of pesticides	yes/no	1.3	0.9	1.9	70	240
APL_003	Organophosphorates	highest tertile of exposure/no exposure	3	0.9	10.1	5	7
APL_003	Pyrenthroids	highest tertile of exposure/no exposure	1.8	1	3.1	23	57
APL_003	Herbicides	yes/no	2.4	0.9	6	8	15

# 21.2. Haematological and biochemical alterations

Fourteen studies examined various haematological and biochemical alterations in relation to pesticide exposure. Main alterations studied were basic haematology and vitamin levels. The sample size ranged between 51 and 1,275. The quality of these studies was modest to low. Most studies reported unadjusted correlation statistics or means between haematological parameters and pesticide exposure and no effect sizes beyond the p values were reported. All studies provided cross-sectional evidence. Despite the fact than many of the reported analyses were statistically significant, results should not be interpreted at this stage due the limited evidence and modest quality associated with these data.

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### 22. Other outcomes

Overall, 30 publications examined the effect of pesticide exposure on other outcomes. Based on our criteria for data synthesis no meta-analysis was performed for those outcomes.

## 22.1. Bone diseases

Three studies examined the effect of pesticide exposure on osteoporosis including 13 different analyses. We identified two European cross-sectional studies and one Asian cohort (median sample size: 176, IQR: 153-908). All studies assess environmental exposure with information on biomarkers of exposure and all studies examined exposure to organochlorines only. Osteoporosis was assessed via ultrasound measurements and bone mineral density. The largest study of 908 women showed that p,p'-DDE was positively associated with bone mineral density, the association remained after adjustment for confounders, but the effect was weak.

## 22.2. Skin diseases

Six studies examined the effect of pesticide exposure on skin lesion (median sample size: 356, IQR 262-2203) including 11 analyses. Four studies used cross-sectional design. Environmental exposure was assessed in 3 studies. The definition of outcome was often skin rash or eczema. The resulst were largely not statistical significant. One prospective study (ID SKD 004) on 5,042 men from the Health Effects of Arsenic Longitudinal Study in Araihazar reported highly significant effect sizes for skin lesions and pesticide use but study also evaluated arsenic exposure and it is difficult to differentiate between the effect of each exposure.

### 22.3. Dental diseases

One study cross-sectional study from America including 496 participants assessed two outcomes. The study assessed environmental exposure with information of biomarkers (ID PER 001). In this study, organochlorine (OC) pesticides were strongly associated with periodontal disease.

### 22.4. Metabolic diseases

One European cross-sectional study assessed the effect of pesticides on metabolic diseases and specifically on levels of various prorfyrins including 8 analyses but no significant results were reported. Environmental exposure was studied using biomarkers for the assessment of exposure.

### 22.5. Men health

One case-control study reported association between pesticide exposure and erectile dysfunction. The study focused on organochlorine pesticides and compared 101 cases with erectile dysfunction to 234 comparable control subjects. The results were no statistically significant and do not provide evidence of an association.

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### 22.6. Gynaecological diseases

In this group we included gynaecological outcomes not included in the previous outcome categories. Four studies are included in this group, three examined endometriosis and one the timing of menopause. The three studies on endometriosis (ID GYN 001, ID GYN 002, ID GYN 003) were all cross-sectional and all examined organochlorines. One out of 12 separate analyses on endometriosis and organochlorines was statistically significant; the highest tertile of aromatic fungicide was associated with a fivefold risk of endometriosis (OR = 5.3; 95% CI, 1.2-23.6) compared to the lowest tertile. This effect size is large and requires independent replication in other prospective studies.

Data from the Agricultural Health Study (AHS) was used to study associations between exposure to pesticides and age at menopause in a prospective investigation of pre-menopausal women. After control for age, smoking status, and past use of oral contraceptives, the median time to menopause increased by approximately 3 months for women who used pesticides (HR 0.87, 95% CI: 0.78, 0.97) and by approximately 5 months for women who used hormonally active pesticides (HR 0.77, 95% CI: 0.65, 0.92). Pesticide use may be associated with a later age at menopause based on these results; however results are prone to false positive bias and independent replication is needed.

# 22.7. Symptoms and general health

Five studies examined general health symptoms such as nausea, fatigue, dizziness, and shortness of breath. The definition of these outcomes is very hard and associated with large measurement errors. Studies were of modest to low quality and all concerned occupational exposures. Some statistically significant results were observed but are far form conclusive at this stage due to heterogeneity of data reported and the limitations associated with these studies.

# 22.8. Kidney diseases

Three studies examined kidney diseases including chronic kidney disease and gallstone disease. One study reported statistically significant results between DDE and DDT residues and gallstone disease.

# 22.9. Benign tumours

One a population-based case-control study on acoustic neuroma found no link between pesticide exposure and acoustic neuroma.

# 22.10. Gastrointestinal diseases

Seven studies examining associations between pesticide exposure and liver enzymes were identified. All studies were cross-sectional or case-control. One study, the National Health and Nutrition Examination Survey (NHANES), examined organochlorines, another one examined exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and paraquat and the remaining studies examined broadly defined pesticide categories. The studies were of modest and low quality and presented only the means of enzymes in exposed and unexposed participants often without adjustments. Almost all studies reported statistically significant results with higher level of liver enzymes (e.g. Gamma-glutamyltransferase (GGT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)) in participants exposed to pesticides. However, due to the low quality of the data and the limited number of studies firm conclusions cannot be drawn and data is only suggestive of associations at this stage.

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### CONCLUSIONS

After an exhaustive and comprehensive search of almost 46,000 scientific publications we identified 602 publications, which examine epidemiologic associations between pesticide exposure and diverse health outcomes. The entire spectrum of health outcomes related to pesticide exposure has not been studied before. Our results show a very wide spectrum including 24 major disease categories. Few environmental exposures have been associated with such a wide range of outcomes. The most prevalent outcomes are cancers and mother and child health outcomes. But other disease categories have received considerable attention such as neurological conditions and reproductive diseases. Despite the large volume of available data and the large number (>6,000) of analyses available, firm conclusions cannot be made for the majority of the outcomes studied. This observation is disappointing especially when one accounts for the large volume of research in the area. However, this observation is in line with previous studies on environmental epidemiology and in particular on pesticides which all acknowledge that such epidemiological studies suffer from many limitations and that the heterogeneity of data is such that does not allows firm conclusions to de made.

The range of categories of pesticide studied is wide but studies very often concentrate on a broadly defined pesticide category, and it is hard to understand which pesticide the population is exposed to. Studies often examine pesticides that have already been banned in western populations and the European Union. The use of biomarkers as means of exposure assessment is infrequent but still available in almost half of the studies. In addition, cohort studies represent a minority of this literature with case control and cross-sectional studies representing an approximately equal proportion of eligible articles. Case-control and cross-sectional evidence does not allow the study of temporal relations and thus are unable to provide support regarding the causality of associations. The assessment of exposure is perhaps the most important methodological limitation of the studies. Studies used different methods for exposure assessment and assignment. Most studies were based on selfreported exposure to pesticides, defined as ever versus never use or as regular versus non-regular use. Such methods suffer from high misclassification rates and especially in the case of retrospective studies where misclassification would be differential with higher exposures reported in participants with disease (recall bias). Above all, such questionnaires might be capable of differentiating subjects with very high and very low exposure levels but are not capable of valid exposure classification across an exposure gradient thus not allowing the study of dose-response relationships. Also, the accuracy of exposure might be high for broad categories of pesticides and commonly used pesticides, but not for specific pesticides. It is important that questionnaires used for exposure assessment are validated. However, studies largely used "home- made" versions of questionnaires, sometimes not giving the information on the actual questions used to assess exposure. In addition, exposure simultaneously in multiple agents is common which may introduce further bias in the results. For example, occupational exposure to pesticides is likely to coexist with exposure to benzene, heavy metals, solvents, suspended particulate matter etc. all of which have adverse health outcomes. It is essential to account for confounding from exposure to multiple agents in order to delineate true associations but this has not been possible in the overwhelming majority of evidence assessed herein.

In addition, the evidence collected and appraised herein is likely to suffer from selective reporting and multiple testing. The studies reported a very wide range of analyses; 602 publications resulted in 6000 analyses. The amount of multiple hypothesis testing is enormous. These analyses need to be adjusted for multiple hypothesis testing else the results suffer from high false positive rate. Even when studies present only one analysis, selective reporting is always a possibility as has been shown in other epidemiological fields as well. In addition, when interpreting results one should also take into account that, especially for certain outcomes (e.g. cancers), the majority of evidence comes from single study populations and the AHS in particular.

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Beyond definition of exposure, the definition of clinical outcomes displayed large variability in eligible epidemiological studies, which can further cause the variability in results. Perhaps most important in this setting is the use of surrogate outcomes examined. Here we observed a great number of surrogate outcomes. Surrogate outcomes are biomarkers or physical measures that are generally accepted as substitutes for or predictors of specific clinical outcomes. However, many times these surrogate outcomes are unvalidated and do not meet the strict definitions of surrogate outcomes. Such outcomes can be defined as possible predictors of clinical outcomes but do not fulfil the criteria for a surrogate outcome. It is essential that the evidence around unvalidated surrogate outcomes are appraised taking into account the implicit assumptions of unvalidated surrogate outcomes.

Acknowledging these limitations we attempted to summarise the evidence retrieved in this report. An added important limitation here is the fact that this review is limited to publications after 2006. This allows us only to review recent evidence and any meta-analysis needs very cautious interpretation, as it does not include all available evidence. Results might be biased if data published after 2006 are different from earlier evidence. To this end, we also provided updated meta-analysis for major outcomes and for those that a relevant meta-analysis published after 2006 was identified. This has only been possible for childhood leukaemia and for Parkinson's disease. For both these outcomes we found significant associations between pesticide exposure and disease in line with previous evidence. Significant summary estimates have also been reported for other outcomes as summarised in Table 25 below. However, as they represent studies form 2006 onwards results should be regarded as suggestive of associations only and limitations especially regarding the heterogeneity of exposure should always been take into consideration.

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### Table 25: Summary of meta-analyses performed in this report

Table 25: Summary of meta-analyses performed f	ii uiis report		
Health outcome	N	Meta-analysis	$I^2$
	studies	result	
Leukemia	6	1.26 (0.93,1.71)	59.4%
Hodgkin's Lymphoma	7	1.29 (0.81, 2.06)	81.6%
Childhood Leukemia (exposure to pesticides	6	1.67 (1.25, 2.23)	81.2%
during pregnancy)			
Childhood Leukemia (exposure to insecticides	5	1.55 (1.14, 2.11)	65%
during pregnancy)			
Childhood Leukemia (exposure to insecticides	9	1.69 (1.35, 2.11)	49.8%
during pregnancy-update Turner 2010)			
Childhood Leukemia (exposure to unspecified	5	2.00 (1.73, 2.30)	39.6%
pesticides during pregnancy)			
Childhood Leukemia (exposure to unspecified	11	1.30 (1.09, 1.56)	26.5%
pesticides during pregnancy-update Turner			
2010)			
Childhood Leukemia (exposure to pesticides	7	1.27 (0.96, 1.69)	61.1%
during childhood)			
Childhood Leukemia (exposure to insecticides	8	1.51 (1.28, 1.78)	0%
during childhood-update Turner 2010)			
Childhood Leukemia (exposure to unspecified	11	1.36 (1.19, 1.55)	0%
pesticides during childhood-update Turner			
2010)			00/
Breast Cancer (DDE exposure)	5	1.13 (0.81, 1.57)	0%
Breast Cancer		1.24 (1.08, 1.43)	0%
Testicular Cancer (DDE exposure)	5	1.40 (0.82, 2.39)	59.5%
Stomach Cancer	6	1.79 (1.30, 2.47)	0%
Liver Cancer	5	2.50 (1.57, 3.98)	25.4%
Cryptorchidism	8	1.19 (0.96, 1.49)	23.9%
Cryptorchidism (DDT exposure)	4	1.47 (0.98, 2.20)	51%
Hypospadias (general pesticide exposure)	6	1.01 (0.74, 1.39)	71.5%
Hypospadias (exposure to specific pesticides)	9	1 (0.84, 1.18)	65.9%
Abortion	6	1.52 (1.09, 2.13)	63.1%
Parkinson's disease	26	1.49 (1.28, 1.73)	54.6%
Parkinson's disease (DDT exposure)	5	1.01 (0.78, 1.30)	0%
Parkinson's disease (paraquat exposure)	9	1.32 (1.09, 1.60)	34.1%
Amyotrophic Lateral Sclerosis	6	1.58 (1.31, 1.90)	10%
Asthma (DDT exposure)	5	1.29 (1.14, 1.45)	0%
Asthma (paraquat exposure)	6	1.40 (0.95, 2.06)	53.3%
Asthma (chlorpyrifos exposure)	5	1.03 (0.82, 1.28)	0%
Type 1 Diabetes (DDE exposure)	8	1.89 (1.25, 2.86)	49%
Type 1 Diabetes (DDT exposure)	6	1.76 (1.20, 2.59)	76.3%
Type 2 Diabetes (DDE exposure)	4	1.29 (1.13, 1.48)	0%

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### RECOMMENDATIONS

As discussed above, the extensive evidence gathered for this report highlights that there is immense amount of information available on pesticide exposure and health outcomes from epidemiological studies. Nonetheless, the quality of this evidence is usually low and many biases are likely to affect the results to an extent that firm conclusions cannot be made. Childhood cancers and Parkinson's disease are the two outcomes for which a corresponding meta-analysis after 2006 was found and for which data are consistent to show an increased risk associated with pesticide exposure. Nonetheless, the exposure needs to be studies further in order to disentangle the effect of specific pesticide classes or even individual pesticides. Effects on other outcomes, such as endocrine disorders, asthma and allergies, diabetes and obesity, are showing increased risk and should be explored further. This report concentrated on examining separately health outcomes. An alternative approach would be to look for pesticide classes, subclasses or even individual pesticides across a range of outcomes. These approaches could highlight whether a pesticide class has a particular detrimental effect across a variety of disease endpoints. Finally, exposure epidemiology has long suffered from exposure measurement and definition and in particular for pesticides this has always been exceptionally difficult to assess and define. Technological advances now enable us to measure in a large scale and agnostic way biomarkers of exposure using high throughput technologies of omics. For example, metabolomic analysis offers a way to capture a whole range of environmental exposures with minimal measurement error and ability to specify the exposure. These approaches are now being developed and are likely to offer much clearer view on the associations between environmental exposures, including dietary exposures, and health outcomes.

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# References

- ALLEN, M. T. & LEVY, L. S. 2013. Parkinson's disease and pesticide exposure--a new assessment. Crit Rev Toxicol, 43, 515-34.
- BAILEY, H. D., ARMSTRONG, B. K., DE KLERK, N. H., FRITSCHI, L., ATTIA, J., SCOTT, R. J., SMIBERT, E. & MILNE, E. 2011. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer, 129, 1678-88.
- BUDNIK, L. T., KLOTH, S., VELASCO-GARRIDO, M. & BAUR, X. 2012. Prostate cancer and toxicity from critical use exemptions of methyl bromide: environmental protection helps protect against human health risks. Environ Health, 11, 5.
- COOPER, G. S. & JONES, S. 2008. Pentachlorophenol and cancer risk: focusing the lens on specific chlorophenols and contaminants. Environ Health Perspect, 116, 1001-8.
- DICK, F. D. 2006. Parkinson's disease and pesticide exposures. Br Med Bull, 79-80, 219-31.
- GOVARTS, E., NIEUWENHUIJSEN, M., SCHOETERS, G., BALLESTER, F., BLOEMEN, K., DE BOER, M., CHEVRIER, C., EGGESBO, M., GUXENS, M., KRAMER, U., LEGLER, J., MARTINEZ, D., PALKOVICOVA, L., PATELAROU, E., RANFT, U., RAUTIO, A., PETERSEN, M. S., SLAMA, R., STIGUM, H., TOFT, G., TRNOVEC, T., VANDENTORREN, S., WEIHE, P., KUPERUS, N. W., WILHELM, M., WITTSIEPE, J. & BONDE, J. P. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect, 120, 162-70.
- IOANNIDIS, J. P., PATSOPOULOS, N. A. & EVANGELOU, E. 2007. Uncertainty in heterogeneity estimates in meta-analyses. BMJ, 335, 914-6.
- ISMAIL, A. A., BODNER, T. E. & ROHLMAN, D. S. 2012. Neurobehavioral performance among agricultural workers and pesticide applicators: a meta-analytic study. Occup Environ Med, 69, 457-64.
- KAMEL, F., UMBACH, D. M., BEDLACK, R. S., RICHARDS, M., WATSON, M., ALAVANJA, M. C., BLAIR, A., HOPPIN, J. A., SCHMIDT, S. & SANDLER, D. P. 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology, 33, 457-62.
- KHANJANI, N., HOVING, J. L., FORBES, A. B. & SIM, M. R. 2007. Systematic review and metaanalysis of cyclodiene insecticides and breast cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 25, 23-52.
- KOUREAS, M., TSAKALOF, A., TSATSAKIS, A. & HADJICHRISTODOULOU, C. 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett, 210, 155-68.
- LAU, J., IOANNIDIS, J. P. & SCHMID, C. H. 1997. Quantitative synthesis in systematic reviews. Ann Intern Med, 127, 820-6.
- LI, A. A., LOWE, K. A., MCINTOSH, L. J. & MINK, P. J. 2012. Evaluation of epidemiology and animal data for risk assessment: chlorpyrifos developmental neurobehavioral outcomes. J Toxicol Environ Health B Crit Rev, 15, 109-84.
- LOPEZ-CERVANTES, M., TORRES-SANCHEZ, L., TOBIAS, A. & LOPEZ-CARRILLO, L. 2004. Dichlorodiphenyldichloroethane burden and breast cancer risk: a meta-analysis of the epidemiologic evidence. Environ Health Perspect, 112, 207-14.

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- MALEK, A. M., BARCHOWSKY, A., BOWSER, R., YOUK, A. & TALBOTT, E. O. 2012. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS. Environ Res, 117, 112-9.
- MERHI, M., RAYNAL, H., CAHUZAC, E., VINSON, F., CRAVEDI, J. P. & GAMET-PAYRASTRE, L. 2007. Occupational exposure to pesticides and risk of hematopoietic cancers: meta-analysis of case-control studies. Cancer Causes Control, 18, 1209-26.
- PEZZOLI, G. & CEREDA, E. 2013. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology, 80, 2035-41.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & PRIYADARSHI, S. S. 2001. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res, 86, 122-7.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & SHRIVASTAVA, S. 2000. A metaanalysis of Parkinson's disease and exposure to pesticides. Neurotoxicology, 21, 435-40.
- SAPHIR, A. 1998. Farmers and cancer: old crop of data gets new scrutiny. J Natl Cancer Inst, 90, 651-3.
- SHIRANGI, A., NIEUWENHUIJSEN, M., VIENNEAU, D. & HOLMAN, C. D. 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. Paediatr Perinat Epidemiol, 25, 172-91.
- SNIJDER, C. A., TE VELDE, E., ROELEVELD, N. & BURDORF, A. 2012. Occupational exposure to chemical substances and time to pregnancy: a systematic review. Hum Reprod Update, 18, 284-300.
- SUTEDJA, N. A., VELDINK, J. H., FISCHER, K., KROMHOUT, H., HEEDERIK, D., HUISMAN, M. H., WOKKE, J. H. & VAN DEN BERG, L. H. 2009. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph Lateral Scler, 10, 302-9.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Environ Health Perspect, 118, 33-41.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Cien Saude Colet, 16, 1915-31.
- VAN DER MARK, M., BROUWER, M., KROMHOUT, H., NIJSSEN, P., HUSS, A. & VERMEULEN, R. 2012. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect, 120, 340-7.
- VAN MAELE-FABRY, G., DUHAYON, S., MERTENS, C. & LISON, D. 2008. Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. Environ Res, 106, 121-37.
- VAN MAELE-FABRY, G., HOET, P., VILAIN, F. & LISON, D. 2012. Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. Environ Int, 46, 30-43.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2010. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control, 21, 787-809.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2011. Residential exposure to pesticides and childhood leukaemia: a systematic review and meta-analysis. Environ Int, 37, 280-91.

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- VINSON, F., MERHI, M., BALDI, I., RAYNAL, H. & GAMET-PAYRASTRE, L. 2011. Exposure to pesticides and risk of childhood cancer: a meta-analysis of recent epidemiological studies. Occup Environ Med, 68, 694-702.
- WIGLE, D. T., TURNER, M. C. & KREWSKI, D. 2009. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ Health Perspect, 117, 1505-13.

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### APPENDICES

#### **APPENDIX I. EXTENDED SEARCH ALGORITHM IN MEDLINE**

Pesticid\* OR Pesticide OR pest control OR "pest control" OR (Chemosteril\* OR Chemosterilant OR Fungicid\* OR fungicide OR Fungicide, Industrial OR Herbicid\* OR Herbicide OR Defoliant\* OR Defoliant, Chemical OR Insect Repellent\*OR Insect Repellent OR Insecticid\* OR Insecticide OR Molluscacid\* OR Molluscacide OR Pesticide Synergist\* OR Pesticide Synergist OR Rodenticid\* OR Rodenticide OR organochlor\* OR organochloride OR organochlorine OR chlorocarbon OR chlorinated hydrocarbon OR chlorinated solvent OR organophosphat\* OR organophosphate OR carbamat\* OR carbamate OR pyrethroid\* OR pyrethroid) OR (1,2-dibromo-3-chloropropane OR 1,3dichloro-1-propene OR 1-(4-ethynylphenyl)-4-propyl-2,6,7-trioxabicyclo(2.2.2)octane OR 1-Methyl-4-phenylpyridiniumOR 2,4,5-Trichlorophenoxyacetic Acid OR 2,4-Dichlorophenoxyacetic AcidOR 2dichlorobenzeneOR 2-Methyl-4-chlorophenoxyacetic Acid OR 2-methyl-4-chlorophenoxyacetic acid dicamba herbicide solution OR 2-phenylphenol OR 3,5,6-trichloro-2-pyridinolOR 4"-epiacetylamino-4"-deoxyavermectin B1 OR 4-dichlorobenzeneOR abamectin OR acephate OR acetochlor OR acifluorfen ORAgent OrangeOR alachlor OR Aldicarb OR Aldrin OR Allethrin OR allosamidin OR alpha-Chlorohydrin OR alpha-naphthyl thiourea OR alpha-naphthylphthalamic acid OR aluminum phosphide OR aminocarb OR amitrazOR AnabasineOR arsenic acidOR Atrazine OR avermectinOR azadirachtin OR AzinphosmethylOR Bacillus thuringiensis protoxinOR bendiocarbOR BenomylOR bentazoneOR benthiocarbOR benzyl benzoate OR bialaphos OR binB protein Bacillus sphaericus OR bioallethrinOR bioresmethrin OR bis(tri-n-butyltin)oxideOR boric acid OR bromacil OR bromadiolone OR bromfenacoumOR bullatacinOR butachlorOR butyl phosphorotrithioate OR Cacodylic Acid OR captafol OR CaptanOR Carbaryl OR Carbofuran OR CarboxinOR Chloranil OR ChlordanOR ChlordeconeOR Chlorfenvinphos OR chlorocresol OR chlorophacinoneOR ChlorphenamidineOR Chlorpropham OR Chlorpyrifos OR chlorsulfuronOR chlortoluronOR cismethrinOR closantel OR CoumaphosOR crotamiton OR cyanazine OR cyclonite OR cyfluthrinOR cyhalothrinOR cyhexatinOR cypermethrinOR cyromazineOR cythioateOR daminozideOR decamethrinOR DEETOR dexon (fungicide)OR diallyl trisulfideOR Diazinon OR Dicamba OR dichlobanilOR Dichlorodiphenyl DichloroethyleneOR DichlorodiphenyldichloroethaneOR dichlorodiphenyltrichloroethane OR DDT OR Dichlorvos OR Dicofol OR dieldrin OR difenacoumOR DimethoateOR dimethyl 4,4'-o-phenylene bis (3-thioallophanate) with carbamic acid ethylene bis (dithio)-mangenese zinc complexOR dimethyl 4-phthalateOR dimethyl phthalateOR Dinitrophenols OR dinosebOR diphenylOR DiquatOR DisulfotonOR DiuronOR doramectin OR EndosulfanOR EndrinOR ethionOR Ethylmercuric Chloride OR Ethylmercury Compounds OR famophos OR fenarimol OR FenitrothionOR fenoxycarb OR fenpropimorphOR Fenthion OR fenvalerate OR fipronil OR fluazifop OR fluazifop-butyl OR fluoroacetic acid OR fluphenacur OR fluridoneOR fluvalinate OR folpet OR FonofosOR glyphosateOR hedolit OR Hempa OR HeptachlorOR Heptachlor Epoxide OR heptenophosOR HexachlorobenzeneOR hexachlorobutadiene OR hexazinoneOR hydramethylnonOR imazalilOR imidaclopridOR insecticidal crystal protein Bacillus ThuringiensisOR iprodioneOR isofenphosOR isoproturonOR IvermectinOR jasplakinolideOR LeptophosOR linaloolOR LindaneOR Linuron ORmalachite greenOR malaoxonOR MalathionOR Maleic HydrazideOR mancozebOR ManebOR mecarzoleOR mecopropOR metalaxylOR metaldehydeOR methamidophosOR methidathionOR MethiocarbOR MethomylOR MethoxychlorOR methyl demetonOR methyl isothiocyanateOR Methyl ParathionOR methylbromfenvinphosOR methyldithiocarbamateOR methyllycaconitineOR metolachlorOR metribuzinOR MevinphosOR milberrycinOR molinateOR MonocrotophosOR monomethylarsonic acidOR N,Ndiethylphenylacetamide OR N-(3,5-dichlorophenyl)succinimideOR N-bromoacetamideOR nhexanalOR Naled OR neem oilOR neosaxitoxinOR Niclosamide OR nitrofenOR nonachlor OR norbormideOR norflurazoneOR nornicotine OR octamethyl pyrophosphoramideOR oryzalinOR

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ParaoxonOR ParaquatOR ParathionOR pendimethalin OR pentachlorobenzeneOR PentachlorophenolOR PermethrinOR phenothrinOR phenthoateOR phentin acetate OR Phenylmercuric Acetate OR phenylmercuric nitrate, basicOR Phenylmercury CompoundsOR Phenylphosphonothioic Acid 2-Ethyl 2-(4-Nitrophenyl) EsterOR Phorate OR phosaloneOR PhosmetOR PhosphamidonOR phosphineOR phosphinothricinOR phoxim OR Picloram OR Piperonyl ButoxideOR pirimicarbOR pirimiphos methylOR precocene IIOR prochlorazOR procymidoneOR profenofosOR PrometryneOR propachlorOR PropanilOR PropoxurOR PyrethrinsOR pyriminil OR quinalphos OR quintozene OR RotenoneOR S,S'-(2-(dimethylamino)-1,3-propanediyl)thiosulfuric acid ester OR SimazineOR sodium chlorateOR spinosadOR sulfamic acidOR sulfometuron methyl OR tebufenozideOR TemefosOR terbutryneOR terbutylazineOR terthienyl OR tetrachloroisophthalonitrileOR TetrachlorvinphosOR tetramethrinOR thallium sulfate OR ThiophanateOR ThiramOR ToxapheneOR triadimefon OR Triallate OR TrichlorfonOR triclopyrOR triflumuron OR Trifluralin OR vinclozolin OR Warfarin OR zinc phosphide OR Zineb OR Zinam)

(LIMITS: HUMAN, 1/1/2006 - 1/10/2012)

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# APPENDIX II. EXPLANATIONS TO THE DATA EXTRACTION DATABASE

Study ID	This is the unique ID of the study given sequentially for each
	study major outcome
PUBMED_ID	This is the PUBMED ID of the study (if not available ID in
	EMBASE was provided and when this was not available the
	title of the study was provided)
First author	First author's last name
Journal	Journal in which the study was published
Year	Year of publication
Country	Country where the study was conducted
Location (continent)	Continent where the study was conducted
Recruitment period	Period during which the study participants were recruited
Exposure Period (preconception,	Growth period in which the pesticide exposure occurred
infancy, childhood, adulthood,	(preconception, pregnancy, infancy, childhood, adolescence,
pregnancy)	adulthood)
Follow-up period	Follow-up calendar period for prospective/ retrospective studies
Follow-up duration (maximum)	Maximum follow-up period in years for prospective/
	retrospective studies
Follow-up duration (years)	Mean or median follow-up period in years for prospective/
(median/mean)	retrospective studies
Study type (cohort, nested case-	The epidemiological study design: cohort, nested case-control,
control, case-control, cross-	case-control, cross-sectional
sectional)	The name of the enviolenced study
Cohort name	The name of the epidemiological study
Age (years) (range/mean/median)	The age of the population studied (preference is to provide the mean or meadian age, when not available the range is
	given). Data is presented in years unless otherwise stated.
Gender (% male)	Percentage of males in study population
Active substance assessed	Pesticide assessed in the study as defined/named in the study
Active substance category	Chemical or functional pesticide category in which the
	pesticide is classified
Authorisation status	Pesticide active substances authorized within EU
	(06/09/2013). Yes/No/NA (NA=not applicable)
Biomarker name	The name of the biomarker of exposure to pesticide (if
	measured)
Control definition	Definition of the control group in case-control studies
Pesticide co-exposure (measured)	Did the study provided information on other co-exposed pesticides? (yes, no)
Population characteristics	Description of the population examined (gender, location,
	disease status)
Type of exposure (occupational,	What is the source of exposure to pesticides: occupational (if
environmental, both)	the exposure is related to a specific occupational activity);
	environmental (if the exposure is not related to any

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Pesticide epidemiology

	occupational activity (e.g. domestic use of pesticides, use of
	pesticides in gardening, exposure related to gardening etc.); both (when both occupation and environmental exposure is
	present).
Type of exposure assessment (direct	Means of measuring pesticide exposure: direct exposure
exposure questionnaire/ biomarker/residential	questionnaire (interview or self-administered); measurement of biomarker in biological fluids; residential history;
history/occupational history/ JEM/	occupational history; Job Exposure Matrix (JEM)
expert evaluation/ environmental	
odeling)	
Exposure definition	Definition of exposure as described in the study
Questionnaire type	Questionnaire type (interview or self administrated) (for
	studies which assessed exposure through questionnaires, else state n/a)
Measurement of biomarker (whole	Body fluid or tissue in which the biomarker was measured
blood, plasma, urine, breast milk,	(whole blood, plasma, urine, breast milk, placenta, nails, hair,
placenta, nails, hair, saliva, adipose	saliva, adipose tissue etc.)
tissue)	Tupo of biochomical accounted for biomarker measurement
Assay type Exposure duration	Type of biochemical assay used for biomarker measurement Duration of exposure to pesticides in years (when available)
Pediatric exposure type (mother,	For studies on child outcomes, describe means of exposure
father, child, combinations)	through self-exposure or parental exposure (mother, father,
	child, combinations)
Pediatric exposure time	For studies on child outcomes, was parental exposure during
(preconception, pregnancy,	preconception, pregnancy or combinations?
combination)	
Health outcome	Health outcome as described in the study
Outcome definition	Health outcome definition used in the study
Disease category Effect estimate type (RR, OR, HR,	Disease category Type of effect estimate for the assessment of pesticide and
beta, MD, SMD)	health outcome relationship (RR, OR, HR, beta, MD, SMD)
Effect (binary, continuous)	Effect estimated on a binary or continuous manner (binary,
× 5. ,	continuous)
Comparison unit (yes/no, unit	The definition of comparison for the calculation of the effect
increase,)	size (yes/no, unit increase etc.)
Effect estimate	Value of effect estimate
SE/SD effect stimate	Standard error/Standard deviation of effect estimate
Lower 95% CI	Lower 95% confidence interval of the effect estimate
Higher 95% Cl	Higher 95% confidence interval of the effect estimate
Adjustment for	Confounders/ variables for which the effect estimate was adjusted for
Controls matched for	Variables for which controls were matched to cases (case
	control studies only)
Sample size	Total number of participants
N cases	Number of cases

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Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Pesticide epidemiology

N controls	Number of controls
Statistical method	Statistical method used to calculate the effect size
Study design (prospective, retrospective, mixed, cross- sectional)	Prospective or retrospective type of study design (prospective, retrospective, mixed, cross-sectional)
Inclusion/exclusion criteria clearly stated (yes, partially, no) Authors mention power calculations (yes, no) Level of detail in describing exposure (high, medium, low) Robust measurement of exposure. (biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	Was the description of study participants (population) inclusion and exclusion criteria detailed? (yes/partially/no) Do the authors mention power calculations in the manuscript preceding or proceeding their statistical analysis (yes/no) Level of detail in which the definition of exposure to pesticides is provided (high/medium/low) Was the measurement of exposure robust: biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)
Were measures of exposure specific? Yes; based on broader, chemically-related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	Were measures of exposure specific? (yes); based on broader, chemically-related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)
Attempt to balance the allocation between the groups (e.g., through stratification, matching) Adjustment performed for	Was an attempt to balance the allocation between the groups in case-control studies either through stratification or matching (yes/no)? Was the effect size adjusted for potential confounders (yes,
potential confounders (yes, some, no)	some, no)?
Assessors blinded to exposure status (for cohort studies)	Were the assessors blinded to exposure status in cohort studies (yes/no/;n/a:not available or not applicable when studies are not cohorts)?
Outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	Were the outcomes assessed using valid and reliable measures implemented consistently across all study participants (yes/no)
Sample size (top [991], middle, bottom quartiles[104])	The size of the sample
Was source of funding acknowledged	Do the authors acknowledge any possible source of funding (yes/no)
Rough quality assessment	Rough quality assessment taking into account the data in all other columns of the quality assessment of data extraction form
COMMENTS	Any comments related to the study that help interpretation of the data extracted

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# APPENDIX III. REFERENCES TO THE DATA EXTRACTION DATABASE

- A.S Al-Sarar, Y. Abo Bakr, G.S Al-Erimah, H.I Hussein, A.E Bayoumi. Hematological and biochemical alterations in occupationally pesticide-exposed workers of Riyadh municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology 3 (4) : 179-185, 2009 ISSN 1819-3420
- Abadi-Korek I, Stark B, Zaizov R, Shaham J. Parental occupational exposure and the risk of acute lymphoblastic leukemia in offspring in israel. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:165-174
- Abdel Rasoul GM, Abou Salem ME, Mechael AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. Neurotoxicology. 2008;29:833-838
- Abdelouahab N, Ainmelk Y, Takser L. Polybrominated diphenyl ethers and sperm quality. Reprod Toxicol. 2011;31:546-550
- Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, Spear PA, Chan HM. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of quebec (canada). Environmental research. 2008;107:380-392
- Abu Sham'a F, Skogstad M, Nijem K, Bjertness E, Kristensen P. Lung function and respiratory symptoms in male palestinian farmers. Arch Environ Occup Health. 2010;65:191-200
- Ahamed M, Anand M, Kumar A, Siddiqui MK. Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. Clinical biochemistry. 2006;39:762-766
- Ahrens W, Mambetova C, Bourdon-Raverdy N, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Zambon P. Occupational exposure to endocrine-disrupting compounds and biliary tract cancer among men. Scand J Work Environ Health. 2007;33:387-396
- Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. Diabetes care. 2011;34:1972-1979
- Al-Saleh I, Al-Doush I, Alsabbaheen A, Mohamed Gel D, Rabbah A. Levels of ddt and its metabolites in placenta, maternal and cord blood and their potential influence on neonatal anthropometric measures. The Science of the total environment. 2012;416:62-74
- Alavanja MCR, Sandler DP, Hoppin JA, Schroeder P, Lynch CF, Blair A, Mahajan R. Fonofos exposure and cancer incidence in the agricultural health study. Environmental Health Perspectives. 2006
- Albers JW, Garabrant DH, Mattsson JL, Burns CJ, Cohen SS, Sima C, Garrison RP, Richardson RJ, Berent S. Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. Toxicological sciences: an official journal of the Society of Toxicology. 2007;97:196-204

Alderton LE, Spector LG, Blair CK, Roesler M, Olshan AF, Robison LL, Ross JA. Child and

111

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

maternal household chemical exposure and the risk of acute leukemia in children with Down's syndrome: A report from the children's oncology group. American journal of epidemiology. 2006;164:212-221

- Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009;43:7909-7915
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, Sunyer J. Thyroid disruption at birth due to prenatal exposure to betahexachlorocyclohexane. Environment international. 2008;34:737-740
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Grimalt JO, Sunyer J. Effects of pcbs, p,p'-ddt, p,p'-dde, hcb and beta-hch on thyroid function in preschool children. Occupational and environmental medicine. 2008;65:452-457
- Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jorgensen E, Kjaerstad MB, Baelum J, Nielsen JB, Skakkebaek NE, Main KM. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 2008;116:566-572
- Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, Silverman DT, Alavanja MC. Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort. International journal of cancer. Journal international du cancer. 2009;124:2495-2500
- Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental ddt exposure in young men living in a malaria area in the limpopo province, south africa. Journal of andrology. 2007;28:423-434
- Araoud M, Neffeti F, Douki W, Najjar MF, Kenani A. Paraoxonase 1 correlates with butyrylcholinesterase and gamma glutamyl transferase in workers chronically exposed to pesticides. Journal of occupational health. 2010;52:383-388
- Arcury TA, Feldman SR, Schulz MR, Vallejos Q, Verma A, Fleischer AB, Jr., Rapp SR, Davis SF, Preisser JS, Quandt SA. Diagnosed skin diseases among migrant farmworkers in North Carolina: Prevalence and risk factors. Journal of agricultural safety and health. 2007;13:407-418
- Arguelles LM, Liu X, Venners SA, Ronnenberg AG, Li Z, Yang F, Yang J, Xu X, Wang X. Serum folate and DDT isomers and metabolites are inversely associated in Chinese women: A cross-sectional analysis. Journal of the American College of Nutrition. 2009;28:380-387
- Aronson KJ, Wilson JW, Hamel M, Diarsvitri W, Fan W, Woolcott C, Heaton JP, Nickel JC, Macneily A, Morales A. Plasma organochlorine levels and prostate cancer risk. Journal of exposure science & environmental epidemiology. 2010;20:434-445
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of ddt and their association with reproductive hormones in adult men from northern thailand. The Science of the total environment. 2006;355:98-105
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand. Environment international. 2006;32:554-559
- Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, Schwarzschild MA, Thun MJ. Pesticide exposure and risk for Parkinson's disease. Annals of neurology.

EFSA supporting publication 2013:EN-497

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2006;60:197-203

- Avivar Oyonarte C., Duran Salas I., Molina Arrebola M.A., Castilla Alcala J.A., Olea Serrano N., Fernandez Cabrera M. Pesticide exposure and decreased sperm count. Revista del Laboratorio Clinico (2010) 3:1 (4-11).
- Axmon A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Zvyezday V, Ludwicki JK, Jonsson BA, Toft G, Bonde JP, Hagmar L. Time to pregnancy as a function of male and female serum concentrations of 2,2'4,4'5,5'-hexachlorobiphenyl (cb-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-dde). Hum Reprod. 2006;21:657-665
- Azmi MA, Naqvi SN, Akhtar K, Moinuddin, Parveen S, Parveen R, Aslam M. Effect of pesticide residues on health and blood parameters of farm workers from rural gadap, karachi, pakistan. Journal of environmental biology / Academy of Environmental Biology, India. 2009;30:747-756
- Baharuddin MR, Sahid IB, Noor MA, Sulaiman N, Othman F. Pesticide risk assessment: A study on inhalation and dermal exposure to 2,4-d and paraquat among malaysian paddy farmers. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:600-607
- Bahena-Medina LA, Torres-Sanchez L, Schnaas L, Cebrian ME, Chavez CH, Osorio-Valencia E, Hernandez RM, Lopez-Carrillo L. Neonatal neurodevelopment and prenatal exposure to dichlorodiphenyldichloroethylene (dde): A cohort study in Mexico. Journal of exposure science & environmental epidemiology. 2011;21:609-614
- Bailey HD, Armstrong BK, de Klerk NH, Fritschi L, Attia J, Scott RJ, Smibert E, Milne E. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. International journal of cancer. Journal international du cancer. 2011;129:1678-1688
- Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, Le ND. Prostate cancer risk and exposure to pesticides in British Columbia farmers. The Prostate. 2011;71:168-183
- Baranska M, Van Amelsvoort L, Birindelli S, Fustinoni S, Corsini E, Liesivuori J, Van Loveren H. Association of pesticide exposure, vaccination response, and interleukin-1 gene polymorphisms. Human & experimental toxicology. 2008;27:709-713
- Barczyk A, Sozanska E, Pierzchala W. [the influence of occupational exposure to pesticides on the frequency of chronic obstructive pulmonary diseases]. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2006;59:596-600
- Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in new jersey. The Science of the total environment. 2010;408:790-795
- Barry KH, Koutros S, Berndt SI, Andreotti G, Hoppin JA, Sandler DP, Burdette LA, Yeager M, Freeman LE, Lubin JH, Ma X, Zheng T, Alavanja MC. Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk. Environ Health Perspect. 2011;119:1726-1732
- Barry KH, Koutros S, Lubin JH, Coble JB, Barone-Adesi F, Beane Freeman LE, Sandler DP, Hoppin JA, Ma X, Zheng T, Alavanja MC. Methyl bromide exposure and cancer risk in the agricultural health study. Cancer causes & control: CCC. 2012;23:807-818

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- Bayrami M, Hashemi T, Malekirad AA, Ashayeri H, Faraji F, Abdollahi M. Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. Toxicology and industrial health. 2012;28:90-96
- Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, Sandler DP, Kamel F. Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. Environ Health Perspect. 2011;119:1610-1615
- Behrens T, Lynge E, Cree I, Lutz JM, Eriksson M, Guenel P, Merletti F, Morales-Suarez-Varela M, Afonso N, Stengrevics A, Fevotte J, Sabroe S, Llopis-Gonzalez A, Gorini G, Hardell L, Stang A, Ahrens W. Pesticide exposure in farming and forestry and the risk of uveal melanoma. Cancer causes & control: CCC. 2012;23:141-151
- Beltrame D, Lo Cascio N, Miotto D, Mapp CE, De Rosa E, Boschetto P. [occupational exposure and chronic heart failure severity]. Giornale italiano di medicina del lavoro ed ergonomia. 2007;29:438-439
- Bergonzi R, De Palma G, Specchia C, Dinolfo M, Tomasi C, Frusca T, Apostoli P. Persistent organochlorine compounds in fetal and maternal tissues: Evaluation of their potential influence on several indicators of fetal growth and health. The Science of the total environment. 2011;409:2888-2893
- Bertrand KA, Spiegelman D, Aster JC, Altshul LM, Korrick SA, Rodig SJ, Zhang SM, Kurth T, Laden F. Plasma organochlorine levels and risk of non-hodgkin lymphoma in a cohort of men. Epidemiology. 2010;21:172-180
- Beseler C, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:1005-1013
- Beseler CL, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures among private pesticide applicators enrolled in the agricultural health study. Environ Health Perspect. 2008;116:1713-1719
- Bhalli JA, Khan QM, Haq MA, Khalid AM, Nasim A. Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis. 2006;21:143-148
- Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, Doody DR, Fish S, Needham LL, Chen C, Schwartz SM. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: A population-based case-control study. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:2012-2018
- Blanco-Munoz J, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Bassol S, Cebrian ME, Lopez-Flores I, Ruiz-Perez I. Effect of exposure to p,p'-dde on male hormone profile in mexican flower growers. Occupational and environmental medicine. 2012;69:5-11
- Blanco-Munoz J, Morales MM, Lacasana M, Aguilar-Garduno C, Bassol S, Cebrian ME. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of morelos, Mexico. Hum Reprod. 2010;25:1787-1795

Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Cause-specific

EFSA supporting publication 2013:EN-497

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mortality of dutch chlorophenoxy herbicide manufacturing workers. Occupational and environmental medicine. 2010;67:24-31

- Boers D, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Campo L, Bosetti C, La Vecchia C, Vergieva T, Tarkowski M, Liesivuori J, Steerenberg P, van Loveren H. Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the europit field studies. Human & experimental toxicology. 2008;27:721-727
- Bonde JP, Toft G, Rylander L, Rignell-Hydbom A, Giwercman A, Spano M, Manicardi GC, Bizzaro D, Ludwicki JK, Zvyezday V, Bonefeld-Jorgensen EC, Pedersen HS, Jonsson BA, Thulstrup AM. Fertility and markers of male reproductive function in Inuit and European populations spanning large contrasts in blood levels of persistent organochlorines. Environ Health Perspect. 2008;116:269-277
- Bonner MR, Coble J, Blair A, Beane Freeman LE, Hoppin JA, Sandler DP, Alavanja MC. Malathion exposure and the incidence of cancer in the agricultural health study. American journal of epidemiology. 2007;166:1023-1034
- Bonner MR, Williams BA, Rusiecki JA, Blair A, Beane Freeman LE, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC. Occupational exposure to terbufos and the incidence of cancer in the agricultural health study. Cancer causes & control: CCC. 2010;21:871-877
- Bonvicini F, Marcello N, Mandrioli J, Pietrini V, Vinceti M. Exposure to pesticides and risk of amyotrophic lateral sclerosis: A population-based case-control study. Annali dell'Istituto superiore di sanita. 2010;46:284-287
- Borkowski WJ, Riederer A, Prapamontol T. Neurological evaluation of newborn infants of mothers working in citrus groves in northern Thailand. International journal of occupational and environmental health. 2011;17:135-143
- Bornman R, de Jager C, Worku Z, Farias P, Reif S. Ddt and urogenital malformations in newborn boys in a malarial area. BJU international. 2010;106:405-411
- Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics. 2010;125:e1270-1277
- Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr DB, Eskenazi B. Prenatal exposure to organophosphate pesticides and iq in 7-year-old children. Environ Health Perspect. 2011;119:1189-1195
- Bräuner EV, Sørensen M, Gaudreau E, LeBlanc A, Eriksen KT, Tjønneland A, Overvad K, Raaschou-Nielsen O. A prospective study of organochlorines in adipose tissue and risk of non-hodgkin lymphoma. Environmental Health Perspectives. 2011;120:105-111
- Brender JD, Felkner M, Suarez L, Canfield MA, Henry JP. Maternal pesticide exposure and neural tube defects in Mexican Americans. Annals of epidemiology. 2010;20:16-22
- Bretveld R, Zielhuis GA, Roeleveld N. Time to pregnancy among female greenhouse workers. Scandinavian Journal of Work, Environment & Health. 2006;32:359-367
- Bretveld RW, Hooiveld M, Zielhuis GA, Pellegrino A, van Rooij IA, Roeleveld N. Reproductive disorders among male and female greenhouse workers. Reprod Toxicol. 2008;25:107-114

Brighina L, Frigerio R, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Farrer

EFSA supporting publication 2013:EN-497

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MJ, Lincoln SJ, Checkoway H, Rocca WA, Maraganore DM. Alpha-synuclein, pesticides, and Parkinson disease: A case-control study. Neurology. 2008;70:1461-1469

- Brooks K, Hasan H, Samineni S, Gangur V, Karmaus W. Placental p,p'dichlorodiphenyldichloroethylene and cord blood immune markers. Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2007;18:621-624
- Brouwers MM, Feitz WF, Roelofs LA, Kiemeney LA, de Gier RP, Roeleveld N. Risk factors for hypospadias. European journal of pediatrics. 2007;166:671-678
- Browne RO, Moyal-Segal LB, Zumsteg D, David Y, Kofman O, Berger A, Soreq H, Friedman A. Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2006;20:1733-1735
- Brucker-Davis F, Ducot B, Wagner-Mahler K, Tommasi C, Ferrari P, Pacini P, Boda-Buccino M, Bongain A, Azuar P, Fenichel P. [environmental pollutants in maternal milk and cryptorchidism]. Gynecologie, obstetrique & fertilite. 2008;36:840-847
- Brucker-Davis F, Ferrari P, Boda-Buccino M, Wagner-Mahler K, Pacini P, Gal J, Azuar P, Fenichel P. Cord blood thyroid tests in boys born with and without cryptorchidism: Correlations with birth parameters and in utero xenobiotics exposure. Thyroid: official journal of the American Thyroid Association. 2011;21:1133-1141
- Brucker-Davis F, Wagner-Mahler K, Bornebusch L, Delattre I, Ferrari P, Gal J, Boda-Buccino M, Pacini P, Tommasi C, Azuar P, Bongain A, Fenichel P. Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from nice area (france). Chemosphere. 2010;81:169-176
- Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A, Kurzenne JY, Mas JC, Fenichel P. Cryptorchidism at birth in nice area (France) is associated with higher prenatal exposure to pcbs and dde, as assessed by colostrum concentrations. Hum Reprod. 2008;23:1708-1718
- Brulls C., Niggemann H., Weissbach W., Dott W., Fischer M., Merk H.F., Blomeke B., Isselstein J., Ilgner, Westhofen M., Wiesmuller G.A.. Pilot study on living conditions and living factors investigated in patients suffering from self-reported multiple chemical sensitivity, fragrance allergies or polyposis nasi. Atemwegs- und Lungenkrankheiten (2008) 34:5 (187-198)
- Buck Louis GM, Rios LI, McLain A, Cooney MA, Kostyniak PJ, Sundaram R. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere. 2011;85:1742-1748
- Burdorf A, Brand T, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA. The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: The generation r study. Occupational and environmental medicine. 2011;68:197-204
- Burns JS, Williams PL, Sergeyev O, Korrick SA, Lee MM, Revich B, Altshul L, Del Prato JT, Humblet O, Patterson DG, Turner WE, Starovoytov M, Hauser R. Serum concentrations of organochlorine pesticides and growth among russian boys. Environ Health Perspect. 2012;120:303-308

Bustamante Montes LP, Waliszewski S, Hernandez-Valero M, Sanin-Aguirre L, Infanzon-Ruiz

EFSA supporting publication 2013:EN-497

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RM, Janas AG. [prenatal exposure to organochlorine pesticides and cryptorchidism]. Ciencia & saude coletiva. 2010;15 Suppl 1:1169-1174

- Carbone P, Giordano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamanca I. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural Sicily. International journal of andrology. 2007;30:3-13
- Carmichael SL, Herring AH, Sjodin A, Jones R, Needham L, Ma C, Ding K, Shaw GM. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80:641-646
- Carozza SE, Li B, Wang Q, Horel S, Cooper S. Agricultural pesticides and risk of childhood cancers. International journal of hygiene and environmental health. 2009;212:186-195
- Cha ES, Lee YK, Moon EK, Kim YB, Lee YJ, Jeong WC, Cho EY, Lee IJ, Hur J, Ha M, Lee WJ. Paraquat application and respiratory health effects among South Korean farmers. Occupational and environmental medicine. 2012;69:398-403
- Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in india. Journal of occupational health. 2009;51:488-497
- Chang CK, Astrakianakis G, Thomas DB, Seixas NS, Ray RM, Gao DL, Wernli KJ, Fitzgibbons ED, Vaughan TL, Checkoway H. Occupational exposures and risks of liver cancer among shanghai female textile workers--a case-cohort study. International journal of epidemiology. 2006;35:361-369
- Chang YL, Li J, Yao SQ, Hu WN, Jiang SF, Guo Z, Yang L, Li DD, Li YM, Liu Y. [a casecontrol study on serum organochlorines residues, genetic polymorphisms of glutathione stransferase t1 and the risks of breast cancer]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2008;29:763-766
- Charles LE, Burchfiel CM, Fekedulegn D, Gu JK, Petrovitch H, Sanderson WT, Masaki K, Rodriguez BL, Andrew ME, Ross GW. Occupational exposure to pesticides, metals, and solvents: The impact on mortality rates in the Honolulu heart program. Work. 2010;37:205-215
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational exposures and movement abnormalities among japanese-american men: The honolulu-asia aging study. Neuroepidemiology. 2006;26:130-139
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Sanderson WT, Petrovitch H. Occupational and other risk factors for hand-grip strength: The honolulu-asia aging study. Occupational and environmental medicine. 2006;63:820-827
- Chatzi L, Alegakis A, Kruger-Krasagakis S, Lionis C. Skin symptoms and work-related skin symptoms among grape farmers in crete, greece. American journal of industrial medicine. 2006;49:77-84
- Chatzi L, Alegakis A, Tzanakis N, Siafakas N, Kogevinas M, Lionis C. Association of allergic rhinitis with pesticide use among grape farmers in crete, greece. Occupational and environmental medicine. 2007;64:417-421

Chen SC, Wong RH, Shiu LJ, Chiou MC, Lee H. Exposure to mosquito coil smoke may be a risk factor for lung cancer in Taiwan. Journal of epidemiology / Japan Epidemiological

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Association. 2008;18:19-25

- Chen Z, Robison L, Giller R, Krailo M, Davis M, Davies S, Shu XO. Environmental exposure to residential pesticides, chemicals, dusts, fumes, and metals, and risk of childhood germ cell tumors. International journal of hygiene and environmental health. 2006;209:31-40
- Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, Durand G, Cordier S. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the pelagie birth cohort. Environ Health Perspect. 2011;119:1034-1041
- Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008;168:298-310
- Chitra GA, Muraleedharan VR, Swaminathan T, Veeraraghavan D. Use of pesticides and its impact on health of farmers in south india. International journal of occupational and environmental health. 2006;12:228-233
- Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-hodgkin lymphoma. Blood. 2006;108:1363-1369
- Christensen CH, Platz EA, Andreotti G, Blair A, Hoppin JA, Koutros S, Lynch CF, Sandler DP, Alavanja MC. Coumaphos exposure and incident cancer among male participants in the agricultural health study (ahs). Environ Health Perspect. 2010;118:92-96
- Cocco P, Brennan P, Ibba A, de Sanjose Llongueras S, Maynadie M, Nieters A, Becker N, Ennas MG, Tocco MG, Boffetta P. Plasma polychlorobiphenyl and organochlorine pesticide level and risk of major lymphoma subtypes. Occupational and environmental medicine. 2008;65:132-140
- Cockburn M, Mills P, Zhang X, Zadnick J, Goldberg D, Ritz B. Prostate cancer and ambient pesticide exposure in agriculturally intensive areas in california. American journal of epidemiology. 2011;173:1280-1288
- Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environ Health Perspect. 2007;115:1442-1447
- Cohn BA, Cirillo PM, Christianson RE. Prenatal ddt exposure and testicular cancer: A nested case-control study. Arch Environ Occup Health. 2010;65:127-134
- Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. Ddt and breast cancer in young women: New data on the significance of age at exposure. Environ Health Perspect. 2007;115:1406-1414
- Cole DC, Wainman B, Sanin LH, Weber JP, Muggah H, Ibrahim S. Environmental contaminant levels and fecundability among non-smoking couples. Reprod Toxicol. 2006;22:13-19
- Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2009;51:1212-1219
- Colt JS, Davis S, Severson RK, Lynch CF, Cozen W, Camann D, Engels EA, Blair A, Hartge P. Residential insecticide use and risk of non-hodgkin's lymphoma. Cancer epidemiology,

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:251-257

- Colt JS, Rothman N, Severson RK, Hartge P, Cerhan JR, Chatterjee N, Cozen W, Morton LM, De Roos AJ, Davis S, Chanock S, Wang SS. Organochlorine exposure, immune gene variation, and risk of non-hodgkin lymphoma. Blood. 2009;113:1899-1905
- Cooney MA, Buck Louis GM, Hediger ML, Vexler A, Kostyniak PJ. Organochlorine pesticides and endometriosis. Reprod Toxicol. 2010;30:365-369
- Cooney MA, Daniels JL, Ross JA, Breslow NE, Pollock BH, Olshan AF. Household pesticides and the risk of wilms tumor. Environmental Health Perspectives. 2006;115:134-137
- Cooper GS, Parks CG, Schur PS, Fraser PA. Occupational and environmental associations with antinuclear antibodies in a general population sample. Journal of toxicology and environmental health. Part A. 2006;69:2063-2069
- Cornelis C, Schoeters G, Kellen E, Buntinx F, Zeegers M. Development of a gis-based indicator for environmental pesticide exposure and its application to a belgian case-control study on bladder cancer. International journal of hygiene and environmental health. 2009;212:172-185
- Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of california. American journal of epidemiology. 2009;169:919-926
- Cote S, Ayotte P, Dodin S, Blanchet C, Mulvad G, Petersen HS, Gingras S, Dewailly E. Plasma organochlorine concentrations and bone ultrasound measurements: A cross-sectional study in peri-and postmenopausal inuit women from greenland. Environmental health: a global access science source. 2006;5:33
- Cox S, Niskar AS, Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. Environ Health Perspect. 2007;115:1747-1752
- Crawford JM, Hoppin JA, Alavanja MC, Blair A, Sandler DP, Kamel F. Hearing loss among licensed pesticide applicators in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:817-826
- Cupul-Uicab LA, Gladen BC, Hernandez-Avila M, Weber JP, Longnecker MP. Dde, a degradation product of ddt, and duration of lactation in a highly exposed area of mexico. Environ Health Perspect. 2008;116:179-183
- Cupul-Uicab LA, Hernandez-Avila M, Terrazas-Medina EA, Pennell ML, Longnecker MP. Prenatal exposure to the major ddt metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) and growth in boys from mexico. Environmental research. 2010;110:595-603
- Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberte C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in quebec, canada. Environmental research. 2008;108:387-392
- Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in inuit adults. Environ Health Perspect. 2009;117:1380-1386

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect. 2009;117:1014-1020
- Dallaire R, Muckle G, Rouget F, Kadhel P, Bataille H, Guldner L, Seurin S, Chajes V, Monfort C, Boucher O, Thome JP, Jacobson SW, Multigner L, Cordier S. Cognitive, visual, and motor development of 7-month-old guadeloupean infants exposed to chlordecone. Environmental research. 2012;118:79-85
- Damgaard IN, Skakkebæk NE, Toppari J, Virtanen HE, Shen H, Schramm K-W, Petersen JH, Jensen TK, Main KM. Persistent pesticides in human breast milk and cryptorchidism. Environmental Health Perspectives. 2006;114:1133-1138
- Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. Pop levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from uppsala, sweden. Environment international. 2010;36:180-187
- Dassanayake T, Gawarammana IB, Weerasinghe V, Dissanayake PS, Pragaash S, Dawson A, Senanayake N. Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2009;120:1693-1698
- Dayton SB, Sandler DP, Blair A, Alavanja M, Beane Freeman LE, Hoppin JA. Pesticide use and myocardial infarction incidence among farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2010;52:693-697
- De Fleurian G, Perrin J, Ecochard R, Dantony E, Lanteaume A, Achard V, Grillo JM, Guichaoua MR, Botta A, Sari-Minodier I. Occupational exposures obtained by questionnaire in clinical practice and their association with semen quality. Journal of andrology. 2009;30:566-579
- de Jager C, Aneck-Hahn NH, Bornman MS, Farias P, Leter G, Eleuteri P, Rescia M, Spano M. Sperm chromatin integrity in ddt-exposed young men living in a malaria area in the limpopo province, south africa. Hum Reprod. 2009;24:2429-2438
- De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, Dombrowski C, Rousseau F, Sanchez VD, Bailey JL. Reduced seminal parameters associated with environmental ddt exposure and p,p'-dde concentrations in men in chiapas, mexico: A crosssectional study. Journal of andrology. 2006;27:16-27
- de Souza A, Medeiros Ados R, de Souza AC, Wink M, Siqueira IR, Ferreira MB, Fernandes L, Loayza Hidalgo MP, Torres IL. [evaluation of the impact of exposure to pesticides on the health of the rural population: Vale do taquari, state of rio grande do sul (brazil)]. Ciencia & saude coletiva. 2011;16:3519-3528
- Delancey JO, Alavanja MC, Coble J, Blair A, Hoppin JA, Austin HD, Beane Freeman LE. Occupational exposure to metribuzin and the incidence of cancer in the agricultural health study. Annals of epidemiology. 2009;19:388-395
- Delport R, Bornman R, MacIntyre UE, Oosthuizen NM, Becker PJ, Aneck-Hahn NH, de Jager C. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to ddt. Environ Health Perspect. 2011;119:647-651

Demers PA, Davies HW, Friesen MC, Hertzman C, Ostry A, Hershler R, Teschke K. Cancer

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

and occupational exposure to pentachlorophenol and tetrachlorophenol (canada). Cancer causes & control: CCC. 2006;17:749-758

- Den Hond E, Dhooge W, Bruckers L, Schoeters G, Nelen V, van de Mieroop E, Koppen G, Bilau M, Schroijen C, Keune H, Baeyens W, van Larebeke N. Internal exposure to pollutants and sexual maturation in flemish adolescents. Journal of exposure science & environmental epidemiology. 2011;21:224-233
- Deng F, Tao FB, Liu DY, Xu YY, Hao JH, Sun Y, Su PY. Effects of growth environments and two environmental endocrine disruptors on children with idiopathic precocious puberty. European journal of endocrinology / European Federation of Endocrine Societies. 2012;166:803-809
- Dennis LK, Lowe JB, Lynch CF, Alavanja MC. Cutaneous melanoma and obesity in the agricultural health study. Annals of epidemiology. 2008;18:214-221
- Dennis LK, Lynch CF, Sandler DP, Alavanja MC. Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. Environ Health Perspect. 2010;118:812-817
- Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, Shepherd S. Pesticide/environmental exposures and parkinson's disease in east texas. Journal of agromedicine. 2008;13:37-48
- Dhooge W, Den Hond E, Koppen G, Bruckers L, Nelen V, Van De Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, Van Larebeke N. Internal exposure to pollutants and body size in flemish adolescents and adults: Associations and dose-response relationships. Environment international. 2010;36:330-337
- Dhooge W, den Hond E, Koppen G, Bruckers L, Nelen V, van de Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, van Larebeke N. Internal exposure to pollutants and sex hormone levels in flemish male adolescents in a cross-sectional study: Associations and dose-response relationships. Journal of exposure science & environmental epidemiology. 2011;21:106-113
- Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, Semple S, Dick S, Counsell C, Mozzoni P, Haites N, Wettinger SB, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A. Environmental risk factors for Parkinson's disease and Parkinsonism: The geoparkinson study. Occupational and environmental medicine. 2007;64:666-672
- Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, Mertens I, Van Gaal L. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring). 2011;19:709-714
- Djordjevic M, Sazdanovic P, Djordjevic G, Jovanovic B. Morbidity in newborns exposed to organophosphorus pesticides. Medicinski pregled. 2010;63:414-417
- Dugas J, Nieuwenhuijsen MJ, Martinez D, Iszatt N, Nelson P, Elliott P. Use of biocides and insect repellents and risk of hypospadias. Occupational and environmental medicine. 2010;67:196-200
- Duk-Hee Lee, Michael W. Steffes, Andreas Sjodin, Richard S. Jones, Larry L. Needham, David R., Jacobs Jr. Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. PLoS ONE 2011; 6(1): e15977

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Eckerman DA, Gimenes LS, de Souza RC, Galvao PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in brazil. Neurotoxicology and teratology. 2007;29:164-175
- Eggesbo M, Stigum H, Longnecker MP, Polder A, Aldrin M, Basso O, Thomsen C, Skaare JU, Becher G, Magnus P. Levels of hexachlorobenzene (hcb) in breast milk in relation to birth weight in a norwegian cohort. Environmental research. 2009;109:559-566
- El-Helaly M, Abdel-Elah K, Haussein A, Shalaby H. Paternal occupational exposures and the risk of congenital malformations--a case-control study. International journal of occupational medicine and environmental health. 2011;24:218-227
- Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, Alperovitch A, Tzourio C. Professional exposure to pesticides and parkinson disease. Annals of neurology. 2009;66:494-504
- Elobeid MA, Padilla MA, Brock DW, Ruden DM, Allison DB. Endocrine disruptors and obesity: An examination of selected persistent organic pollutants in the nhanes 1999-2002 data. International journal of environmental research and public health. 2010;7:2988-3005
- Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal organophosphate metabolite and organochlorine levels and performance on the brazelton neonatal behavioral assessment scale in a multiethnic pregnancy cohort. American journal of epidemiology. 2007;165:1397-1404
- Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 2011;119:1182-1188
- English RG, Perry M, Lee MM, Hoffman E, Delport S, Dalvie MA. Farm residence and reproductive health among boys in rural South Africa. Environment international. 2012;47:73-79
- Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for nonhodgkin lymphoma including histopathological subgroup analysis. International journal of cancer. Journal international du cancer. 2008;123:1657-1663
- Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, Jewell NP. In utero exposure to dichlorodiphenyltrichloroethane (ddt) and dichlorodiphenyldichloroethylene (dde) and neurodevelopment among young mexican american children. Pediatrics. 2006;118:233-241
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate pesticide exposure and neurodevelopment in young mexican-american children. Environ Health Perspect. 2007;115:792-798
- Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Jr., Mainous AG, 3rd. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and ddt with diabetes in the 1999-2002 national health and nutrition examination survey. Environmental research. 2007;103:413-418
- Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination survey. Environment international. 2010;36:398-401

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

F. Giordano, V. Dell'Orco, G. Galante, F. Giannandrea,

- Fang F, Quinlan P, Ye W, Barber MK, Umbach DM, Sandler DP, Kamel F. Workplace exposures and the risk of amyotrophic lateral sclerosis. Environ Health Perspect. 2009;117:1387-1392
- Farooq U, Joshi M, Nookala V, Cheriyath P, Fischman D, Graber NJ, Stellman SD, Muscat J. Self-reported exposure to pesticides in residential settings and risk of breast cancer: A casecontrol study. Environmental health : a global access science source. 2010;9:30
- Farr SL, Cai J, Savitz DA, Sandler DP, Hoppin JA, Cooper GS. Pesticide exposure and timing of menopause: The agricultural health study. American journal of epidemiology. 2006;163:731-742
- Fatemeh Tohidia, Farzaneh Farrokhib, Ali Taravatic. Effects of pesticide on the thyroid hormones of pesticide sprayers living in Mazandaran. Abstracts. doi:10.1016/j.clinbiochem.2011.08.1063
- Fear NT, Hey K, Vincent T, Murphy M. Paternal occupation and neural tube defects: A casecontrol study based on the oxford record linkage study register. Paediatric and perinatal epidemiology. 2007;21:163-168
- Fear NT, Vincent TJ, King JC, MacCarthy A, Bunch KJ, Murphy MF. Wilms tumour and paternal occupation: An analysis of data from the national registry of childhood tumours. Pediatric blood & cancer. 2009;53:28-32
- Feldman AL, Johansson AL, Nise G, Gatz M, Pedersen NL, Wirdefeldt K. Occupational exposure in parkinsonian disorders: A 43-year prospective cohort study in men. Parkinsonism & related disorders. 2011;17:677-682
- Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: Results of a casecontrol study. Birth defects research. Part A, Clinical and molecular teratology. 2008;82:98-105
- Feng Hong qi, Yang Lin, Guo Ling, Huang Wen li, Xu Bo nan, LI Zhao Xiang, Wang Tong. A case control study on the risk factors of Yunnan endemic sudden cardiac death. Chin J Endemiol Jul 20 2005 Vol 24 No.4 ; 1000-4955 2005 04-0414-03
- Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, Hubbard A, Barr DB. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environmental Health Perspectives. 2005;114:597-602
- Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. In utero exposure to ddt and performance on the brazelton neonatal behavioral assessment scale. Neurotoxicology. 2007;28:471-477
- Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p'-dde, hcb, pcbs and reproductive hormones among men of reproductive age. Reprod Toxicol. 2012;34:429-435
- Fernandez MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez JM, Cruz M, Olea-Serrano F, Olea N. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: A nested case-control study.

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Environ Health Perspect. 2007;115 Suppl 1:8-14

- Fieten KB, Kromhout H, Heederik D, van Wendel de Joode B. Pesticide exposure and respiratory health of indigenous women in costa rica. American journal of epidemiology. 2009;169:1500-1506
- Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Jr., Checkoway H. Occupational factors and risk of Parkinson's disease: A populationbased case-control study. American journal of industrial medicine. 2010;53:217-223
- Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, Cheng CW. Pesticide exposure on southwestern Taiwanese with mnsod and nqo1 polymorphisms is associated with increased risk of parkinson's disease. Clinica chimica acta; international journal of clinical chemistry. 2007;378:136-141
- Fortes C, Mastroeni S, Melchi F, Pilla MA, Alotto M, Antonelli G, Camaione D, Bolli S, Luchetti E, Pasquini P. The association between residential pesticide use and cutaneous melanoma. Eur J Cancer. 2007;43:1066-1075
- Freeman LE, Rusiecki JA, Hoppin JA, Lubin JH, Koutros S, Andreotti G, Zahm SH, Hines CJ, Coble JB, Barone-Adesi F, Sloan J, Sandler DP, Blair A, Alavanja MC. Atrazine and cancer incidence among pesticide applicators in the agricultural health study (1994-2007). Environ Health Perspect. 2011;119:1253-1259
- Freire C, Lopez-Espinosa MJ, Fernandez M, Molina-Molina JM, Prada R, Olea N. Prenatal exposure to organochlorine pesticides and tsh status in newborns from southern spain. The Science of the total environment. 2011;409:3281-3287
- Freire C, Ramos R, Amaya E, Fernandez MF, Santiago-Fernandez P, Lopez-Espinosa MJ, Arrebola JP, Olea N. Newborn tsh concentration and its association with cognitive development in healthy boys. European journal of endocrinology / European Federation of Endocrine Societies. 2010;163:901-909
- Friesen MC, Davies HW, Teschke K, Ostry AS, Hertzman C, Demers PA. Impact of the specificity of the exposure metric on exposure-response relationships. Epidemiology. 2007;18:88-94
- Frigerio R, Sanft KR, Grossardt BR, Peterson BJ, Elbaz A, Bower JH, Ahlskog JE, de Andrade M, Maraganore DM, Rocca WA. Chemical exposures and Parkinson's disease: A population-based case-control study. Movement disorders: official journal of the Movement Disorder Society. 2006;21:1688-1692
- Fritschi L, Glass DC, Tabrizi JS, Leavy JE, Ambrosini GL. Occupational risk factors for prostate cancer and benign prostatic hyperplasia: A case-control study in Western Australia. Occupational and environmental medicine. 2007;64:60-65
- Gabel P, Jensen MS, Andersen HR, Baelum J, Thulstrup AM, Bonde JP, Toft G. The risk of cryptorchidism among sons of women working in horticulture in Denmark: A cohort study. Environmental health: a global access science source. 2011;10:100
- Gallagher RP, Macarthur AC, Lee TK, Weber JP, Leblanc A, Mark Elwood J, Borugian M, Abanto Z, Spinelli JJ. Plasma levels of polychlorinated biphenyls and risk of cutaneous malignant melanoma: A preliminary study. International journal of cancer. Journal international du cancer. 2011;128:1872-1880

Ganesh B, Sushama S, Monika S, Suvarna P. A case-control study of risk factors for lung

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

cancer in mumbai, India. Asian Pacific journal of cancer prevention: APJCP. 2011;12:357-362

- Garced S, Torres-Sanchez L, Cebrian ME, Claudio L, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and child growth during the first year of life. Environmental research. 2012;113:58-62
- Gascon M, Vrijheid M, Martinez D, Ballester F, Basterrechea M, Blarduni E, Esplugues A, Vizcaino E, Grimalt JO, Morales E, Sunyer J. Pre-natal exposure to dichlorodiphenyldichloroethylene and infant lower respiratory tract infections and wheeze. The European respiratory journal. 2012;39:1188-1196
- Gaspari L, Paris F, Jandel C, Kalfa N, Orsini M, Daures JP, Sultan C. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: A nested case-control study. Hum Reprod. 2011;26:3155-3162
- Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B. Well-water consumption and parkinson's disease in rural california. Environ Health Perspect. 2009;117:1912-1918
- Gatto NM, Longnecker MP, Press MF, Sullivan-Halley J, McKean-Cowdin R, Bernstein L. Serum organochlorines and breast cancer: A case-control study among african-american women. Cancer causes & control: CCC. 2007;18:29-39
- German D, Roy A, Shalat S, Buckley B, Gearing M, Levey A, Richardson J. A ddt metabolite is elevated in the serum of alzheimer's disease patients. Alzheimer's & Dementia. 2012;8:P496
- Gian S. Jhangri, Colin L. Soskolne, Giovanni Pagano, Gerardo Botte, Patrizia Di Cintio. Alcohol and tobacco variables in the assessment of internal validity in an
- Giannandrea F, Gandini L, Paoli D, Turci R, Figa-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:780-787
- Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figa-Talamanca I. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth defects research. Part A, Clinical and molecular teratology. 2010;88:241-250
- Giordano F, Dell'Orco V, Giannandrea F, Lauria L, Valente P, Figa-Talamanca I. Mortality in a cohort of pesticide applicators in an urban setting: Sixty years of follow-up. International journal of immunopathology and pharmacology. 2006;19:61-65
- Giwercman A, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, Lindh C, Pedersen HS, Ludwicki JK, Lesovoy V, Shvets M, Spano M, Manicardi GC, Bizzaro D, Bonefeld-Jorgensen EC, Bonde JP. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three european cohorts. Environmental Health Perspectives. 2006;114:1348-1353
- Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S. Immune cell counts and risks of respiratory infections among infants exposed pre- and postnatally to organochlorine compounds: A prospective study. Environmental health : a global access science source. 2008;7:62

Gold LS, Ward MH, Dosemeci M, De Roos AJ. Systemic autoimmune disease mortality and

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupational exposures. Arthritis and rheumatism. 2007;56:3189-3201

- Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the agricultural health study. American journal of epidemiology. 2010;171:455-464
- Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of pcb congeners and chlorinated pesticides. Environ Health Perspect. 2011;119:319-325
- Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, Morse G, Carpenter DO. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native american men. Environ Health Perspect. 2009;117:1454-1460
- Grandjean P, Harari R, Barr DB, Debes F. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in ecuadorian school children. Pediatrics. 2006;117:e546-556
- Greenburg DL, Rusiecki J, Koutros S, Dosemeci M, Patel R, Hines CJ, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to captan in the agricultural health study. Cancer causes & control : CCC. 2008;19:1401-1407
- Guillette EA, Conard C, Lares F, Aguilar MG, McLachlan J, Guillette LJ. Altered breast development in young girls from an agricultural environment. Environmental Health Perspectives. 2005;114:471-475
- Gunnar Toft, Allan Flyvbjerg and Jens Peter Bonde. A GlobalAccess Science Source. Environmental Health 2006; 5: 32 32
- Guodong D, Pei W, Ying T, Jun Z, Yu G, Xiaojin W, Rong S, Guoquan W, Xiaoming S. Organophosphate pesticide exposure and neurodevelopment in young shanghai children. Environmental science & technology. 2012;46:2911-2917
- H Yu, X Liu, K Kezios, O Kalantzi, YWang, M Petreas, J-S Park, P Cirillio, B Cohn, P Factor-Litvak. Prenatal organochlorine exposure maternal thyroid function and neuromotor development. Am J Epidemiol. 2011;173(Suppl):S1–S316
- Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the national health and nutrition examination survey, 1999-2002. Environ Health Perspect. 2007;115:1204-1209
- Ha MH, Lee DH, Son HK, Park SK, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999-2002. Journal of human hypertension. 2009;23:274-286
- Hadjigeorgiou GM, Stefanidis I, Dardiotis E, Aggellakis K, Sakkas GK, Xiromerisiou G, Konitsiotis S, Paterakis K, Poultsidi A, Tsimourtou V, Ralli S, Gourgoulianis K, Zintzaras E. Low rls prevalence and awareness in central greece: An epidemiological survey. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2007;14:1275-1280
- Han Y, Xia Y, Han J, Zhou J, Wang S, Zhu P, Zhao R, Jin N, Song L, Wang X. The relationship of 3-pba pyrethroids metabolite and male reproductive hormones among non-

EFSA supporting publication 2013:EN-497

<sup>126</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupational exposure males. Chemosphere. 2008;72:785-790

- Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of parkinson's disease: A family-based case-control study. BMC neurology. 2008;8:6
- Handal AJ, Harlow SD, Breilh J, Lozoff B. Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. Epidemiology. 2008;19:851-859
- Handal AJ, Lozoff B, Breilh J, Harlow SD. Neurobehavioral development in children with potential exposure to pesticides. Epidemiology. 2007;18:312-320
- Harari R, Julvez J, Murata K, Barr D, Bellinger DC, Debes F, Grandjean P. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect. 2010;118:890-896
- Hardell L, Andersson SO, Carlberg M, Bohr L, van Bavel B, Lindstrom G, Bjornfoth H, Ginman C. Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:700-707
- Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. International journal of andrology. 2006;29:228-234
- Hardell L, Carlberg M, Hardell K, Bjornfoth H, Wickbom G, Ionescu M, van Bavel B, Lindstrom G. Decreased survival in pancreatic cancer patients with high concentrations of organochlorines in adipose tissue. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2007;61:659-664
- Hardell. Concentrations of organohalogen compounds and titres of antibodies to epstein-barr virus antigens and the risk for non-hodgkin lymphoma. Oncology Reports. 2009;21
- Harley KG, Marks AR, Bradman A, Barr DB, Eskenazi B. Ddt exposure, work in agriculture, and time to pregnancy among farmworkers in california. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:1335-1342
- Hashemi N, Mirsadraee M, Shakeri MT, Varasteh AR. Prevalence of work-related respiratory symptoms in iranian farmers. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2006;13:198-202
- Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, Welsh-Bohmer KA. Occupational exposure to pesticides increases the risk of incident ad: The cache county study. Neurology. 2010;74:1524-1530
- Herin F, Boutet-Robinet E, Levant A, Dulaurent S, Manika M, Galatry-Bouju F, Caron P, Soulat JM. Thyroid function tests in persons with occupational exposure to fipronil. Thyroid : official journal of the American Thyroid Association. 2011;21:701-706
- Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. Inhalation toxicology. 2008;20:839-849

Hernandez-Morales AL, Zonana-Nacach A, Zaragoza-Sandoval VM. [associated risk factors in

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

acute leukemia in children. A cases and controls study]. Revista medica del Instituto Mexicano del Seguro Social. 2009;47:497-503

- Hodgson S, Thomas L, Fattore E, Lind PM, Alfven T, Hellstrom L, Hakansson H, Carubelli G, Fanelli R, Jarup L. Bone mineral density changes in relation to environmental pcb exposure. Environ Health Perspect. 2008;116:1162-1166
- Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA, Demers PA, Blair A. Exposure to multiple pesticides and risk of non-hodgkin lymphoma in men from six canadian provinces. International journal of environmental research and public health. 2011;8:2320-2330
- Hoppin JA, Umbach DM, Kullman GJ, Henneberger PK, London SJ, Alavanja MC, Sandler DP. Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the agricultural health study. Occupational and environmental medicine. 2007;64:334-341
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the agricultural health study. American journal of respiratory and critical care medicine. 2008;177:11-18
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MC, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the agricultural health study. The European respiratory journal. 2009;34:1296-1303
- Hoppin JA, Umbach DM, London SJ, Lynch CF, Alavanja MC, Sandler DP. Pesticides and adult respiratory outcomes in the agricultural health study. Annals of the New York Academy of Sciences. 2006;1076:343-354
- Hoppin JA, Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP. Pesticide use and chronic bronchitis among farmers in the agricultural health study. American journal of industrial medicine. 2007;50:969-979
- Horton MK, Rundle A, Camann DE, Boyd Barr D, Rauh VA, Whyatt RM. Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. Pediatrics. 2011;127:e699-706
- Hossain F, Ali O, D'Souza UJ, Naing DK. Effects of pesticide use on semen quality among farmers in rural areas of sabah, malaysia. Journal of occupational health. 2010;52:353-360
- Hou L, Lee WJ, Rusiecki J, Hoppin JA, Blair A, Bonner MR, Lubin JH, Samanic C, Sandler DP, Dosemeci M, Alavanja MC. Pendimethalin exposure and cancer incidence among pesticide applicators. Epidemiology. 2006;17:302-307
- Huisman M.H.B., De Jong D.E., Van Doormaa P.T.C., Vermeulen R., Heederick D., Kromhout H., Schelhaas H.J., Van Der Kooi A.J., De VISSER M., Veldink J.H., Van Den Berg A.H. Exogenous risk factors in ALS: A population-based case-control study. Amyotrophic Lateral Sclerosis (2011) 12 SUPPL. 1 (23).
- Issaragrisil S, Kaufman DW, Anderson T, Chansung K, Leaverton PE, Shapiro S, Young NS. The epidemiology of aplastic anemia in thailand. Blood. 2006;107:1299-1307
- Itoh H, Iwasaki M, Hanaoka T, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S. Serum organochlorines and breast cancer risk in japanese women: A casecontrol study. Cancer causes & control : CCC. 2009;20:567-580

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Iwasaki M, Inoue M, Sasazuki S, Kurahashi N, Itoh H, Usuda M, Tsugane S. Plasma organochlorine levels and subsequent risk of breast cancer among japanese women: A nested case-control study. The Science of the total environment. 2008;402:176-183
- J R Suarez-Lopez, J H Himes, D R Jacobs, Jr., B H Alexander, D Lazovich, M Gunnar. Secondary pesticide exposure is associated with head circumference and growth in children. Am J Epidemiol. 2012;175(11 Suppl):S1–S145
- Jansson C, Plato N, Johansson AL, Nyren O, Lagergren J. Airborne occupational exposures and risk of oesophageal and cardia adenocarcinoma. Occupational and environmental medicine. 2006;63:107-112
- Jeebhay MF, Baatjies R, Chang YS, Kim YK, Kim YY, Major V, Lopata AL. Risk factors for allergy due to the two-spotted spider mite (tetranychus urticae) among table grape farm workers. International archives of allergy and immunology. 2007;144:143-149
- Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, Wang S, Wang X. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in chinese men. Reprod Toxicol. 2011;31:171-176
- Jimenez-Jimenez FJ, de Toledo-Heras M, Alonso-Navarro H, Ayuso-Peralta L, Arevalo-Serrano J, Ballesteros-Barranco A, Puertas I, Jabbour-Wadih T, Barcenilla B. Environmental risk factors for essential tremor. European neurology. 2007;58:106-113
- Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland inuit. Diabetologia. 2008;51:1416-1422
- Jurewicz J, Hanke W, Makowiec-Dabrowska T. [low risk of reproductive disorders among female greenhouse workers--safe work conditions or health selection for the light work?]. Medycyna pracy. 2008;59:123-131
- Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. Epidemiology. 2012;23:689-698
- Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I. Maternal ddt exposures in relation to fetal and 5-year growth. Epidemiology. 2006;17:692-700
- Kallioniemi MK, Simola AJ, Kymalainen HR, Vesala HT, Louhelainen JK. Mental symptoms among Finnish farm entrepreneurs. Annals of agricultural and environmental medicine: AAEM. 2009;16:159-168
- Kamalesh Das, Chiranjib Nag and Mrinalkanti Ghosh. Familial, Environmental, and Occupational Risk Factors in Development of Amyotrophic Lateral Sclerosis. North American Journal of Medical Sciences 2012.
- Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed pesticide applicators in the agricultural health study. Human & experimental toxicology. 2007;26:243-250
- Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported parkinson's disease in the agricultural health study. American journal of epidemiology. 2007;165:364-374

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, Hoppin JA, Dosemeci M, Coble J, Lubin J, Blair A, Alavanja M. Cancer incidence among pesticide applicators exposed to trifluralin in the agricultural health study. Environmental research. 2008;107:271-276
- Karami S, Boffetta P, Rothman N, Hung RJ, Stewart T, Zaridze D, Navritalova M, Mates D, Janout V, Kollarova H, Bencko V, Szeszenia-Dabrowska N, Holcatova I, Mukeria A, Gromiec J, Chanock SJ, Brennan P, Chow WH, Moore LE. Renal cell carcinoma, occupational pesticide exposure and modification by glutathione s-transferase polymorphisms. Carcinogenesis. 2008;29:1567-1571
- Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH. Hodgkin lymphoma and pesticides exposure in men: A Canadian case-control study. Journal of agromedicine. 2012;17:30-39
- Kaufman DW, Anderson TE, Issaragrisil S. Risk factors for leukemia in Thailand. Annals of hematology. 2009;88:1079-1088
- Kelada SN, Checkoway H, Kardia SL, Carlson CS, Costa-Mallen P, Eaton DL, Firestone J, Powers KM, Swanson PD, Franklin GM, Longstreth WT, Jr., Weller TS, Afsharinejad Z, Costa LG. 5' and 3' region variability in the dopamine transporter gene (slc6a3), pesticide exposure and parkinson's disease risk: A hypothesis-generating study. Human molecular genetics. 2006;15:3055-3062
- Khanjani N, Sim MR. Maternal contamination with dichlorodiphenyltrichloroethane and reproductive outcomes in an Australian population. Environmental research. 2006;101:373-379
- Khanjani N, Sim MR. Reproductive outcomes of maternal contamination with cyclodiene insecticides, hexachlorobenzene and beta-benzene hexachloride. The Science of the total environment. 2006;368:557-564
- Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M. Gst polymorphisms, interaction with smoking and pesticide use, and risk for parkinson's disease in a japanese population. Parkinsonism & related disorders. 2010;16:447-452
- Koutros S, Andreotti G, Berndt SI, Hughes Barry K, Lubin JH, Hoppin JA, Kamel F, Sandler DP, Burdette LA, Yuenger J, Yeager M, Alavanja MC, Freeman LE. Xenobioticmetabolizing gene variants, pesticide use, and the risk of prostate cancer. Pharmacogenetics and genomics. 2011;21:615-623
- Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. Heterocyclic aromatic amine pesticide use and human cancer risk: Results from the u.S. Agricultural health study. International journal of cancer. Journal international du cancer. 2009;124:1206-1212
- Koutros S, Mahajan R, Zheng T, Hoppin JA, Ma X, Lynch CF, Blair A, Alavanja MC. Dichlorvos exposure and human cancer risk: Results from the agricultural health study. Cancer causes & control: CCC. 2008;19:59-65
- Kouznetsova M, Huang X, Ma J, Lessner L, Carpenter DO. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. Environmental Health

EFSA supporting publication 2013:EN-497

<sup>130</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Perspectives. 2006;115:75-79

- Kumar V, Yadav CS, Singh S, Goel S, Ahmed RS, Gupta S, Grover RK, Banerjee BD. Cyp 1a1 polymorphism and organochlorine pesticides levels in the etiology of prostate cancer. Chemosphere. 2010;81:464-468
- L. Lind, D. Lee, D.R. Jacobs, S. Salihovic, B. vanBavel, P.M. Lind. Are persistent organic pollutants associated with obesity, the metabolic syndrome or both? Abstracts / Toxicology Letters 205S (2011) S60–S179
- Lacasana M, Lopez-Flores I, Rodriguez-Barranco M, Aguilar-Garduno C, Blanco-Munoz J, Perez-Mendez O, Gamboa R, Bassol S, Cebrian ME. Association between organophosphate pesticides exposure and thyroid hormones in floriculture workers. Toxicology and applied pharmacology. 2010;243:19-26
- Lacasana M, Vazquez-Grameix H, Borja-Aburto VH, Blanco-Munoz J, Romieu I, Aguilar-Garduno C, Garcia AM. Maternal and paternal occupational exposure to agricultural work and the risk of an encephaly. Occupational and environmental medicine. 2006;63:649-656
- Laden F, Bertrand KA, Altshul L, Aster JC, Korrick SA, Sagiv SK. Plasma organochlorine levels and risk of non-hodgkin lymphoma in the nurses' health study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010;19:1381-1384
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV, Alavanja MC. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the agricultural health study. Blood. 2009;113:6386-6391
- Langer P, Kocan A, Drobna B, Susienkova K, Radikova Z, Huckova M, Imrich R, Ksinantova L, Klimes I. Polychlorinated biphenyls and testosterone: Age and congener related correlation approach in heavily exposed males. Endocrine regulations. 2010;44:109-114
- Langer P, Kocan A, Tajtakova M, Koska J, Radikova Z, Ksinantova L, Imrich R, Huckova M, Drobna B, Gasperikova D, Sebokova E, Klimes I. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: Outcome of transgenerational transmission? Chemosphere. 2008;73:1145-1150
- Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Radikova Z, Koska J, Ksinantova L, Huckova M, Imrich R, Wimmerova S, Gasperikova D, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Fish from industrially polluted freshwater as the main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere. 2007;67:S379-385
- Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. Chemosphere. 2007;69:118-127
- Lauria L, Settimi L, Spinelli A, Figa-Talamanca I. Exposure to pesticides and time to pregnancy among female greenhouse workers. Reprod Toxicol. 2006;22:425-430
- Lee DH, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and gamma glutamyltransferase: Results from the national health and

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

examination survey 1999-2002. Clinical chemistry. 2006;52:1825-1827

- Lee DH, Jacobs DR, Jr., Steffes M. Association of organochlorine pesticides with peripheral neuropathy in patients with diabetes or impaired fasting glucose. Diabetes. 2008;57:3108-3111
- Lee DH, Jacobs DR, Kocher T. Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. Environ Health Perspect. 2008;116:1558-1562
- Lee DH, Jacobs DR, Porta M. Association of serum concentrations of persistent organic pollutants with the prevalence of learning disability and attention deficit disorder. Journal of epidemiology and community health. 2007;61:591-596
- Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetes care. 2007;30:622-628
- Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetologia. 2007;50:1841-1851
- Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr. A strong doseresponse relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. Diabetes care. 2006;29:1638-1644
- Lee DH, Lee IK, Steffes M, Jacobs DR, Jr. Extended analyses of the association between serum concentrations of persistent organic pollutants and diabetes. Diabetes care. 2007;30:1596-1598
- Lee DH, Lind L, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind PM. Associations of persistent organic pollutants with abdominal obesity in the elderly: The prospective investigation of the vasculature in uppsala seniors (pivus) study. Environment international. 2012;40:170-178
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Background exposure to persistent organic pollutants predicts stroke in the elderly. Environment international. 2012;47:115-120
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: The prospective investigation of the vasculature in Uppsala seniors (pivus) study. Diabetes care. 2011;34:1778-1784
- Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect. 2007;115:883-888
- Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose of some persistent organic pollutants predicts type 2 diabetes: A nested case-control study. Environ Health Perspect. 2010;118:1235-1242

Lee WJ, Alavanja MC, Hoppin JA, Rusiecki JA, Kamel F, Blair A, Sandler DP. Mortality

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

among pesticide applicators exposed to chlorpyrifos in the agricultural health study. Environ Health Perspect. 2007;115:528-534

- Lee WJ, Baccarelli A, Tretiakova M, Gorbanev S, Lomtev A, Klimkina I, Tchibissov V, Averkina O, Dosemeci M. Pesticide exposure and lung cancer mortality in leningrad province in russia. Environment international. 2006;32:412-416
- Lee WJ, Purdue MP, Stewart P, Schenk M, De Roos AJ, Cerhan JR, Severson RK, Cozen W, Hartge P, Blair A. Asthma history, occupational exposure to pesticides and the risk of nonhodgkin's lymphoma. International journal of cancer. Journal international du cancer. 2006;118:3174-3176
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC. Pesticide use and colorectal cancer risk in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:339-346
- LeVan TD, Koh WP, Lee HP, Koh D, Yu MC, London SJ. Vapor, dust, and smoke exposure in relation to adult-onset asthma and chronic respiratory symptoms: The singapore chinese health study. American journal of epidemiology. 2006;163:1118-1128
- Li J, Lu Y, Shi Y, Wang T, Wang G, Luo W, Jiao W, Chen C, Yan F. Environmental pollution by persistent toxic substances and health risk in an industrial area of china. Journal of Environmental Sciences. 2011;23:1359-1367
- Li JY, Li H, Tao P, Lei FM. [serum organochlorines pesticides level of non-occupational exposure women and risk of breast cancer:A case-control study]. Wei sheng yan jiu = Journal of hygiene research. 2006;35:391-394
- Li JY, Wu DS, Yang F, Zeng HY, Lei FM, Zhou WD, Li H, Tao P. [study on serum organochlorines pesticides (ddts) level, cyp1a1 genetic polymorphism and risk of breast cancer: A case control study]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:217-222
- Lifeng T, Shoulin W, Junmin J, Xuezhao S, Yannan L, Qianli W, Longsheng C. Effects of fenvalerate exposure on semen quality among occupational workers. Contraception. 2006;73:92-96
- Lim JS, Son HK, Park SK, Jacobs DR, Jr., Lee DH. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond). 2011;35:744-747
- Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (pops) and carotid atherosclerosis in the elderly. Environ Health Perspect. 2012;120:38-43
- Liu B, Jung KH, Horton MK, Camann DE, Liu X, Reardon AM, Perzanowski MS, Zhang H, Perera FP, Whyatt RM, Miller RL. Prenatal exposure to pesticide ingredient piperonyl butoxide and childhood cough in an urban cohort. Environment international. 2012;48:156-161
- Lizardi PS, O'Rourke MK, Morris RJ. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of pediatric psychology. 2008;33:91-101
- Lo AC, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, Omar S, Hamilton SR, Greenson JK, Abbruzzese JL. Lifestyle, occupational, and reproductive

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

factors in relation to pancreatic cancer risk. Pancreas. 2007;35:120-129

- Lo AC, Soliman AS, Khaled HM, Aboelyazid A, Greenson JK. Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. Diseases of the colon and rectum. 2010;53:830-837
- Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, Hernandez-Avila M. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) in relation to anogenital distance in male newborns from chiapas, mexico. American journal of epidemiology. 2007;165:1015-1022
- Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Zock JP, Pollan M. Occupation, exposure to chemicals, sensitizing agents, and risk of multiple myeloma in Sweden. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:3123-3127
- Lopez-Espinosa MJ, Granada A, Carreno J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from southern spain and some related factors. Placenta. 2007;28:631-638
- Lopez-Espinosa MJ, Murcia M, Iniguez C, Vizcaino E, Llop S, Vioque J, Grimalt JO, Rebagliato M, Ballester F. Prenatal exposure to organochlorine compounds and birth size. Pediatrics. 2011;128:e127-134
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, Grimalt JO, Ballester F. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. Journal of exposure science & environmental epidemiology. 2010;20:579-588
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, Marco A, Rebagliato M, Grimalt JO, Ballester F. Association between thyroid hormone levels and 4,4'-dde concentrations in pregnant women (valencia, spain). Environmental research. 2009;109:479-485
- Louis ED, Factor-Litvak P, Parides M, Andrews L, Santella RM, Wolff MS. Organochlorine pesticide exposure in essential tremor: A case-control study using biological and occupational exposure assessments. Neurotoxicology. 2006;27:579-586
- Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. American journal of public health. 2011;101:63-70
- Lu JL. Comparison of pesticide exposure and physical examination, neurological assessment, and laboratory findings between full-time and part-time vegetable farmers in the Philippines. Environmental health and preventive medicine. 2009;14:345-352
- Lu JL. Occupational safety of farmers in the vegetable industry. International journal of occupational safety and ergonomics: JOSE. 2011;17:445-453
- Luis D Boada, Manuel Zumbado, Luis Alberto Henríquez-Hernández, Maira Almeida-González, Eva E Álvarez-León, Lluis Serra-Majem and Octavio P Luzardo. Complex organochlorine pesticide mixtures as determinant factor for breast cancer risk: a populationbased case–control study in theCanary Islands (Spain). Environmental Health 2012, 11:28

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Lv L, Lin G, Gao X, Wu C, Dai J, Yang Y, Zou H, Sun H, Gu M, Chen X, Fu H, Bao L. Casecontrol study of risk factors of myelodysplastic syndromes according to world health organization classification in a chinese population. American journal of hematology. 2011;86:163-169
- Lv L, Lin GW, Wang XQ, Bao LM, Zou HJ. [a case-control study of risk factors for myelodysplastic syndromes]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2007;25:705-709
- Lynch SM, Mahajan R, Beane Freeman LE, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to butylate in the agricultural health study (ahs). Environmental research. 2009;109:860-868
- Lynch SM, Rusiecki JA, Blair A, Dosemeci M, Lubin J, Sandler D, Hoppin JA, Lynch CF, Alavanja MCR. Cancer incidence among pesticide applicators exposed to cyanazine in the agricultural health study. Environmental Health Perspectives. 2006;114:1248-1252
- MacCarthy A, Bunch KJ, Fear NT, King JC, Vincent TJ, Murphy MF. Paternal occupation and neuroblastoma: A case-control study based on cancer registry data for Great Britain 1962-1999. British journal of cancer. 2010;102:615-619
- MacFarlane E, Simpson P, Benke G, Sim MR. Suicide in australian pesticide-exposed workers. Occup Med (Lond). 2011;61:259-264
- Mackenzie Ross SJ, Brewin CR, Curran HV, Furlong CE, Abraham-Smith KM, Harrison V. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicology and teratology. 2010;32:452-459
- Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect. 2007;115:1780-1786
- Mahajan R, Blair A, Coble J, Lynch CF, Hoppin JA, Sandler DP, Alavanja MC. Carbaryl exposure and incident cancer in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:1799-1805
- Mahajan R, Bonner MR, Hoppin JA, Alavanja MCR. Phorate exposure and incidence of cancer in the agricultural health study. Environmental Health Perspectives. 2006;114:1205-1209
- Maluf E, Hamerschlak N, Cavalcanti AB, Junior AA, Eluf-Neto J, Falcao RP, Lorand-Metze IG, Goldenberg D, Santana CL, Rodrigues Dde O, Passos LN, Rosenfeld LG, Pitta M, Loggetto S, Ribeiro AA, Velloso ED, Kondo AT, Coelho EO, Pintao MC, de Souza HM, Borbolla JR, Pasquini R. Incidence and risk factors of aplastic anemia in latin american countries: The latin case-control study. Haematologica. 2009;94:1220-1226
- Manfo FPT, Moundipa PF, Déchaud H, Tchana AlN, Nantia EA, Zabot M-T, Pugeat M. Effect of agropesticides use on male reproductive function: A study on farmers in djutitsa (cameroon). Environmental Toxicology. 2012;27:423-432
- Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and parkinson disease. Epidemiology. 2010;21:87-94

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Marina Krstevska Konstantinova. Organochlorine pesticides in Macedonain girls

- Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. Organophosphate pesticide exposure and attention in young mexican-american children: The chamacos study. Environ Health Perspect. 2010;118:1768-1774
- Marzouk D.A., El Gaafary M.M., El Damaty S.I., Sabbour S.M., Mecky F.A.S., Saker M., Sayed A.M., Fahim H.I., Anwar W.A.. Breast cancer and hormonal intake among Egyptian females. European Journal of Oncology (2009) 14:1 (37-51).
- Matmurodov R.J., Khalimova K.M., Raimova M.M. Polymorphism of the genes GSTM1, GSTT1, and environmental factors in the development of Parkinson's disease among representatives of the Uzbek nationality. European Journal of Neurology (2011) 18 SUPPL. 2 (501).
- McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ. Environmental exposure to polychlorinated biphenyls and p,p'-dde and sperm sex-chromosome disomy. Environ Health Perspect. 2012;120:535-540
- McDuffie HH, Quail J, Ghosh S, Pahwa P. Host factors, occupation, and testicular cancer in saskatchewan, canada: 1979-2002. Journal of agricultural safety and health. 2007;13:247-258
- McElroy JA, Gangnon RE, Newcomb PA, Kanarek MS, Anderson HA, Brook JV, Trentham-Dietz A, Remington PL. Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in wisconsin. Journal of exposure science & environmental epidemiology. 2007;17:207-214
- McGlynn KA, Abnet CC, Zhang M, Sun XD, Fan JH, O'Brien TR, Wei WQ, Ortiz-Conde BA, Dawsey SM, Weber JP, Taylor PR, Katki H, Mark SD, Qiao YL. Serum concentrations of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (ddt) and 1,1-dichloro-2,2-bis(pchlorophenyl)ethylene (dde) and risk of primary liver cancer. Journal of the National Cancer Institute. 2006;98:1005-1010
- McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of the National Cancer Institute. 2008;100:663-671
- McHugh MK, Kachroo S, Liu M, D'Amelio AM, Jr., Dong Q, Hong WK, Greisinger AJ, Spitz MR, Etzel CJ. Assessing environmental and occupational risk factors for lung cancer in mexican-americans. Cancer causes & control: CCC. 2010;21:2157-2164
- Meeker JD, Altshul L, Hauser R. Serum pcbs, p,p'-dde and hcb predict thyroid hormone levels in men. Environmental research. 2007;104:296-304
- Meeker JD, Barr DB, Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Hum Reprod. 2008;23:1932-1940
- Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol. 2009;27:155-160
- Meeker JD, Barr DB, Hauser R. Thyroid hormones in relation to urinary metabolites of nonpersistent insecticides in men of reproductive age. Reprod Toxicol. 2006;22:437-442
- Meeker JD, Ravi SR, Barr DB, Hauser R. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod Toxicol. 2008;25:184-191

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Meeker JD, Ryan L, Barr DB, Hauser R. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 2006;17:61-68
- Melkonian S, Argos M, Pierce BL, Chen Y, Islam T, Ahmed A, Syed EH, Parvez F, Graziano J, Rathouz PJ, Ahsan H. A prospective study of the synergistic effects of arsenic exposure and smoking, sun exposure, fertilizer use, and pesticide use on risk of premalignant skin lesions in Bangladeshi men. American journal of epidemiology. 2011;173:183-191
- Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, Fochs S, Sunyer J. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect. 2011;119:272-278
- Menegaux F, Baruchel A, Bertrand Y, Lescoeur B, Leverger G, Nelken B, Sommelet D, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood acute leukaemia. Occupational and environmental medicine. 2006;63:131-134
- Merletti F, Richiardi L, Bertoni F, Ahrens W, Buemi A, Costa-Santos C, Eriksson M, Guenel P, Kaerlev L, Jockel KH, Llopis-Gonzalez A, Merler E, Miranda A, Morales-Suarez-Varela MM, Olsson H, Fletcher T, Olsen J. Occupational factors and risk of adult bone sarcomas: A multicentric case-control study in europe. International journal of cancer. Journal international du cancer. 2006;118:721-727
- Messaros BM, Rossano MG, Liu G, Diamond MP, Friderici K, Nummy-Jernigan K, Daly D, Puscheck E, Paneth N, Wirth JJ. Negative effects of serum p,p'-dde on sperm parameters and modification by genetic polymorphisms. Environmental research. 2009;109:457-464
- Meyer A, Alexandre PC, Chrisman Jde R, Markowitz SB, Koifman RJ, Koifman S. Esophageal cancer among brazilian agricultural workers: Case-control study based on death certificates. International journal of hygiene and environmental health. 2011;214:151-155
- Meyer KJ, Reif JS, Veeramachaneni DNR, Luben TJ, Mosley BS, Nuckols JR. Agricultural pesticide use and hypospadias in eastern arkansas. Environmental Health Perspectives. 2006;114:1589-1595
- Meyer TE, Coker AL, Sanderson M, Symanski E. A case-control study of farming and prostate cancer in african-american and caucasian men. Occupational and environmental medicine. 2007;64:155-160
- Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: An overview and some results of the italian multicenter case-control study on hematolymphopoietic malignancies. Annals of the New York Academy of Sciences. 2006;1076:366-377
- Mills KT, Blair A, Freeman LE, Sandler DP, Hoppin JA. Pesticides and myocardial infarction incidence and mortality among male pesticide applicators in the agricultural health study. American journal of epidemiology. 2009;170:892-900
- Mills PK, Yang RC. Agricultural exposures and gastric cancer risk in Hispanic farm workers in california. Environmental research. 2007;104:282-289
- Min JY, Cho JS, Lee KJ, Park JB, Park SG, Kim JY, Min KB. Potential role for organochlorine pesticides in the prevalence of peripheral arterial diseases in obese persons: Results from the national health and nutrition examination survey 1999-2004. Atherosclerosis. 2011;218:200-206
- Miyake Y, Tanaka K, Masuzaki Y, Sato N, Ikeda Y, Chisaki Y, Arakawa M. Organochlorine concentrations in breast milk and prevalence of allergic disorders in japanese women.

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Chemosphere. 2011;85:374-378

- Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP, Weiderpass E, Partanen T. Parental occupational exposure to pesticides and the risk of childhood leukemia in costa rica. Scandinavian Journal of Work, Environment & Health. 2007;33:293-303
- Montgomery MP, Kamel F, Saldana TM, Alavanja MC, Sandler DP. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993-2003. American journal of epidemiology. 2008;167:1235-1246
- Morahan JM, Pamphlett R. Amyotrophic lateral sclerosis and exposure to environmental toxins: An Australian case-control study. Neuroepidemiology. 2006;27:130-135
- Mozzachio AM, Rusiecki JA, Hoppin JA, Mahajan R, Patel R, Beane-Freeman L, Alavanja MC. Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study. Environmental research. 2008;108:400-403
- Mueller BA, Kuehn CM, Shapiro-Mendoza CK, Tomashek KM. Fetal deaths and proximity to hazardous waste sites in washington state. Environ Health Perspect. 2007;115:776-780
- Multigner L, Kadhel P, Pascal M, Huc-Terki F, Kercret H, Massart C, Janky E, Auger J, Jegou B. Parallel assessment of male reproductive function in workers and wild rats exposed to pesticides in banana plantations in guadeloupe. Environmental health: a global access science source. 2008;7:40
- Multigner L, Ndong JR, Giusti A, Romana M, Delacroix-Maillard H, Cordier S, Jegou B, Thome JP, Blanchet P. Chlordecone exposure and risk of prostate cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010;28:3457-3462
- N. Murgia, M. Dell'Omo, G. Muzi, G. Brugnami, M. Biancalana,
- Nagayama J, Kohno H, Kunisue T, Kataoka K, Shimomura H, Tanabe S, Konishi S. Concentrations of organochlorine pollutants in mothers who gave birth to neonates with congenital hypothyroidism. Chemosphere. 2007;68:972-976
- Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, Yanagawa T, Fukushige J, Watanabe T. Immunologic effects of perinatal exposure to dioxins, pcbs and organochlorine pesticides in japanese infants. Chemosphere. 2007;67:S393-398
- Naidoo S, London L, Burdorf A, Naidoo R, Kromhout H. Spontaneous miscarriages and infant deaths among female farmers in rural south africa. Scand J Work Environ Health. 2011;37:227-236
- Narendra M, Kavitha G, Helah Kiranmai A, Raghava Rao N, Varadacharyulu NC. Chronic exposure to pyrethroid-based allethrin and prallethrin mosquito repellents alters plasma biochemical profile. Chemosphere. 2008;73:360-364
- Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environmental science & technology. 2011;45:1680-1687
- Neundorfer B. . Solvents and PARKINSON'S disease. Padiatrische Praxis (2008) 72:3 (508-510).

Nicolas Lebas, Louise Nadon, Mounia Rhazi, Hugues Richard, Marie Desy, Marie-Elise Parent. Exposure to occupational and domestic pesticides, and prostate cancer risk:

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

preliminary findings from a case-control study in Montreal, Canada.

- Nicolle-Mir L. Exposure to pesticides and thyroid diseases. Environmement, Risques et Sante (2010) 9:5 (381-382).
- Nicolle-Mir L. Occupational factors and Parkinson's disease. Environmement, Risques et Sante (2010) 9:5 (382-383).
- Nordby KC, Irgens LM, Kristensen P. Immunological exposures in Norwegian agriculture and pre-eclampsia. Paediatric and perinatal epidemiology. 2006;20:462-470
- Norlaily H, Azidah AK, Asrenee AR, Rohayah H, Juwita S. Proportion of dementia and its associated factors among elderly patients attending outpatient clinics of universiti sains malaysia hospital. The Medical journal of Malaysia. 2009;64:140-145
- Ochoa-Acuna H, Frankenberger J, Hahn L, Carbajo C. Drinking-water herbicide exposure in indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Perspect. 2009;117:1619-1624
- Ociepa-Zawal M, Rubis B, Wawrzynczak D, Wachowiak R, Trzeciak WH. Accumulation of environmental estrogens in adipose tissue of breast cancer patients. Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering. 2010;45:305-312
- Onishchenko GG, Mamaev IA, Guseinov GK. [impact of the area burden of agrochemicals on tuberculosis morbidity and mortality]. Problemy tuberkuleza i boleznei legkikh. 2006:30-33
- Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X, Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: Results of a french case-control study. Occupational and environmental medicine. 2009;66:291-298
- Orsi L, Troussard X, Monnereau A, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Clavel J. Occupation and lymphoid malignancies: Results from a french case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:1339-1350
- Ostrea EM, Jr., Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012;33:669-675
- Ozen S, Darcan S, Bayindir P, Karasulu E, Simsek DG, Gurler T. Effects of pesticides used in agriculture on the development of precocious puberty. Environmental monitoring and assessment. 2012;184:4223-4232
- P. Monica Lind, Samira Salihovic, Bert van Bavel, Lars Lind. Circulating levels of pp-DDE and hypertension. Abstracts / Toxicology Letters 211S (2012) S43–S216
- Padmaja R. Jonnalagadda\*, A. Y. E. Prasad, Katla Ashok Reddy, Challa Suresh, M. Vishnu, Vardhana Rao, Goparaju Ramya and D. Raghunatha Rao. Biochemical alterations of certain health parameters in cotton growing farmers exposed to organophosphorous and pyrethroid insecticides. African Journal of Biotechnology Vol. 9(49), pp. 8369-8377, 6 December, 2010

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McDuffie HH, McLaughlin JR. Multiple myeloma and exposure to pesticides: A Canadian case-control study. Journal of agromedicine. 2012;17:40-50
- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR. Soft-tissue sarcoma and pesticides exposure in men: Results of a Canadian case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:1279-1286
- Pahwa P, Karunanayake CP, Spinelli JJ, Dosman JA, McDuffie HH. Ethnicity and incidence of Hodgkin lymphoma in canadian population. BMC cancer. 2009;9:141
- Pahwa P, McDuffie HH, Dosman JA, McLaughlin JR, Spinelli JJ, Robson D, Fincham S. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:264-274
- Pamphlett R. Exposure to environmental toxins and the risk of sporadic motor neuron disease: An expanded Australian case-control study. European journal of neurology: the official journal of the European Federation of Neurological Societies. 2012;19:1343-1348
- Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: An analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect. 2009;117:488-494
- Pan IJ, Daniels JL, Herring AH, Rogan WJ, Siega-Riz AM, Goldman BD, Sjodin A. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: An analysis of the pregnancy, infection, and nutrition babies study. Paediatric and perinatal epidemiology. 2010;24:262-271
- Pant N, Kumar R, Mathur N, Srivastava SP, Saxena DK, Gujrati VR. Chlorinated pesticide concentration in semen of fertile and infertile men and correlation with sperm quality. Environmental toxicology and pharmacology. 2007;23:135-139
- Park SK, Kang D, Beane-Freeman L, Blair A, Hoppin JA, Sandler DP, Lynch CF, Knott C, Gwak J, Alavanja M. Cancer incidence among paraquat exposed applicators in the agricultural health study: Prospective cohort study. International journal of occupational and environmental health. 2009;15:274-281
- Park SK, Son HK, Lee SK, Kang JH, Chang YS, Jacobs DR, Lee DH. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among nondiabetic adults. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2010;43:1-8
- Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J, Sarto G, Howard BV. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the women's health initiative observational study. Arthritis care & research. 2011;63:184-194
- Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (ewas) on type 2 diabetes mellitus. PloS one. 2010;5:e10746

Pathak R, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Banerjee BD.

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Maternal and cord blood levels of organochlorine pesticides: Association with preterm labor. Clinical biochemistry. 2009;42:746-749

- Pathak R, Mustafa M, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Association between recurrent miscarriages and organochlorine pesticide levels. Clinical biochemistry. 2010;43:131-135
- Pathak R, Mustafa MD, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Intra uterine growth retardation: Association with organochlorine pesticide residue levels and oxidative stress markers. Reprod Toxicol. 2011;31:534-539
- Patil JA, Patil AJ, Sontakke AV, Govindwar SP. Occupational pesticides exposure of sprayers of grape gardens in western Maharashtra (india): Effects on liver and kidney function. Journal of basic and clinical physiology and pharmacology. 2009;20:335-355
- Pearce MS, Hammal DM, Dorak MT, McNally RJ, Parker L. Paternal occupational exposure to pesticides or herbicides as risk factors for cancer in children and young adults: A casecontrol study from the north of england. Arch Environ Occup Health. 2006;61:138-144
- Pekmezovic T, Suvajdzic Vukovic N, Kisic D, Grgurevic A, Bogdanovic A, Gotic M, Bakrac M, Brkic N. A case-control study of myelodysplastic syndromes in belgrade (serbia montenegro). Annals of hematology. 2006;85:514-519
- Pereira D, Garrett C. [risk factors for Parkinson disease: An epidemiologic study]. Acta medica portuguesa. 2010;23:15-24
- Perez-Herrera N, Polanco-Minaya H, Salazar-Arredondo E, Solis-Heredia MJ, Hernandez-Ochoa I, Rojas-Garcia E, Alvarado-Mejia J, Borja-Aburto VH, Quintanilla-Vega B. Pon1q192r genetic polymorphism modifies organophosphorous pesticide effects on semen quality and DNA integrity in agricultural workers from southern mexico. Toxicology and applied pharmacology. 2008;230:261-268
- Perry MJ, Ouyang F, Korrick SA, Venners SA, Chen C, Xu X, Lasley BL, Wang X. A prospective study of serum ddt and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age. American journal of epidemiology. 2006;164:1056-1064
- Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, Barr DB, Xu X. Organophosphorous pesticide exposures and sperm quality. Reprod Toxicol. 2011;31:75-79
- Persson EC, Graubard BI, Evans AA, London WT, Weber JP, LeBlanc A, Chen G, Lin W, McGlynn KA. Dichlorodiphenyltrichloroethane and risk of hepatocellular carcinoma. International journal of cancer. Journal international du cancer. 2012;131:2078-2084
- Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, Jorgensen PJ, Budtz-Jorgensen E, Grandjean P. Impact of dietary exposure to food contaminants on the risk of parkinson's disease. Neurotoxicology. 2008;29:584-590
- Petit C, Blangiardo M, Richardson S, Coquet F, Chevrier C, Cordier S. Association of environmental insecticide exposure and fetal growth with a bayesian model including multiple exposure sources: The pelagie mother-child cohort. American journal of epidemiology. 2012;175:1182-1190
- Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R, Cordier S. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort

EFSA supporting publication 2013:EN-497

<sup>141</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

study in Brittany, France. Environmental health: a global access science source. 2010;9:71

- Philibert A, Schwartz H, Mergler D. An exploratory study of diabetes in a first nation community with respect to serum concentrations of p,p'-dde and pcbs and fish consumption. International journal of environmental research and public health. 2009;6:3179-3189
- Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environmental research. 2007;105:364-369
- Polsky JY, Aronson KJ, Heaton JP, Adams MA. Pesticides and polychlorinated biphenyls as potential risk factors for erectile dysfunction. Journal of andrology. 2007;28:28-37
- Pombo-de-Oliveira MS, Koifman S. Infant acute leukemia and maternal exposures during pregnancy. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:2336-2341
- Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, Ferro A, Ingelido AM, Maggi A, Panici PB, De Felip E. Endometriosis and organochlorinated environmental pollutants: A case-control study on italian women of reproductive age. Environ Health Perspect. 2009;117:1070-1075
- Postuma RB, Montplaisir JY, Pelletier A, Dauvilliers Y, Oertel W, Iranzo A, Ferini-Strambi L, Arnulf I, Hogl B, Manni R, Miyamoto T, Mayer G, Stiasny-Kolster K, Puligheddu M, Ju Y, Jennum P, Sonka K, Santamaria J, Fantini ML, Zucconi M, Leu-Semenescu S, Frauscher B, Terzaghi M, Miyamoto M, Unger MM, Cochen De Cock V, Wolfson C. Environmental risk factors for rem sleep behavior disorder: A multicenter case-control study. Neurology. 2012;79:428-434
- Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, Kaufman D. Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific journal of public health / Asia-Pacific Academic Consortium for Public Health. 2011;23:369-377
- Prochazka M, Feychting M, Ahlbom A, Edwards CG, Nise G, Plato N, Schwartzbaum JA, Forssen UM. Occupational exposures and risk of acoustic neuroma. Occupational and environmental medicine. 2010;67:766-771
- Provost D, Cantagrel A, Lebailly P, Jaffre A, Loyant V, Loiseau H, Vital A, Brochard P, Baldi I. Brain tumours and exposure to pesticides: A case-control study in southwestern France. Occupational and environmental medicine. 2007;64:509-514
- Puertas R, Lopez-Espinosa MJ, Cruz F, Ramos R, Freire C, Perez-Garcia M, Abril A, Julvez J, Salvatierra M, Campoy C, Olea N. Prenatal exposure to mirex impairs neurodevelopment at age of 4 years. Neurotoxicology. 2010;31:154-160
- Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, Barr DB, Blair A, Rothman N, McGlynn KA. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. Environ Health Perspect. 2009;117:1514-1519
- Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;120:642-649
- Qiu XQ, Zhong QA, Zeng XY, Li YH, Nie SF. [a case-control study on congenital heart diseases with methylenetetrahydrofolate reductase gene, cystathionine beta-synthase gene,

EFSA supporting publication 2013:EN-497

<sup>142</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

and environmental factors]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:260-263

- Quaranta MG, Porpora MG, Mattioli B, Giordani L, Libri I, Ingelido AM, Cerenzia P, Di Felice A, Abballe A, De Felip E, Viora M. Impaired nk-cell-mediated cytotoxic activity and cytokine production in patients with endometriosis: A possible role for pcbs and dde. Life sciences. 2006;79:491-498
- Quiros-Alcala L, Alkon AD, Boyce WT, Lippert S, Davis NV, Bradman A, Barr DB, Eskenazi B. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. Neurotoxicology. 2011;32:646-655
- Qureshi MM, Hayden D, Urbinelli L, Ferrante K, Newhall K, Myers D, Hilgenberg S, Smart R, Brown RH, Cudkowicz ME. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (als). Amyotrophic lateral sclerosis: official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2006;7:173-182
- R. Alarcon, M. Requena, A.F. Hernández, T. Parrón. The relationship between breast cancer and pesticide exposure in regions with differing pesticide use levels. Abstracts / Toxicology Letters 205S (2011) S180–S300
- Ragab M, Elzayadi AR, Hamdy H, Badran H, Shawky S, Emara S. 692 non viral risk factors in development of hcc among egyptian chronic liver disease patients. Journal of Hepatology. 2012;56:S274
- Rastogi SK, Singh VK, Kesavachandran C, Jyoti, Siddiqui MK, Mathur N, Bharti RS. Monitoring of plasma butyrylcholinesterase activity and hematological parameters in pesticide sprayers. Indian journal of occupational and environmental medicine. 2008;12:29-32
- Rau AT, Coutinho A, Avabratha KS, Rau AR, Warrier RP. Pesticide (endosulfan) levels in the bone marrow of children with hematological malignancies. Indian pediatrics. 2012;49:113-117
- Rauch SA, Braun JM, Barr DB, Calafat AM, Khoury J, Montesano AM, Yolton K, Lanphear BP. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012;120:1055-1060
- Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, Whyatt R. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 2011;119:1196-1201
- Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006;118:e1845-1859
- Recio-Vega R, Ocampo-Gomez G, Borja-Aburto VH, Moran-Martinez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: Association between sperm parameters and urinary pesticide levels. Journal of applied toxicology: JAT. 2008;28:674-680
- Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proceedings of the National Academy of Sciences of the United States of America. 2011;108:12770-

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

12775

- Ribas-Fito N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-dde) in relation to child growth. International journal of epidemiology. 2006;35:853-858
- Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. Environ Health Perspect. 2007;115:447-450
- Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, Sunyer J. In utero exposure to background concentrations of ddt and cognitive functioning among preschoolers. American journal of epidemiology. 2006;164:955-962
- Richardson DB, Terschuren C, Hoffmann W. Occupational risk factors for non-hodgkin's lymphoma: A population-based case-control study in northern Germany. American journal of industrial medicine. 2008;51:258-268
- Richardson JR, Shalat SL, Buckley B, Winnik B, O'Suilleabhain P, Diaz-Arrastia R, Reisch J, German DC. Elevated serum pesticide levels and risk of parkinson disease. Archives of neurology. 2009;66:870-875
- Rignell-Hydbom A, Elfving M, Ivarsson SA, Lindh C, Jonsson BA, Olofsson P, Rylander L. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants in relation to risk of type 1 diabetes. PloS one. 2010;5:e11281
- Rignell-Hydbom A, Lidfeldt J, Kiviranta H, Rantakokko P, Samsioe G, Agardh CD, Rylander L. Exposure to p,p'-dde: A risk factor for type 2 diabetes. PloS one. 2009;4:e7503
- Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. Human & experimental toxicology. 2007;26:447-452
- Rignell-Hydbom A, Skerfving S, Lundh T, Lindh CH, Elmstahl S, Bjellerup P, Junsson BA, Strumberg U, Akesson A. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. Environmental research. 2009;109:991-996
- Riu E, Monso E, Marin A, Magarolas R, Radon K, Morera J, Andreo F, Nowak D. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. American journal of rhinology. 2008;22:361-364
- Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California central valley. Environ Health Perspect. 2007;115:1482-1489
- Rocheleau CM, Romitti PA, Sanderson WT, Sun L, Lawson CC, Waters MA, Stewart PA, Olney RS, Reefhuis J. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth defects research. Part A, Clinical and molecular teratology. 2011;91:927-936
- Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral performance of adult and adolescent agricultural workers. Neurotoxicology. 2007;28:374-380
- Rojas-Garcia AE, Medina-Diaz IM, Robledo-Marenco Mde L, Barron-Vivanco BS, Giron-Perez MI, Velazquez-Fernandez JB, Gonzalez-Arias CA, Albores-Medina A, Quintanilla-

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Vega B, Ostrosky-Wegman P, Rojas-Garcia MC, Perez-Herrera NE, Lopez-Flores JF. Hematological, biochemical effects, and self-reported symptoms in pesticide retailers. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:517-521

- Roldan-Tapia L, Nieto-Escamez FA, del Aguila EM, Laynez F, Parron T, Sanchez-Santed F. Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicology and teratology. 2006;28:694-703
- Romero Ramos R, Romero Gutierrez G, Abortes Monroy I, Medina Sanchez HG. [risk factors associated to female infertility]. Ginecologia y obstetricia de Mexico. 2008;76:717-721
- Rosano A, Gemelli V, Giovannelli C, Paciotti G, Sabatucci A, Spagnolo A. [fertility changes in women working in greenhouses]. La Medicina del lavoro. 2009;100:448-454
- Rossman MD, Thompson B, Frederick M, Iannuzzi MC, Rybicki BA, Pander JP, Newman LS, Rose C, Magira E, Monos D. Hla and environmental interactions in sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders. 2008;25:125-132
- Rosso AL, Hovinga ME, Rorke-Adams LB, Spector LG, Bunin GR. A case-control study of childhood brain tumors and fathers' hobbies: A children's oncology group study. Cancer causes & control: CCC. 2008;19:1201-1207
- Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and nonagricultural hispanic workers. Environmental Health Perspectives. 2006;114:691-696
- Rubin CH, Lanier A, Kieszak S, Brock JW, Koller KR, Strosnider H, Needham L, Zahm S, Harpster A. Breast cancer among alaska native women potentially exposed to environmental organochlorine chemicals. International journal of circumpolar health. 2006;65:18-27
- Rudant J, Menegaux F, Leverger G, Baruchel A, Nelken B, Bertrand Y, Patte C, Pacquement H, Verite C, Robert A, Michel G, Margueritte G, Gandemer V, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood hematopoietic malignancies: The escale study (sfce). Environ Health Perspect. 2007;115:1787-1793
- Ruder AM, Carreon T, Butler MA, Calvert GM, Davis-King KE, Waters MA, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. Exposure to farm crops, livestock, and farm tasks and risk of glioma: The upper midwest health study. American journal of epidemiology. 2009;169:1479-1491
- Ruder AM, Waters MA, Carreon T, Butler MA, Davis-King KE, Calvert GM, Schulte PA, Ward EM, Connally LB, Lu J, Wall D, Zivkovich Z, Heineman EF, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of primary intracranial gliomas in farm and rural residents. Journal of agricultural safety and health. 2006;12:255-274
- Ruder AM, Yiin JH. Mortality of us pentachlorophenol production workers through 2005. Chemosphere. 2011;83:851-861
- Rugbjerg K, Harris MA, Shen H, Marion SA, Tsui JK, Teschke K. Pesticide exposure and risk of Parkinson's disease--a population-based case-control study evaluating the potential for recall bias. Scand J Work Environ Health. 2011;37:427-436

## Rull RP, Gunier R, Von Behren J, Hertz A, Crouse V, Buffler PA, Reynolds P. Residential

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environmental research. 2009;109:891-899

- Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. American journal of epidemiology. 2006;163:743-753
- Rusiecki JA, Hou L, Lee WJ, Blair A, Dosemeci M, Lubin JH, Bonner M, Samanic C, Hoppin JA, Sandler DP, Alavanja MC. Cancer incidence among pesticide applicators exposed to metolachlor in the agricultural health study. International journal of cancer. Journal international du cancer. 2006;118:3118-3123
- Rusiecki JA, Patel R, Koutros S, Beane-Freeman L, Landgren O, Bonner MR, Coble J, Lubin J, Blair A, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to permethrin in the agricultural health study. Environ Health Perspect. 2009;117:581-586
- Rylander L, Wallin E, Jonssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between cb-153 and p,p'-dde and hormone levels in serum in middle-aged and elderly men. Chemosphere. 2006;65:375-381
- Sagiv SK, Nugent JK, Brazelton TB, Choi AL, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and measures of behavior in infancy using the neonatal behavioral assessment scale (nbas). Environ Health Perspect. 2008;116:666-673
- Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. Environ Health Perspect. 2012;120:904-909
- Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. American journal of epidemiology. 2010;171:593-601
- Sagiv SK, Tolbert PE, Altshul LM, Korrick SA. Organochlorine exposures during pregnancy and infant size at birth. Epidemiology. 2007;18:120-129
- Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: A case-control study in Lebanon. Journal of epidemiology and community health. 2006;60:256-261
- Salameh PR, Waked M, Baldi I, Brochard P, Saleh BA. Chronic bronchitis and pesticide exposure: A case-control study in Lebanon. European journal of epidemiology. 2006;21:681-688
- Saldana TM, Basso O, Baird DD, Hoppin JA, Weinberg CR, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and hypertensive disorders during pregnancy. Environ Health Perspect. 2009;117:1393-1396
- Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. Diabetes care. 2007;30:529-534
- Samanic C, Rusiecki J, Dosemeci M, Hou L, Hoppin JA, Sandler DP, Lubin J, Blair A, Alavanja MCR. Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study. Environmental Health Perspectives. 2006;114:1521-1526
- Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD. Occupational exposure to pesticides and risk of adult brain tumors. American journal of epidemiology.

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

2008;167:976-985

- Sanchez A.T., Olivera R.M.P., Sanchez G.M.D.O., Dorantes G.L.. Pemphigus vulgaris. Epidemiological study and analysis of possible risk factors of mortality. Dermatologia Revista Mexicana (2006) 50:2 (50-53).
- Santibanez M, Alguacil J, de la Hera MG, Navarrete-Munoz EM, Llorca J, Aragones N, Kauppinen T, Vioque J. Occupational exposures and risk of stomach cancer by histological type. Occupational and environmental medicine. 2012;69:268-275
- Santibanez M, Vioque J, Alguacil J, de la Hera MG, Moreno-Osset E, Carrato A, Porta M, Kauppinen T. Occupational exposures and risk of pancreatic cancer. European journal of epidemiology. 2010;25:721-730
- Sanyal J, Chakraborty DP, Sarkar B, Banerjee TK, Mukherjee SC, Ray BC, Rao VR. Environmental and familial risk factors of Parkinson's disease: Case-control study. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 2010;37:637-642
- Sartor SG, Eluf-Neto J, Travier N, Wunsch Filho V, Arcuri AS, Kowalski LP, Boffetta P. [occupational risks for laryngeal cancer: A case-control study]. Cadernos de saude publica. 2007;23:1473-1481
- Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, Hoppin JA. Maternal pesticide use and birth weight in the agricultural health study. Journal of agromedicine. 2010;15:127-136
- Sawada N, Iwasaki M, Inoue M, Itoh H, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: A nested casecontrol study. Environ Health Perspect. 2010;118:659-665
- Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'- dde, and other toxicants in akwesasne mohawk youth. Environ Health Perspect. 2008;116:806-813
- Schell LM, Gallo MV, Ravenscroft J, DeCaprio AP. Persistent organic pollutants and antithyroid peroxidase levels in akwesasne mohawk young adults. Environmental research. 2009;109:86-92
- Schmeisser N, Behrens T, Mester B, Gottlieb A, Langner I, Ahrens W. Local cluster of germ cell cancer in a cohort of male automotive workers in germany not explained by previous or concurrent activities and exposures in farming and forestry. Cancer epidemiology. 2011;35:73-77
- Schmeisser N, Kaerlev L, Bourdon-Raverdy N, Ganry O, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Zambon P, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Ahrens W. Occupational exposure to pesticides and bile tract carcinoma in men: Results from a european multicenter case-control study. Cancer causes & control: CCC. 2010;21:1493-1502
- Schreinemachers DM. Perturbation of lipids and glucose metabolism associated with previous 2,4-d exposure: A cross-sectional study of nhanes iii data, 1988-1994. Environmental health: a global access science source. 2010;9:11
- Semchuk KM, Rosenberg AM, McDuffie HH, Cessna AJ, Pahwa P, Irvine DG. Antinuclear antibodies and bromoxynil exposure in a rural sample. Journal of toxicology and

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

environmental health. Part A. 2007;70:638-657

- Settimi L, Spinelli A, Lauria L, Miceli G, Pupp N, Angotzi G, Fedi A, Donati S, Miligi L, Osborn J, Figa-Talamanca I. Spontaneous abortion and maternal work in greenhouses. American journal of industrial medicine. 2008;51:290-295
- Seyed Jalal Emam, Maryam Salehcheh, Mohammad Hossein Haghighizadeh, Seyed Mohammad Hosein Mousavi Jazayeri. Occupational exposure to pesticides among farmers. Pak J Med Sci 2012;28(1):120-123
- Sharma E, Mustafa M, Pathak R, Guleria K, Ahmed RS, Vaid NB, Banerjee BD. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. European journal of obstetrics, gynecology, and reproductive biology. 2012;161:163-169
- Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in cyp1a1, gstmi, gstt1 genes and organochlorine pesticides in the etiology of hypospadias. Human & experimental toxicology. 2011;30:1464-1474
- Shim YK, Mlynarek SP, van Wijngaarden E. Parental exposure to pesticides and childhood brain cancer: U.S. Atlantic coast childhood brain cancer study. Environ Health Perspect. 2009;117:1002-1006
- Shirangi A, Fritschi L, Holman CD. Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. Occupational and environmental medicine. 2008;65:719-725
- Siddharth M, Datta SK, Bansal S, Mustafa M, Banerjee BD, Kalra OP, Tripathi AK. Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. Journal of biochemical and molecular toxicology. 2012;26:241-247
- Silva SR, Martins JL, Seixas S, Silva DC, Lemos SP, Lemos PV. [congenital defects and exposure to pesticides in sao francisco valley]. Revista brasileira de ginecologia e obstetricia : revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia. 2011;33:20-26
- Skeie GO, Muller B, Haugarvoll K, Larsen JP, Tysnes OB. Differential effect of environmental risk factors on postural instability gait difficulties and tremor dominant Parkinson's disease. Movement disorders: official journal of the Movement Disorder Society. 2010;25:1847-1852
- Slager RE, Poole JA, LeVan TD, Sandler DP, Alavanja MC, Hoppin JA. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the agricultural health study. Occupational and environmental medicine. 2009;66:718-724
- Slager RE, Simpson SL, Levan TD, Poole JA, Sandler DP, Hoppin JA. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. Journal of toxicology and environmental health. Part A. 2010;73:1382-1393
- Slater ME, Linabery AM, Spector LG, Johnson KJ, Hilden JM, Heerema NA, Robison LL, Ross JA. Maternal exposure to household chemicals and risk of infant leukemia: A report from the children's oncology group. Cancer causes & control : CCC. 2011;22:1197-1204
- Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6

148

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

years. Acta Paediatr. 2008;97:1465-1469

- Snijder CA, Roeleveld N, Te Velde E, Steegers EA, Raat H, Hofman A, Jaddoe VW, Burdorf A. Occupational exposure to chemicals and fetal growth: The generation r study. Hum Reprod. 2012;27:910-920
- Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27:1510-1517
- Soldin OP, Nsouli-Maktabi H, Genkinger JM, Loffredo CA, Ortega-Garcia JA, Colantino D, Barr DB, Luban NL, Shad AT, Nelson D. Pediatric acute lymphoblastic leukemia and exposure to pesticides. Therapeutic drug monitoring. 2009;31:495-501
- Solomon C, Poole J, Palmer KT, Peveler R, Coggon D. Neuropsychiatric symptoms in past users of sheep dip and other pesticides. Occupational and environmental medicine. 2007;64:259-266
- Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, Jacobs DR, Jr., Lee DH. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in korea. Environment international. 2010;36:410-414
- Spinelli JJ, Ng CH, Weber JP, Connors JM, Gascoyne RD, Lai AS, Brooks-Wilson AR, Le ND, Berry BR, Gallagher RP. Organochlorines and risk of non-hodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;121:2767-2775
- Spix C, Schulze-Rath R, Kaatsch P, Blettner M. Case-control study on risk factors for leukaemia and brain tumours in children under 5 years in germany. Klinische Padiatrie. 2009;221:362-368
- Stallones L. Suicide and potential occupational exposure to pesticides, colorado 1990-1999. Journal of agromedicine. 2006;11:107-112
- Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Hoppin JA. Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the agricultural health study. Neurotoxicology and teratology. 2012;34:168-176
- Starks SE, Hoppin JA, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Gerr F. Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the agricultural health study. Environ Health Perspect. 2012;120:515-520
- Steerenberg P, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Vergieva T, Zaikov C, Pennanen S, Liesivuori J, Van Loveren H. Toxicological evaluation of the immune function of pesticide workers, a european wide assessment. Human & experimental toxicology. 2008;27:701-707
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal pcb exposure and intelligence (iq) in 9-year-old children. Environ Health Perspect. 2008;116:1416-1422
- Strom SS, Yamamura Y, Flores-Sandoval FN, Pettaway CA, Lopez DS. Prostate cancer in mexican-americans: Identification of risk factors. The Prostate. 2008;68:563-570
- Strom SS, Yamamura Y, Kantarijian HM, Cortes-Franco JE. Obesity, weight gain, and risk of chronic myeloid leukemia. Cancer epidemiology, biomarkers & prevention: a publication of

EFSA supporting publication 2013:EN-497

<sup>149</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18:1501-1506

- Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spano M, Toft G, Bonde JP, Jonsson BA, Rignell-Hydbom A, Rylander L, Giwercman A, Pedersen HS, Bonefeld-Jorgensen EC, Ludwicki JK, Lesovoy V, Sakkas D, Bizzaro D. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of cb-153 and p,p'dde in european and inuit populations. Reproduction. 2006;132:949-958
- Stuetz W, McGready R, Cho T, Prapamontol T, Biesalski HK, Stepniewska K, Nosten F. Relation of ddt residues to plasma retinol, alpha-tocopherol, and beta-carotene during pregnancy and malaria infection: A case-control study in karen women in northern thailand. The Science of the total environment. 2006;363:78-86
- Su Y, Dai Y, Lin Y, Gao X, Han Y, Zhao B. Serum organochlorine pesticide residues and risk of gallstone disease: A case-control study in xiamen. Annals of epidemiology. 2012;22:592-597
- Subahir MN, Shah SA, Zainuddin ZM. Risk factors for prostate cancer in universiti kebangsaan Malaysia medical centre: A case-control study. Asian Pacific journal of cancer prevention: APJCP. 2009;10:1015-1020
- Sunyer J, Alvarez-Pedrerol M, To-Figueras J, Ribas-Fito N, Grimalt JO, Herrero C. Urinary porphyrin excretion in children is associated with exposure to organochlorine compounds. Environ Health Perspect. 2008;116:1407-1410
- Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de Cid R, Anto JM, Torrent M. Early life environment, neurodevelopment and the interrelation with atopy. Environmental research. 2010;110:733-738
- Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goni F, Basterrechea M, Vrijheid M, Guerra S, Anto JM. Dde in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology. 2010;21:729-735
- Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2006;36:1236-1241
- Sutoluk Z, Kekec Z, Daglioglu N, Hant I. Association of chronic pesticide exposure with serum cholinesterase levels and pulmonary functions. Archives of Environmental & Occupational Health. 2011;66:95-99
- Swaen G, van Amelsvoort L, Boers D, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Pennanen S, Liesivuori J, Colosio C, van Loveren H. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: Results from the europit field study. Human & experimental toxicology. 2008;27:715-720
- Swan SH. Semen quality in fertile us men in relation to geographical area and pesticide exposure. International journal of andrology. 2006;29:62-68; discussion 105-108
- Tadevosyan N.S., Tadevosyan A.E., Petrosyan M.S. Pesticides application in agricultural of Armenia and their impact on reproductive function in humans. THE NEW ARMENIAN MEDICAL JOURNALVol. 3 (2009), N 2, 41 48

Tagiyeva N, Devereux G, Semple S, Sherriff A, Henderson J, Elias P, Ayres JG. Parental

EFSA supporting publication 2013:EN-497

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The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupation is a risk factor for childhood wheeze and asthma. The European respiratory journal. 2010;35:987-993

- Tan X.H. Risk factors for Parkinson disease and the path analysis: One-to-one paired design. Neural Regeneration Research (2007) 2:2 (117-120)
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011;119:866-872
- Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW. Occupation and risk of Parkinsonism: A multicenter case-control study. Archives of neurology. 2009;66:1106-1113
- Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. Reported residential pesticide use and breast cancer risk on long island, New York. American journal of epidemiology. 2007;165:643-651
- Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. Epidemiological study of high cancer among rural agricultural community of punjab in northern india. International journal of environmental research and public health. 2008;5:399-407
- Tiido T, Rignell-Hydbom A, Jönsson BAG, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvyezday V, Spano M, Manicardi G-C, Bizzaro D, Bonefeld-Jørgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A. Impact of pcb and p, p'-dde contaminants on human sperm y:X chromosome ratio: Studies in three european populations and the inuit population in greenland. Environmental Health Perspectives. 2005;114:718-724
- Toft G, Axmon A, Lindh CH, Giwercman A, Bonde JP. Menstrual cycle characteristics in european and inuit women exposed to persistent organochlorine pollutants. Hum Reprod. 2008;23:193-200
- Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, Lindh CH, Pedersen HS, Ludwicki JK, Lesovoy V, Hagmar L, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Thulstrup AM, Bonde JP. Semen quality and exposure to persistent organochlorine pollutants. Epidemiology. 2006;17:450-458
- Toft G, Thulstrup AM, Jonsson BA, Pedersen HS, Ludwicki JK, Zvezday V, Bonde JP. Fetal loss and maternal serum levels of 2,2',4,4',5,5'-hexachlorbiphenyl (cb-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-dde) exposure: A cohort study in greenland and two european populations. Environmental health: a global access science source. 2010;9:22
- Ton TG, Longstreth WT, Jr., Koepsell TD. Environmental toxins and risk of narcolepsy among people with hla dqb1\*0602. Environmental research. 2010;110:565-570
- Tondel M, Lindh J, Jonsson P, Vrethem M, Persson B. Occupational determinants of cryptogenic polyneuropathy. Neuroepidemiology. 2006;26:187-194
- Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen Hernandez M, Garcia-Hernandez RM, Del Rio-Garcia C, Wolff MS, Lopez-Carrillo L. In utero p,p'-dde exposure and infant neurodevelopment: A perinatal cohort in mexico. Environ Health Perspect. 2007;115:435-439

EFSA supporting publication 2013:EN-497

<sup>151</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Torres-Sanchez L, Schnaas L, Cebrian ME, Hernandez Mdel C, Valencia EO, Garcia Hernandez RM, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and neurodevelopment: A follow-up from 12 to 30 months of age. Neurotoxicology. 2009;30:1162-1165
- Torres-Sanchez L, Zepeda M, Cebrian ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, Lopez-Carrillo L. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Annals of the New York Academy of Sciences. 2008;1140:155-162
- Trabert B, Longnecker MP, Brock JW, Klebanoff MA, McGlynn KA. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ Health Perspect. 2012;120:478-482
- Tsai J, Kaye WE, Bove FJ. Wilms' tumor and exposures to residential and occupational hazardous chemicals. International journal of hygiene and environmental health. 2006;209:57-64
- Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Van Trong P, Nhung NT. Impacts of pesticide use on semen characteristics among rice farmers in kienxuong district, thaibinh province, vietnam. The Southeast Asian journal of tropical medicine and public health. 2007;38:569-575
- Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. Environ Health Perspect. 2009;117:1076-1082
- Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and dde in adults. Environ Health Perspect. 2007;115:1197-1203
- Twum C, Wei Y. The association between urinary concentrations of dichlorophenol pesticides and obesity in children. Reviews on environmental health. 2011;26:215-219
- Ubaidullaeva KM. [the clinical and functional features of chronic obstructive lung disease in patients with organic chlorine pesticides in blood]. Problemy tuberkuleza i boleznei legkikh. 2006:21-23
- Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, Drobna B, Trnovec T, Susienkova K, Labudova V, Gasperikova D, Langer P, Klimes I. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia. 2010;53:899-906
- Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Mdr1 gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16:1172-1177
- Vajdic CM, Fritschi L, Grulich AE, Kaldor JM, Benke G, Kricker A, Hughes AM, Turner JJ, Milliken S, Goumas C, Armstrong BK. Atopy, exposure to pesticides and risk of nonhodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;120:2271-2274
- Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP, Hoppin JA. Chronic bronchitis among nonsmoking farm women in the agricultural health

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EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:574-583

- Valikhani M, Kavusi S, Chams-Davatchi C, Daneshpazhooh M, Barzegari M, Ghiasi M, Abedini R. Pemphigus and associated environmental factors: A case-control study. Clinical and experimental dermatology. 2007;32:256-260
- Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. Prenatal concentrations of polychlorinated biphenyls, dde, and ddt and overweight in children: A prospective birth cohort study. Environ Health Perspect. 2012;120:451-457
- van Amelsvoort L, Mohren D, Slangen J, Swaen G, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Liesivuori J, Tarkowski M, Colosio C, van Loveren H. Immune effects and exposure to ethylenebisdithiocarbamate pesticides in re-entry workers in the netherlands. Human & experimental toxicology. 2008;27:693-699
- van Balen E, Font R, Cavalle N, Font L, Garcia-Villanueva M, Benavente Y, Brennan P, de Sanjose S. Exposure to non-arsenic pesticides is associated with lymphoma among farmers in spain. Occupational and environmental medicine. 2006;63:663-668
- van Bemmel DM, Visvanathan K, Beane Freeman LE, Coble J, Hoppin JA, Alavanja MC. Sethyl-n,n-dipropylthiocarbamate exposure and cancer incidence among male pesticide applicators in the agricultural health study: A prospective cohort. Environ Health Perspect. 2008;116:1541-1546
- Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, Schoeters G, Desager K. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 2009;117:122-126
- Vidal JS, Vidailhet M, Derkinderen P, de Gaillarbois TD, Tzourio C, Alperovitch A. Risk factors for progressive supranuclear palsy: A case-control study in France. Journal of neurology, neurosurgery, and psychiatry. 2009;80:1271-1274
- Vidal JS, Vidailhet M, Elbaz A, Derkinderen P, Tzourio C, Alperovitch A. Risk factors of multiple system atrophy: A case-control study in french patients. Movement disorders: official journal of the Movement Disorder Society. 2008;23:797-803
- Viel JF, Floret N, Deconinck E, Focant JF, De Pauw E, Cahn JY. Increased risk of non-hodgkin lymphoma and serum organochlorine concentrations among neighbors of a municipal solid waste incinerator. Environment international. 2011;37:449-453
- Villarejo D, McCurdy SA. The California agricultural workers health survey. Journal of agricultural safety and health. 2008;14:135-146
- Villeneuve S, Cyr D, Lynge E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Fevotte J, Guenel P. Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: A case-control study in europe. Occupational and environmental medicine. 2010;67:837-844
- Vlajinac HD, Sipetic SB, Maksimovic JM, Marinkovic JM, Dzoljic ED, Ratkov IS, Kostic VS. Environmental factors and Parkinson's disease: A case-control study in belgrade, serbia. The International journal of neuroscience. 2010;120:361-367

Waggoner JK, Kullman GJ, Henneberger PK, Umbach DM, Blair A, Alavanja MC, Kamel F, Lynch CF, Knott C, London SJ, Hines CJ, Thomas KW, Sandler DP, Lubin JH, Beane

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EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Freeman LE, Hoppin JA. Mortality in the agricultural health study, 1993-2007. American journal of epidemiology. 2011;173:71-83

- Walker KM, Carozza S, Cooper S, Elgethun K. Childhood cancer in texas counties with moderate to intense agricultural activity. Journal of agricultural safety and health. 2007;13:9-24
- Waller SA, Paul K, Peterson SE, Hitti JE. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in washington state. American journal of obstetrics and gynecology. 2010;202:241 e241-246
- Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. European journal of epidemiology. 2011;26:547-555
- Wang J, Zhu Y, Cai X, Yu J, Yang X, Cheng J. Abnormal glucose regulation in pyrethroid pesticide factory workers. Chemosphere. 2011;82:1080-1082
- Wang P, Tian Y, Wang XJ, Gao Y, Shi R, Wang GQ, Hu GH, Shen XM. Organophosphate pesticide exposure and perinatal outcomes in shanghai, china. Environment international. 2012;42:100-104
- Wanigasuriya KP, Peiris-John RJ, Wickremasinghe R. Chronic kidney disease of unknown aetiology in Sri Lanka: Is cadmium a likely cause? BMC nephrology. 2011;12:32
- Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ Health Perspect. 2009;117:1007-1013
- Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, Altshul L, Ascherio A. Persistent organochlorine pesticides in serum and risk of parkinson disease. Neurology. 2010;74:1055-1061
- Weisskopf MG, Morozova N, O'Reilly EJ, McCullough ML, Calle EE, Thun MJ, Ascherio A. Prospective study of chemical exposures and amyotrophic lateral sclerosis. Journal of neurology, neurosurgery, and psychiatry. 2009;80:558-561
- Weselak M, Arbuckle TE, Wigle DT, Krewski D. In utero pesticide exposure and childhood morbidity. Environmental research. 2007;103:79-86
- Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D. Pre- and post-conception pesticide exposure and the risk of birth defects in an ontario farm population. Reprod Toxicol. 2008;25:472-480
- Wickerham EL, Lozoff B, Shao J, Kaciroti N, Xia Y, Meeker JD. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment international. 2012;47:80-85
- Wohlfahrt-Veje C, Andersen HR, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Smaller genitals at school age in boys whose mothers were exposed to non-persistent pesticides in early pregnancy. International journal of andrology. 2012;35:265-272
- Wohlfahrt-Veje C, Andersen HR, Schmidt IM, Aksglaede L, Sorensen K, Juul A, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Early breast development in girls after prenatal exposure to non-persistent pesticides. International journal of andrology. 2012;35:273-282
- Wohlfahrt-Veje C, Main KM, Schmidt IM, Boas M, Jensen TK, Grandjean P, Skakkebaek NE, Andersen HR. Lower birth weight and increased body fat at school age in children

EFSA supporting publication 2013:EN-497

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prenatally exposed to modern pesticides: A prospective study. Environmental health : a global access science source. 2011;10:79

- Wohlfahrt-Veje C., Main K.M., Schmidt I.M., Jensen T.K., Grandjean P., Skakkebaek N.E., Andersen H.R. Effects of prenatal exposure to modern pesticides on birth weight, growth and body composition in childhood; Interactions with maternal smoking and PON1 genepolymorphisms. Hormone Research in Paediatrics (2011) 76 SUPPL. 2 (232-233).
- Wojtyniak BJ, Rabczenko D, Jonsson BA, Zvezday V, Pedersen HS, Rylander L, Toft G, Ludwicki JK, Goralczyk K, Lesovaya A, Hagmar L, Bonde JP. Association of maternal serum concentrations of 2,2', 4,4'5,5'-hexachlorobiphenyl (cb-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-dde) levels with birth weight, gestational age and preterm births in inuit and european populations. Environmental health : a global access science source. 2010;9:56
- Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. Environmental exposures and puberty in inner-city girls. Environmental research. 2008;107:393-400
- Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, Wetmur J. Prenatal pesticide and pcb exposures and birth outcomes. Pediatric research. 2007;61:243-250
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of acute myeloid leukemia in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:112-128
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of non-hodgkin lymphoid neoplasms in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:129-146
- Wu P.-L., Dai B.-T., Yu Z.-H., Yu J., Xian Y., Su Y.-C. Dependablity investigation of the risk factors of childhood leukaemia. Chinese Journal of Evidence-Based Medicine (2010) 10:9 (1037-1040).
- Wu T, Bhanegaonkar AJ, Flowers JW. Blood concentrations of selected volatile organic compounds and neurobehavioral performance in a population-based sample. Arch Environ Occup Health. 2006;61:17-25
- Xia Y, Han Y, Wu B, Wang S, Gu A, Lu N, Bo J, Song L, Jin N, Wang X. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertility and sterility. 2008;89:1743-1750
- Xu JX, Hoshida Y, Yang WI, Inohara H, Kubo T, Kim GE, Yoon JH, Kojya S, Bandoh N, Harabuchi Y, Tsutsumi K, Koizuka I, Jia XS, Kirihata M, Tsukuma H, Aozasa K. Life-style and environmental factors in the development of nasal nk/t-cell lymphoma: A case-control study in east asia. International journal of cancer. Journal international du cancer. 2007;120:406-410
- Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR. Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in u.S. Adults. Environ Health Perspect. 2010;118:60-66
- Xu X, Nembhard WN, Kan H, Kearney G, Zhang ZJ, Talbott EO. Urinary trichlorophenol levels and increased risk of attention deficit hyperactivity disorder among us school-aged children. Occupational and environmental medicine. 2011;68:557-561

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- Yang JH, Lee YM, Bae SG, Jacobs DR, Jr., Lee DH. Associations between organochlorine pesticides and vitamin d deficiency in the u.S. Population. PloS one. 2012;7:e30093
- Yang Yang, Zeng Li-Xia, Yan Hong. Analysis of risk factors of birth defects in Shaanxi Province. Journal of XI'an Jiaotong University (Medical Sciences) 2011; Vol. 32 No. 1
- Yiin JH, Ruder AM, Stewart PA, Waters MA, Carreon T, Butler MA, Calvert GM, Davis-King KE, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of pesticide applicators and risk of glioma. Environmental health: a global access science source. 2012;11:39
- Yucra S, Gasco M, Rubio J, Gonzales GF. Semen quality in peruvian pesticide applicators: Association between urinary organophosphate metabolites and semen parameters. Environmental health: a global access science source. 2008;7:59
- Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in peruvian pesticide sprayers. International journal of occupational and environmental health. 2006;12:355-361
- Zakerinia M, Namdari M, Amirghofran S. The relationship between exposure to pesticides and the occurrence of lymphoid neoplasm. Iranian Red Crescent medical journal. 2012;14:337-344
- Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. Muscle invasive bladder cancer in upper egypt: The shift in risk factors and tumor characteristics. BMC cancer. 2008;8:250
- Zhang Y, Zhu S, Gao Y, Wang XJ, Chen T, Yang Y, Wang GQ, Hu GH, Shi R, Jin P, Tian Y. [a case-control study on correlation of pesticide exposure with childhood acute leukemia]. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2011;45:41-46
- Zhu JL, Hjollund NH, Andersen AM, Olsen J. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: A study within the danish national birth cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:347-352
- Zota AR, Aschengrau A, Rudel RA, Brody JG. Self-reported chemicals exposure, beliefs about disease causation, and risk of breast cancer in the cape cod breast cancer and environment study: A case-control study. Environmental health: a global access science source. 2010;9:40
- Zschiedrich K, Konig IR, Bruggemann N, Kock N, Kasten M, Leenders KL, Kostic V, Vieregge P, Ziegler A, Klein C, Lohmann K. Mdr1 variants and risk of parkinson disease. Association with pesticide exposure? Journal of neurology. 2009;256:115-120

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#### **GLOSSARY AND ABBREVIATIONS**

AHS: Agricultural Health Study

Beta estimate: coefficient of linear regression

Bias: A systemic inaccuracy in data due to the characteristics of the process employed in the creation, collection, manipulation and presentation of the data or due to faulty sample design of the estimating technique

Biomarker: A measurable substance or characteristic in the human body that can be used to monitor the presence of a chemical in the body, biological responses, or adverse health effects. Biomarkers of exposure are used to assess the amount of a chemical that is present within the body.

Blinded outcome assessment: Individuals who assess the exposure are blinded to the health outcome status of the participants.

CARDIA: The "Coronary Artery Risk Development In Young Adults" study, a multi-center, population-based study.

Case-control study: A type of observational study in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute. Case-control studies are retrospective, as the exposure status is assessed retrospectively.

Case reports: Detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of individual patients.

Case series: descriptive study that tracks patients with a known exposure given similar treatment or examines their medical records for exposure and outcome. These studies lack control groups.

Center-specific analysis: Analysis per centre in studies, which have participants, recruited from more than one centre.

CHAMACOS: The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS). A prospective birth cohort aimed at studying the association of pesticides and other environmental agents on the health of pregnant women and their children living in the Salinas Valley, California.

#### CI: Confidence Interval

Cohort study: A longitudinal/prospective study, which analyses risk factors and follows a group of people who do not have the disease until participants develop the disease(s) of interest

Confounders: Extraneous variables in a statistical model that correlate (positively or negatively) with both the dependent variable (exposure) and the independent variable (outcome)

Cross-sectional study: A study that involves observation of all of participants at one specific point in time, exposure and outcome are measured in the same time point.

Ecological study: Studies in which the unit of observation is the population or community. Disease rates and exposures are measured in each of a series of populations and their relation is examined.

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Effect (binary/continuous): Outcome is binary (dichotomous, e.g. cancer (yes/no)) or continuous (e.g. systolic blood pressure (120mmHg)).

Effect estimate/ size: A measure of the strength of association

ESCALE: The "Etude sur les cancers de l'enfant" study, a national registry-based case-control study

Funnel plots: graph designed to check the existence of publication bias in systematic reviews and meta-analyses

Heterogeneity: meta-analysis is used to estimate a combined effect from a group of similar studies. However, the individual estimates of treatment effect will vary by chance; some variation is expected. The question is whether there is more variation than would be expected by chance alone. When this excessive variation occurs, it is called heterogeneity

#### HR: Hazard Ratio

 $I^2$ : measure of the consistency between trials in a meta-analysis, it is a measurement of heterogeneity and takes values form 0 (no heterogeneity) to 1 (extreme heterogeneity)

INUENDO: "INUENDO—Biopersistent organochlorines in diet and human fertility" Epidemiological studies of time to pregnancy and semen quality in Inuit and European populations", a European project on fertility that was supported by the European Commission to the 5th Framework Programme Quality of Life and Management of Living Resources, Key Action 4 on Environment and Health (Contract no. QLK4-CT-2001-00202) (http://www.inuendo.dk).

IRR: Incidence rate ratio

IQR: Interquartile Range

JEM :Job Exposure Matrix

MD: Mean Difference

Meta-analysis: The process or technique of synthesizing research results by using various statistical methods to retrieve, select, and combine results from previous separate but related studies.

Multiple testing: Testing many hypotheses, which are not a priori defined or based on a priori hypothesis.

Misclassification: Bias in an estimate arising from measurement error

Multivariable models: Statistical models with more than one dependent variable. These models typically adjust for a number of confounders the analysis of interest.

Nested case-control study: In a nested case-control study, cases of a disease that occur in a defined cohort are identified and, for each, a specified number of matched controls is selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case

NHANES: National Health and Nutrition Examination Survey

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Observational study: an observational study draws inferences about the possible effect of a treatment on subjects, where the assignment of subjects into a treated group versus a control group is outside the control of the investigator

OR: Odds ratio

Pooled effect estimate: Summary effect estimate of the meta-analysis, the result of meta-analysis

POPs: Persistent Organic Pollutants

Prospective study: An epidemiologic study in which the groups of individuals (cohorts) are selected on the bases of factors that are to be examined for possible effects on some outcome

Publication bias: Bias arisen from the tendency for <u>researchers</u>, editors, and pharmaceutical companies to handle the reporting of experimental results that are *positive* (i.e. showing a <u>significant</u> finding) differently from results that are <u>negative</u> (i.e. supporting the <u>null hypothesis</u>) or inconclusive.

Recall bias: Systematic errors due to differences in accuracy or completeness of recall to memory of past events or experiences.

Residual confounding: Residual confounding occurs when a confounder has not been adequately adjusted for in the analysis (usually because the confounder is not known)

Retrospective study: an epidemiologic study in which participating individuals are classified as either having some outcome (cases) or lacking it (controls); the outcome may be a specific disease, and the persons' histories are examined for specific factors that might be associated with that outcome

Reverse causality: Reverse causality refers to the direction of cause-and-effect, it is not known whether the exposure has led to the outcome or the outcome has led to the exposure.

RR: Relative Risk

Narrative review: An article written to consider the critical points of current knowledge including substantive findings, as well as theoretical and methodological contributions to a particular topic

SD: Standard Deviation

SE: Standard Error

Surrogate outcome: A laboratory measurement or physical sign that is used in trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the exposure

Systematic reviews: Reviews of the evidence on a clearly formulated question that use systematic and explicit methods to identify, select and critically appraise relevant primary research, and to extract and analyse data from the studies that are included in the review

Type-I error: The incorrect rejection of a true null hypothesis

UFW: United Farm Workers

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#### 外部科学報告書

## 農薬ばく露と健康影響に関連する疫学研究に関する文献レビュー<sup>1</sup>

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#### 概要

我々は、2006 年以降に発表された農薬ばく露と健康影響との関連を調査した疫学研究の系統的かつ 広範な文献レビューを行った。43,259 件の引用文献を検索し、非常に多様な影響を調査した 603 件の 論文を同定し、6,000 件以上の農薬ばく露と健康影響との間の分析結果を提示した。さまざまな影響を 23 の主要な疾患カテゴリーに分類した。研究の中で最も多いのは、発がん影響(N=164)と子どもの健 康に関連した影響(N=84)であった。研究の大部分は症例対照研究と横断研究(N=222)で、農薬への 職業ばく露(N=329)を調査したものであった。さまざまな農薬の定義を用いて研究が行われ、広範囲 かつ多様な農薬が研究されていたため、これらの情報を研究間で調和させることは非常に難しい。利 用可能なデータの量が多く、分析の数も多い(6,000以上)にもかかわらず、研究された結果の大部分 について確固たる結論を出すことはできなかった。これは、この分野の研究量の多さを考慮すると、特 に残念な結果である。しかし、この結果は環境疫学、特に農薬に関するこれまでの研究と一致してお り、疫学研究には多くの限界があり、データの不均一性があるために確固たる結論を出すことができ ないことを認めている。我々はまた、主要な影響及び2006年以降に発表された関連するメタアナリシ スが確認されたものについて、更新されたメタアナリシスを実施した。これは小児白血病とパーキン ソン病についてのみ可能である。これらの影響については、以前のエビデンスに沿って、農薬ばく露と 疾患との間に有意な関連があることが分かった。

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キーワード 農薬;疫学研究;農薬ばく露;健康影響;死亡率;症例対照研究;コホート研究

#### 免責事項

本文書は、上記の著者として特定された機関によって作成され、採用されたものである。この作業は、欧州食品安全機関と著者 との間の契約に基づき、入札手続きを経て落札された著者のみが行ったものである。本文書は、欧州食品安全機関が従う情報公 開の原則に従って公表されている。当局が採用した成果物とはみなされない。欧州食品安全機関は、著者の権利を損なうことな く、本文書で取り上げられた問題及び結論に関して、その権利、見解及び立場を留保する。

<sup>&</sup>lt;sup>1</sup> 質問番号 EFSA-Q-2012-00372 このアウトプットに関連するお問い合わせは <u>pesticides.ppr@efsa.europa.eu</u> までお願いしま す。提案された引用。Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I, 2013. 農薬へのばく露と健康へ の影響を関連付ける疫学研究に関する文献レビュー。EFSA 支援出版物 2013:EN-497, 159 pp. Available online: www.efsa.europa.eu/publications

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### 背景と参考文献

ここ数年の間に、農薬ばく露とヒトへの有害健康影響との関連を調査する疫学研究が豊富に行われ るようになった。これらの研究では、例えば、吸入、経口、経皮及び経胎盤による農薬ばく露が、様々 な臓器や組織のがん、小児の神経発達障害、アレルギー、生殖能力の低下(男性と女性)、先天異常、 パーキンソン病などの原因となる、あるいはその可能性が示唆されていることが立証されている。

しかし、農薬ばく露に起因する多くの有害健康影響については、矛盾した、あるいは曖昧な研究も存 在する。研究は一般的にデザイン(例:症例対照研究とコホート研究)、サンプルサイズ、そして多く の場合、ばく露量は実際の測定値ではなく、むしろ推定値または想定値である。

2006年1月1日から2012年3月31日までの関連出版物を網羅した包括的な最新の文献収集とレビューを実施し、これらの研究の質も評価すべきである。

今回の調達手続きによる契約の目的は以下の通りである。

目的1:農薬ばく露とヒトへの有害健康影響との関連が調査された科学的出版物を収集・集積する。

目的2:収集された各研究の質的側面(調査のコーナーポイントなど)をレビューし、評価する。

目的3:疫学研究のデータベースと報告書の提供。

本契約は、EFSAが次の者に授与した。イオアニナ大学医学部衛生・疫学部(Ioannina, Grecce) 契約者: イオアニナ医科大学衛生・疫学部、イオアニナ大学、(Ioannina, Grecce)。 契約タイトル: 農薬へのばく露と健康影響を関連付ける疫学研究の文献調査 契約番号: CFT/EFSA/PRAS/2012/04 - CT 01

### 序文と目的

本プロジェクトは、すでに確立されている、あるいは示唆されているヒトにおける有害影響との関 連についての理解を深めるために、農薬ばく露と健康関連影響との関連を調査するために実施された 疫学研究を体系的に収集し、レビューし、評価することを目的としている。このレビューでは、特に食 い違いの原因を調査することに焦点を当て、職業を通じた、あるいは一般集団におけるすべてのばく 露タイプに焦点を当てている。特に、農薬ばく露とヒトへの有害健康影響との関連が調査された科学 的出版物を収集した。利用可能なエビデンスは、その質的側面に関してレビューと評価が行われてい る。最後に、農薬の有害健康影響を調査した研究のデータベースを作成した。

最終報告書は、健康影響カテゴリーを中心に構成され、データ抽出データベースとリンクしている。 方法のセクションでは、文献レビューと研究の選択プロセスで使用した検索基準と検索戦略の詳細な 文書を提供している。このセクションでは、選択されたばく露とばく露指標及び検討された代用及び 臨床影響に関する詳細な文書を用いた分析的枠組みについても記述している。対象となる研究の完全 なリストとデータ抽出データベースの内容を含めた文献検索の結果を提示する。また、検討した影響 と農薬の結果及び文献レビューの結果に基づく結論を提示する。

#### 背景と目的

農薬は、昆虫、真菌、野鼠、不快生物、雑草などの作物にダメージを与えることができる有害生物に 対して広く使用されており、長年にわたり、損失を防止または削減し、作物の品質を向上させるために 使用されている。2006年と2007年には、世界で約52億ポンドの農薬が使用された。しかし、農薬は広範 囲に使用されており、それに関連して農薬の使用による利益があるにもかかわらず、これらの化学物 質は対象生物に有害影響を及ぼすように設計されているため、ヒトへの有害健康影響が懸念されてい る。実際、農薬の使用とがん、神経変性疾患、先天異常などの健康有害影響との間にはエビデンスがあ るが、これまでのところ結果には一貫性がなく、いくつかの農薬について確固たる結論を出すことは できていない。

本レビューの目的は、農薬ばく露と健康関連影響との関連を調査するために実施された疫学研究を 体系的に収集し、レビューし、評価することである。このレビューでは、特に食い違いの原因を調査す ることに重点を置いて、職業を通じた、または一般集団におけるすべてのばく露タイプを対象として いる。特に、農薬ばく露とヒトへの有害健康影響との関連が調査された科学的出版物を収集し、まとめ た。利用可能なエビデンスは質的側面に関してレビューされ評価され、妥当な研究からデータが抽出 されている。最後に、結果の継続的な更新を容易にする目的で、農薬の有害健康影響を調査した研究の データベースを作成した。

前述の目的は、特に環境疫学と農薬ばく露の方法論的課題と、膨大な量の査読付き文献のため、刺激的な課題を構成している。

### 材料と方法

### 1. 検索戦略

包括的な文献検索を実施し、農薬ばく露と健康影響に関する査読付きの原著研究を対象とした。検 索戦略は、2006年1月1日から2012年9月30日までに発表された観察疫学研究で、重要なばく露期間(妊 娠前、妊娠期、小児期、成人期)における農薬ばく露と健康関連影響との関係を調査したものを特定す るように設計された。検索戦略は、主にMEDLINE(1950年から現在まで)、EMBASE(Excerpta Medica Database、1980年から現在まで)データベースを使用し、TOXNET(Toxicology Data Network、U.S. National Library of Medicine 2012)、OpenSigle(2012)及びProQuest Digital Dissertations and Theseses(2012)を補足検索として使用した。

### 2. MEDLINE 及び EMBASE における原著論文の検索アルゴリズム

本システマティックレビューは、農薬へのばく露と関連した臨床影響または臨床影響の代用となる 有効なバイオマーカーを調査した研究を特定することを目的とした。最大限の感度を得るために、我々 が開発した検索アルゴリズムには影響に関連する検索用語は含まれていない。検索アルゴリズムの構 築にあたっては、MEDLINEMESHの用語とEMBASEの農薬分類ツリーを用いて同定された農薬関連の用語に 集中した。MEDLINEでは、農薬と農薬(薬理作用)のMESH用語を調べた。同様に、EMBASE Entreeインデ ックスの農薬用語を調べた。農薬のカテゴリー(例えば、殺虫剤、除草剤、殺菌剤など)と、文献に記 載されている特定の農薬名、または薬理学的用語(例えば、DDTまたはジクロロジフェニルトリクロロ エタン)を網羅的に調べた。また、過去10年間の農薬ばく露に関する出版されたシステマティックレビ ューで使用された検索用語を調べ、追加の用語を調べた。

最初に構築したアルゴリズムは、前述のすべての用語を含む長いものであった。異なる検索を試験 的に行い、精度への影響を最小限に抑えながらアルゴリズムの感度を向上させるために検索を短縮し た。すべての検索はヒトに限定し、2006年1月1日以降の出版日に限定した。

農薬薬理学的名称のMESHデータベースから提供された農薬名の長いリストは、MEDLINEの農薬関連 語検索(pesticid\* OR pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields] OR "pesticides"[Pharmacological Action])の上位2,270件の引用のみを提供していた。そ のうち2,270件の引用のうち200件を調査したところ、疫学研究は含まれておらず、農薬の物性や化学 構造に関する化学的研究を参照していることが判明した。そこで、一般用語を含む検索アルゴリズム を採用した。アルゴリズムは、データベースがMEDLINEとEMBASEを同時に検索する機能を提供している ため、EMBASEで構築した(下記のテキストボックスを参照)。以下のようなアルゴリズムを構築した。

### 表 1: EMBASE 及び MEDLINE の検索アルゴリズム

pesticid\* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid\* OR 'fungicide'/exp OR herbicid\* OR 'herbicide'/exp OR insecticid\* OR 'insecticide'/exp OR molluscacid\* OR'molluscacide'/exp OR molluscicid\* OR 'molluscacide'/exp OR rodenticid\* OR 'rodenticide'/exp OR carbamat\* OR 'carbamate'/exp OR pyrethroid\* OR 'pyrethroid'/exp OR 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp AND [humans]/lim AND [2006-2013]/py

このアルゴリズムにより、EMBASE と MEDLINE を合わせて 43,259 件の引用が行われた。そのうち 14,539 件は EMBASE に固有のものであった。このアルゴリズムには、分割オプションを使用して Emtree エン トリとして、またテキストワードとして使用された農薬関連の用語とサブカテゴリがすべて含まれて いる。分割オプションを使用すると、用語が Emtree のシソーラス内でより具体的な、またはより狭い インデックス用語を持つ場合、それらも検索の一部として自動的に検索される。有機塩素、グリホサー ト、パラコート、マネブなどの用語は、分割オプションの農薬ツリーの一部であり、検索されるため、 除外された。これらの用語を含めると、同じ結果が得られる。以下の図 1 は、EMBASE で検索された用 語のインデックスツリーの例を示している。

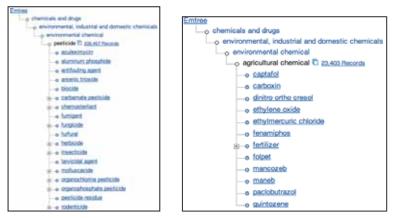


図 1: Emtree の分類ツリーの例

### 3. 補足検索

また、毒性学、有害化学物質、環境衛生、有害物質排出に関するデータベースを掲載しているTOXNET のデータベースを検索し、MEDLINEやEMBASEでの前回の検索で見逃した情報を特定した。生物医学文献 (Toxicology Literature Online (TOXLINE)及びDevelopmental Toxicology Literature (DART))の 文献を検索するデータベースのみを使用した。残りのTOXNETデータベースは、化学物質ごとの化学的 データ、毒物学的データ、環境データのサマリーを提供しており、今回の検索には関係なかった。 TOXLINEとDARTについては、「Pesticide OR Pesticides」という一般的な用語を使用したが、農薬のサ ブカテゴリを含む検索アルゴリズムが長くなっても、同定された参考文献の数にわずかな影響しかな かったためである。検索は2006年以降の出版日に限定し、MEDLINEで同定された文献は除外した。検索 語の化学物質の同義語を特定する機能を有効にした。全体では、TOXLINEから893件、DARTから34件の文 献が検索された。

また、ヨーロッパで出された灰色文献[非商業出版物](論文)の書誌的参考文献700,000件を収録したSystem for Information on Grey Literature in Europe (OpenSigle)も調べた。2006年以降に出

版された農薬(検索語pesticid\*)に関する書誌的文献はなかった。

また、ProQuest Digital Dissertations and Thesesデータベースを検索するための検索アルゴリズ ムを構築した。MEDLINEやEMBASEで検索した結果、学術雑誌に掲載された論文は検索対象から除外した。 検索語を"pesticide\* AND health "とし、特定の主題(環境科学 OR 公衆衛生 OR 環境保健 OR 疫学 OR 農薬 OR 栄養 OR 労働衛生)と、2006年から2012年までの出版日に限定して検索を行った。この検 索戦略の結果、1,713件の検索結果が得られた。主題制限が行われていない場合(12,135)、または「健 康」という用語が最初のアルゴリズムから除外されている場合(18,195)には、多くの検索結果が得ら れた。

最後に、特定されたすべての妥当な研究とシステマティックレビューの参考文献リストをデータ抽 出の際にスキャンして追加の参考文献を探した。

#### 4. 文献システマティックレビューとメタアナリシスの検索

また、特定の健康影響に関連したシステマティックレビューやメタアナリシスを対象とした検索を 行った。MEDLINEでは、4件以上の研究が確認された健康影響を対象としたレビューに限定し、健康影響 名と「システマティックレビュー OR メタアナリシス」というキーワードを用いて、論文のタイトル または要約に限定して検索を行った。

#### 5. 本報告書の構成

本報告書は、健康影響のカテゴリーを中心に構成されており、各健康影響のグループの結果を個別 に提供している。最後に一般的な結論のセクションが提示されている。また、各健康影響のセクション の最後には、読みやすいように表と図を掲載している。また、本文中では各対象論文の ID 番号を参 照している。これらは、本報告書に別ファイルとして提供されているデータ抽出データベースの各健 康影響グループの ID に対応している。IDは、特定の健康影響の略語と研究番号で定義されている。

# 表2:検索されたリソース、検索語及び特定された参照先の概要

データ			
ベース	検索条件	Limits	文献数
MEDLINE	pesticid* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'herbicide'/exp OR insecticid* OR 'insecticide'/exp OR molluscacid* OR 'molluscacide'/exp OR molluscicid* OR 'molluscicide'/exp OR rodenticid* OR 'rodenticide'/exp OR carbamat* OR 'carbamate'/exp OR pyrethroid* OR 'pyrethroid'/exp OR 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp pesticid* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'herbicide'/exp OR insecticid* OR 'insecticide'/exp OR molluscacid* OR 'molluscicide'/exp OR molluscacid* OR 'molluscicide'/exp OR rodenticid* OR 'rodenticide'/exp OR carbamat* OR 'rodenticide'/exp OR pyrethroid* OR 'molluscicide'/exp OR carbamat* OR 'rodenticide'/exp OR carbamat* OR 'rodenticide'/exp OR pyrethroid* OR 'pyrethroid'/exp OR pyrethroid* OR 'pyrethroid'/exp OR pyrethroid* OR	ヒト, 公開日: 2006~2012 ヒト, 公開日: 2006~2012 ELINE で確認された文献は無	28, 729
EMBASE	chemical'/exp	No. 公開日:2006~2012年、MEDLINE	14, 530
TOXLINE	Pesticide OR Pesticides	で確認された文献は無い。 公開日:2006~2012 年、MEDLINE	893
DART	Pesticide OR Pesticides	で確認された文献は無い。	34
OpenSigle	Pesticide*	掲載時期:2006年~2012年 掲載時期:2006年~2012年、 主題(環境科学、公衆衛生、環 境保健、疫学、農薬、栄養、労 働衛生)、専門的学術誌に掲載	0
ProQuest	Pesticide* AND health	された論文は無い。	1,713 合計: 45,899

#### 6. 研究の選択

様々なデータベースの文献検索で特定されたすべてのタイトルをスクリーニングし、農薬と代替健 康影響を含む健康影響との関連を評価した研究を特定した。次に、選択したタイトルのすべての要約 を重複してスクリーニングし、農薬ばく露と代替健康影響を含むあらゆる健康影響との関連を評価す る疫学研究を特定した。主要研究とシステマティックレビューまたはメタアナリシスの両方を選択す る。要約審査の段階で妥当性基準を満たす可能性のある論文を検索し、全文論文を重複して審査して 妥当性を確認した。すべての全文論文の却下理由は記録されている。

#### 6.1. 全文論文の妥当性基準

農薬ばく露と健康関連影響との関連を評価する観察研究を対象とした。コホート研究、横断研究、症 例対照研究を対象とした。2006 年 1 月 1 日から 2012 年 9 月 30 日までに発表されたヒトを対象 とした研究を対象とした。動物を対象とした研究及びヒトの細胞を対象とした研究は除外した。言語、 集団、地理的制限はなかった。エビデンスの全体性を高めるために、すべての種類の農薬を考慮した。 農薬へのばく露は、研究参加者による農薬の使用報告、または政府登録データ(自己管理質問紙、面接 官管理質問紙、職業ばく露マトリックス (JEM))、居住状況(農薬ばく露への近接性)、農薬ばく露 に関連するバイオマーカーの検出、または各研究で定義されたその他の手段によって定義されたもの とした。妥当な健康関連影響は、腫瘍形成やパーキンソン病などの「主要な」臨床影響、神経認知スケ ールなどの臨床代替健康影響、肝酵素などの臨床影響との関連が確立されている代用実験的影響とし た。

ナラティブレビュー、症例集積、症例報告(対照集団のない研究)は除外した。また、農薬中毒また は不慮の高用量農薬ばく露による健康関連の影響を評価する研究も除外した。論文で報告された十分 な量的情報(効果推定値など)が入手できない研究は除外し、効果量や関連の尺度を計算できるように した。異なる追跡期間で同じ研究に関連する報告があり、同じ結果を調査している場合は、データの重 複を避けるために、追跡期間が長い方の研究を保持した。また、肥料に言及した研究(アルゴリズム用 語「農薬」から分解)や、抗凝固剤のワルファリンや疥癬の治療に用いられる薬剤など、様々な病状の 治療に用いられる物質の副作用に言及した研究も除外した。農薬・除草剤に含まれる溶剤などの非有 効成分は対象外とした。化学戦経験者に対するオレンジ剤の様々な影響を調査した研究は、極高用量 ばく露の事例であるため除外した。最後に、ばく露とばく露のバイオマーカーとの関連を調査した研 究も、健康への影響を調査していないため、対象外とした。最後に、EFSAとの協議により、ヒ素、アル ファ及びベータへキサクロロシクロへキサン(HCH)、鉛、ポリ塩化ビフェニル(PCB)を含むダイオキ シン類などの農薬へのばく露を調査した研究/分析は、本プロジェクトには関係がないと考えられる ため除外した。

システマティックレビュー及びメタアナリシスについては、評価された農薬、ばく露域、影響にかか わらず、健康関連影響に対する農薬ばく露の影響をシステマティックに評価したすべてのシステマテ ィックレビュー及びメタアナリシスを調査した。系統的アプローチ(系統的文献検索、対象とした研究 の方法論的特徴の評価、メタアナリシスが実施された場合には重み付け要約推定値の使用や不均一性 の形式的評価を含む標準的な分析ツールの使用)が承認されているすべての出版物を対象とした。ナ ラティブレビューは除外する。

### 6.2. 品質管理対策

パイロット文献検索はすべて重複して実施した。さらに、すべてのタイトル検索の最初の500件の結 果は重複して行い、研究者間で結果を比較し、高いレベルの一致を示した。同意度のカッパ統計量は 0.78であった。2人の独立した研究グループのメンバーが、要約スクリーニング、全文スクリーニング、 データ抽出を重複して行った。すべての不一致は、合意または第三者の判定によって解決した。

### 6.3. データ抽出データベース

全工程を通じて実行されたデータ抽出項目と品質評価項目を用いて、データ抽出データベースの構 築及び確認を行った。データ抽出データベースは7つのドメイン:参考文献、期間、試験特性、ばく露 評価、影響、統計分析、品質評価(別々のファイル)で構成されている。最初の 6 つの領域は、対象 となる研究の全文から直接抽出された情報に関連しており、主に定量的統合のための研究を選択し、 定量的統合を支援するために使用される。研究は、調査された各影響と調査された各ばく露について、 データベースの一行を提供している。研究が様々なばく露の定義を提示している場合には、最も包括 的なばく露の定義をデータ抽出のために選択し、それに続いてサンプルサイズが最も大きいものを選 択する。しかし、同じ化学物質の同定に使用される異なるバイオマーカーの定量的情報を含む研究、例 えばジクロロジフェニルトリクロロエタン (DDT)の p,p'-DDT と p,p'-DDE のように、それらはすべ て別々の行で報告されている。研究がサブグループでデータを提示している場合(例:男性と女性) は、データがサブグループでのみ提示されている場合は複合論争が提示されている場合を除き、主分 析(グループ全体)のみを抽出している。異なる農薬クラスと異なる健康影響に関する分析は、個別に 抽出されている。付録IIでは、データ抽出データベースで使用されたすべての項目を説明している。

データ抽出フォームは、妥当で体系的な手順を経て検証された。具体的には、データベースから無作 為に選択された研究の情報を抽出するブラインドループの後、様々なバージョンのフォームを検証し た。データベースの網羅性を維持しつつ、最大の一致度が得られるものを選択した。各項目について は、2名の研究者がそれぞれ独立して抽出し、不一致は議論によって解決している。

#### 6.4. 品質評価

データ抽出データベースの最後の部分は、対象となる各論文の方法論的評価に関するものである。 ここでは、研究デザイン、研究集団、ばく露の定義の詳細度、ばく露測定の方法、測定の特異性などに 焦点を当てている。これらはばく露評価の疫学において問われる重要な問題である。我々はまた、マッ チングモデルや多変量モデル、盲検化されたばく露評価、十分に定義された有効な影響評価によって 交絡因子を考慮するための努力にも焦点を当ててきた。また、資金源が認められているかどうかにも 注目した。方法論的評価の要素は RTI 項目バンクから調査した。RTI 項目バンクは、システマティッ ク・エビデンスレビューに含まれる介入またはばく露の観察研究のバイアスのリスクと精度を評価す るための実用的で妥当性のある項目バンクである。質問はばく露評価を反映するように適応されてい る。質的評価の質問では、質的評価への回答を一貫して緑、オレンジ、赤で色分けし、緑はバイアスの リスクが低く、赤はバイアスのリスクが高いことを表している。以下の表 3 は、それぞれどの回答が 低リスク、高リスクと考えられたかを説明したものである。しかし、品質評価の質問は、各研究に関連 するバイアスのリスクを示唆しているに過ぎないので、注意して解釈する必要がある。この品質評価 のスコアが高くても、バイアスのリスクが高い研究もあれば、その逆もあるかもしれない。最後の列 は、研究の全体的な質を低、中、高で評価するために作成された。この分類は、表3で説明した方法論 的評価の質問に対する回答に基づいている。

### 表3:対象研究の方法論的評価

省間	高リスク	低リスク
研究デザイン(有望、回顧的、混合、断面的)	回顧的、混合、該 当なし	有望
除外基準が明確に記載されている(はい、部分的に、いいえ)	いいえ	はい
著者は電力計算について言及しています(はい、いいえ)		はい
暴露の記述の詳細レベル(高、中、低)	低	高
暴露のロパストな測定(パイオマーカー(有):小面積生態学的尺度、 職種、アンケート(部分的):大面積生態学的尺度(無)に基づいてい る。)	いいえ	はい
曝露の尺度は特定のものだったか?はい:より広範な化学的に関連したグループに基づいて(部分的)、多様な化学的および毒性学的特性の 広範なグループに基づいて(いいえ)。	いいえ	はい
グループ間の配分のバランスを図る (層別化、マッチングなど)。	いいえ	はい
潜在的な交絡因子の調整を行った(はい、いくつか、いいえ)。	いいえ	はい
被ばく状態に盲検化された評価者(コホート研究の場合)	いいえ	はい
有効かつ信頼性の高い尺度を用いて評価された結果は、すべての研究	いいえ	はい
参加者に一貫して実施されているか?		
サンプルサイズ	低	最大
ラフな品質評価	6 以上の回答で高リ	6 以上の回答で低リス
	スク	ク

### 6.5. 定量的な統合

結果の定量的な統合は、調査された影響ごとに4件以上の研究があり、発表されたエビデンス間に実 質的な不均一性がない場合にのみ試みられた。不均一性の存在と程度は、I2(0%から100%の範囲)で 評価した(Ioannidis 2007)。固定効果モデルとランダム効果モデルを用いて、RR/ORの推定値をまと めた(Lau 1997)。固定効果モデルでは、共通の基礎となる効果が存在し、観察された変動は偶然のみ に起因すると想定している。不均一でない場合、固定効果モデルとランダム効果モデルでは同じ結果 が得られる。出版バイアスは、ファンネルプロットと結果の視覚的精査を用いて評価した。

5件以上の妥当研究がある各影響について、定量的な統合を試みた。比較するグループが相互に排他 的でない場合には、同じメタアナリシスにおいて、同じコホート研究からのデータはなかった。4件以 上の研究がある各影響については、結果を比較し、以前の研究の背景の中で我々の知見を解釈するた めに、以前に発表されたメタアナリシスも探した。メタアナリシスは、a)文献レビューで同定されたシ ステマティックレビュー及びメタアナリシス及びb)調査対象の各影響について公表されているメタア ナリシスを同定するために、PUBMDBDでの標的検索によって発見された。メタアナリシスのa) 2006年ま でに発表された研究が含まれており、b)影響及びばく露の定義が本報告書で使用されている定義と同 等である場合には、以前に発表されたメタアナリシスを我々の結果で更新するよう試みた。最後に、10 件以上の研究がメタアナリシスに含まれている場合に非対称性を視覚的精査するためにファンネルプ ロットをプロットした。 結果

#### 7. 全体の結果

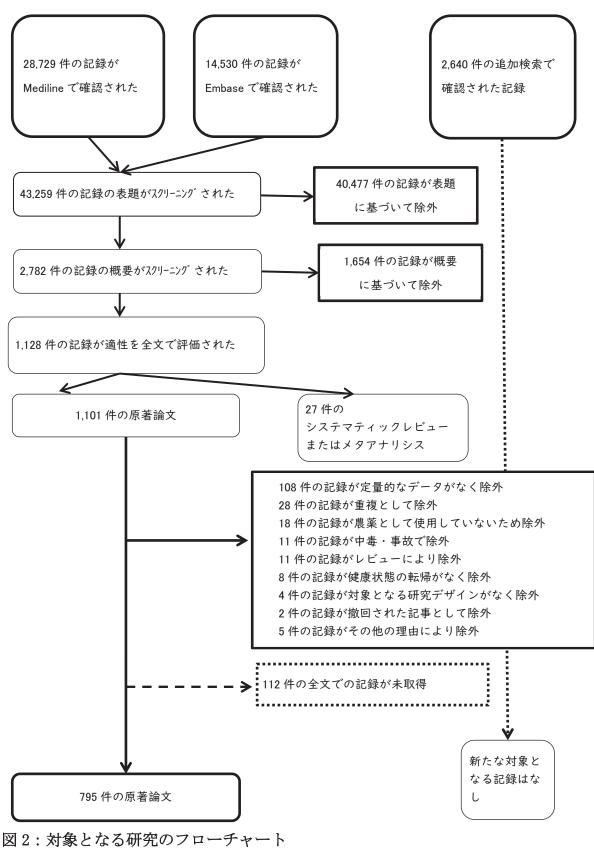
このセクションでは、スクリーニングされ、妥当と判断された研究の数、除外された研究の数とそれ に対応する理由を示すフローチャートを含む文献レビューの結果に焦点を当てている。また、同定さ れた研究とその主な特徴の概要についても説明する。

#### 7.1. 個々の研究の選択プロセス

検索された43,259件の引用のうち、40,477件がタイトルのスクリーニングレベルで除外された。残 りの2,782タイトルのうち、さらに1,654タイトルが要約スクリーニングで除外された。その結果、1,128 件の引用文献のうち、1,101件が原著論文であり、27件がシステマティックレビューまたはメタアナリ シスであることが判明した。1,101 本の原著論文のうち 184 本が除外された(図 2)。また、全文(ま たは学会発表のための要約)がオンラインで見つからない出版物(101 件)については、著者への手紙 や蔵書からの調査により全文を検索したが、要約のみから情報を抽出した58研究については、これが 不可能であった。

全文レベルで除外した主な理由は次の通りである:定量的な情報/データがない(これらは主に、農薬と健康影響との関連に関する定量的な情報を提示していない要約発表またはコメント/論説であった、n=108);記録の重複(n=28)、農薬の使用が示唆されていない(n=18)、中毒または不慮の極高 用量ばく露に関する研究(n=11)、主要データのないレビュー(n=11)、健康影響に関するデータがない(n=8)。補足検索では、大量の政策文書、助成金申請書、研究が既に検索されたため、追加の参考 文献を提供することはできなかった。識別された研究の参照リスト、特に識別されたシステマティッ クレビューの参照リストからの補足検索は、データ抽出の間も継続され、新たに識別された研究は、現 在の妥当な研究リストに追加される。全文スクリーニングとデータ抽出の際に、さらに301研究が除外 された。除外の主な理由は、ポリ塩化ビフェニル(PCB)などの妥当な農薬がなかったことであった(図 2)。全体として、602の個別の出版物が本レビューに含めることができた。これらの602の出版物は、 6,479の異なる分析に対応しており、データ抽出データベースにも存在する。

このアウトプットに関連するお問い合わせは pesticides.ppr@efsa.europa.eu までお願いします。 提案された引用。Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I, 2013. 農薬へのばく露と健康への影 響を関連付ける疫学研究に関する文献レビュー。EFSA 支援出版物 2013:EN-497, 159 pp Available online: <u>www.efsa.europa.eu/publications</u>



### 7.2. エビデンスマップ表と調査した影響

我々は、幅広い病態生理をカバーする多種多様な評価された影響を調査した。このデータベースに は、評価された臨床研究上の質問にアプローチするために承認された様々な方法論を反映して、「ハー ド」な臨床影響と多くの代替健康影響が存在している。我々は、異なる影響を23の主要な疾患カテゴリ ーに分類した(表4及び図3)。研究の中で最も多いのは、発がん(N=164)と小児の健康に関連する影 響(N=84)である。表4はエビデンスマップ表に対応し、2006 年から 2012 年の間に農薬ばく露に関 連したすべての影響とその頻度を記述したプロジェクトの影響マッピングを示したものである。

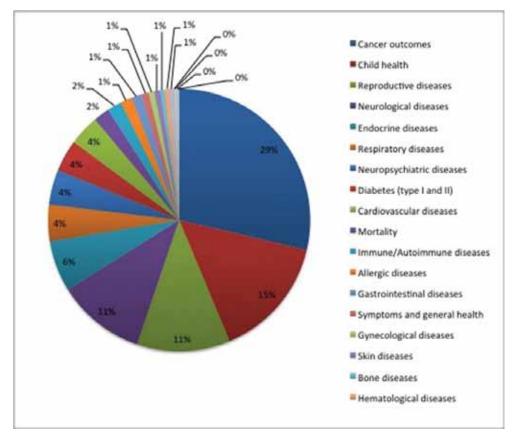


図3:対象となる論文のうち、主要な影響カテゴリーとその影響を調査した研究の 割合

### 表4:対象となる研究で調査されたすべての主要な影響カテゴリーを含むエビデン

ス	7	v	プ	表

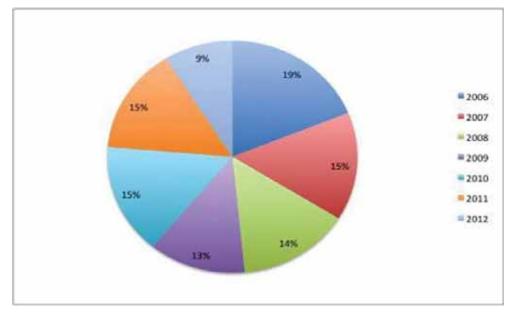
	ودور برقي مسمي
主な影響	研究数
発がん	164
小児の健康	84
生殖器疾患	64
神経疾患	61
内分泌疾患	35
精神・精神運動発達	32
呼吸器系疾患	25
精神神経疾患	15
糖尿病(I型・II型)	22
循環器疾患	31
血液疾患	15
死亡率	11
免疫・自己免疫疾患	10
アレルギー性疾患	8
消化器疾患	7
症状と全身の健康	5
婦人科系の疾患	4
皮膚疾患	4
骨の疾患	3
腎臓の疾患	3
良性腫瘍	1
歯科疾患	1
男性の健康	1
代謝性疾患	1

#### 7.3. 対象となる研究の特徴

対象となる研究は 2006 年から 2012 年までに発表されたものである。対象研究の発表年の分布を 見ると、過去 5 年間でほぼ均等に分布していることがわかる(図 4)。特筆すべきは、現場で実施さ れた今日までの観察研究の中で最大のものである Agricultural Health Study (AHS)の様々な報告書 の結果がかなりの割合で存在することである。実際、AHSの出版物 (n=42) は、含まれている研究の7% を占めている。その他の22件の研究は、国民健康・栄養調査 (NHANES) コホートの横断研究である。

研究の大部分は症例対照研究(N=222)と横断研究(図5)であり、農薬への職業ばく露(N=329)を 調査していた。研究のほぼ半数(N=285)はアメリカを拠点とした研究であった(図6)。農薬の評価方 法は、バイオマーカーの測定、または自記式質問紙の使用が最も多かった(図7)。約半数(N=261)の 研究が方法論的評価で「高」に分類された。

広範囲で多様な農薬が研究されており、様々な農薬の定義を用いた研究が行われている。また、公表 されている文献のかなりの割合が、欧州連合(EU)や先進国のほとんどで使用が承認されていない農薬 に焦点を当てていることも予想される。このような研究は、農薬の長期残留の根拠や、開発途上国での 継続的な農薬使用の根拠に関わるものであることを認識している。例えば、DDTとその代謝物のみに焦 点を当てた研究は、対象となる研究のほぼ10%を占めている。



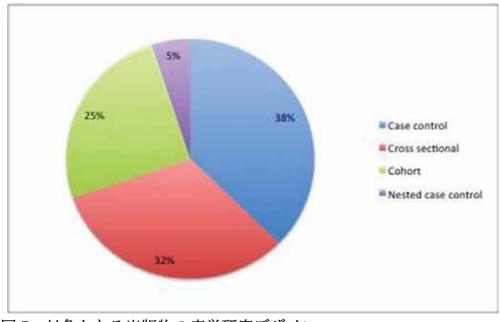


図 4:2006 年から 2012 年までの出版年度ごとの対象論文の割合

図5:対象となる出版物の疫学研究デザイン

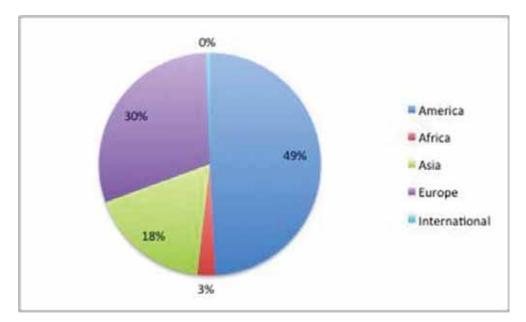


図6:対象となる疫学研究が実施された地域(大陸)の状況

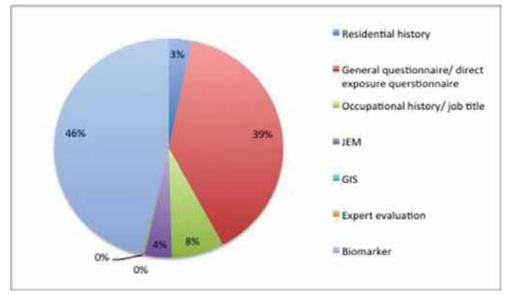


図7:対象となる疫学研究におけるばく露評価の方法

7.4. システマティックレビューとメタアナリシスのシステマティック文献レビュー 検索戦略を通じて、システマティックレビューとメタアナリシスも同定した。全体として、2006年以 降に出版された21の異なる妥当なレビューが同定された。調査した影響を以下の表5に示す。ほとんど のレビューではがんに関連した影響を調査しており、いくつかのレビューでは農薬と調査した影響と の間に明確な関連があると主張していた。これらのレビューは、個々の研究とともに、関連する影響カ テゴリーで議論されている。

## 表5: 文献レビューで確認されたシステマティックレビューとメタアナリシスのリ スト

· 影響	研究数	著者が主張する関連	著者・雑誌・出版年
筋萎縮性側索硬化症	3	No	Sutedja NA et al, 2009 Kamel F et al, 2012 Malek et al, 2012
がん	11		
乳がん	1	No	Khanjani N et al, 2007
小児がん	2	Yes	Infante-Rivard C et al, 2007 Vinson F et al, 2011
小児白血病	6	Yes	Wingle DT et al, 2009 Turner et al, 2010 Van Maele-Fabry G et al, 2010 Van Maele-Fabry G et al, 2011 Bailey HD et al, 2011 Turner MC et al, 2011
多発性がん	1	Yes	Cooper et al, 2008
前立腺がん	1	Yes	Budnik LT et al, 2012
複合的健康影響	1	Yes	Koureas M et al, 2012
神経行動学	2	No	Ismail AA et al, 2012 Li AA et al, 2012
パーキンソン病	2	Yes	Van der Mark M et al, 2012 Van Maele Fabry G et al, 2012
生殖性	1	No	Shirangi A, 2011
妊娠までの期間	1	Yes	Snijder CA et al, 2012

### 8. 発がん

全体では164の出版物が農薬ばく露の発がんへの影響を調査し、2,000以上の個別の分析に貢献した。 他の影響に見られるように、ばく露の定義の多様性は驚くべきものであり、データ統合に特別な問題 をもたらしている。164件のうち36件のみが前向きコホート研究であり、他の13件はコホート内症例対 照研究であった。エビデンスの圧倒的多数は後ろ向き症例対照分析から来ており、ばく露測定におい てリコールバイアスがかかりやすい。また、49の前向き分析のうち、30(61%)は同じ前向き研究であ るAgricultural Health Study (AHS)からのものであり、この前向きコホートを超えたエビデンスは 限られている。これは重要な観察であり、前向きな関連のエビデンスの60%は単一の集団から得られ ているという事実を強調している。分析のサンプルサイズはしばしば小さく、参加者数は24~82,596 人(中央値301人)であった。さらに、33の研究ではばく露のバイオマーカーに関する情報が得られて おり、職業ばく露マトリックス(JEM)を用いて職業ばく露を評価したのは7件のみであった。研究に共 通する制限事項としては、サンプルサイズが小さいこと、ばく露が自己申告であること、多重検定によ る高い偽陽性率の可能性(多重検定を調整せずに複数の仮説を検定しているため、結果が偽陽性にな る可能性がある)及び後ろ向きなデザインなどが挙げられる。多くの研究で有機塩素系殺虫剤を調査 しており、多種多様な農薬が評価された。

調査されたさまざまながんのカテゴリーを、各影響カテゴリーに寄与した研究の数及び定量的統合 の推奨事項とともに表6に示す。データの不均一性と同定された研究数が少ないため、データの統計的 統合(メタアナリシス)は一部のがんサブグループについてのみ実施された。

### 8.1. 造血器新生物

### 8.1.1. 白血病

全体では、26件の研究(及び2件の要約)が農薬ばく露と様々な形態の自血病との関連を調査した。 これら26件の研究のうち14件はAHSからの報告であり、結果が重複していたり、異なる農薬群の調査が 行われていたりした。DDEに関する2件の研究(ID CAN\_063、ID CAN\_064)のみが住居ばく露を調査して おり、残りの研究はすべて農薬への職業ばく露を調査したものである。99の異なる分析のうち12の研 究は、6.1と0.2の間にあるすべての研究の効果量で統計的に有意であった。統計的に有意な結果が得 られたのは7つの研究であり、AHSを除くすべての研究の品質は中等度から低度であった。表7 は、同 じ農薬クラスに関する情報を報告した研究の結果をまとめたものである。結果の大部分は、有意では なく、効果量が小さいものであった。図8は、ランダム効果メタアナリシスを示しており、各研究で最 大のサンプルサイズで分析を行っている。このメタアナリシスでは、有意ではない統合効果(OR 1.26、 95% CI 0.93、1.71)が得られ、中等度の不均一性を有していた。農薬への職業ばく露と自血病に関す る以前のメタアナリシスは、2008年と2007年に発表されている(Merhi 2007, Van Maele-Fabry 2008)。 以前のメタアナリシスからの全体的な要約効果推定値は、農薬への職業ばく露とすべての造血器がん との間には、弱いながらも有意に明確な関連があることを示唆していた。しかし、両報告とも、ばく露 情報や造血器腫瘍の他のリスク因子に関する十分なデータが不足していること、ばく露の定義や白血 病型の定義が不明確であることなど、幅広い限界があることを認めている。

#### 8.1.2. ホジキンリンパ腫

7件の研究が農薬ばく露とホジキンリンパ腫との関連を調査した。すべての研究は質問紙を用いてば く露を評価しており、1件の研究はサンプル数が多く、すべての研究は後ろ向きであった。広範囲の農 薬クラスが調査されたが、結果の意味のある統合はできなかった。75の個別分析のうち12の分析が統 計的に有意であり、すべての分析において効果量は8.4から0.4の範囲であった。我々は、最大のサンプ ルサイズを持つAgricultural Health Study (AHS)分析のみを残して、ランダム効果メタアナリシス を試みた。その結果は統計的に有意ではなく、各研究で調査された農薬クラスが異なることに起因す る可能性がある高い不均一性を有していた(図9)。

### 8.1.3. その他のリンパ腫

ホジキンリンパ腫以外のリンパ腫については、非常に多様な定義が44件の研究で使用されており、 そのうち21件は農業健康調査(Agricultural Health Study: AHS)からの報告で、2件はBC(ブリティ ッシュコロンビア州)製材所の労働者コホート研究からの報告であった。研究ではリンパ腫及びリン パ増殖性症候群(ID CAN\_047、ID CAN\_049、ID CAN\_074)の広範な定義が調査され、他の研究では濾胞 性リンパ腫、びまん性大細胞リンパ腫及び末梢性T細胞リンパ腫のより具体的な定義が調査された。21 件の研究では、農薬ばく露と非ホジキンリンパ腫の幅広い定義との間の効果量が報告された。これら の研究のうち5件は前向き(ID CAN\_063、ID CAN\_064、ID CAN\_067、ID CAN\_118、ID CAN\_121)であり、 7件はばく露のバイオマーカーとの関連を調査した(ID CAN\_056、ID CAN\_057、ID CAN\_064、ID CAN\_065、 ID CAN\_067、ID CAN\_060、ID CAN\_052)。しかし、それ以降の分析はすべて有機塩素系農薬に関するも のであり、関連を示す確固たるエビデンスがないまま、有意な結果が得られたのはわずかであった(全 35 回の分析のうち 6 回の分析)。AHSでは、ブチル酸塩の使用と非ホジキンリンパ腫との間に大きな 有意な効果量が観察された(RR 2.94、95% 1.49~5.96、p=0.002;高ばく露と非ばく露との間に有意な 効果量が観察された)。しかし、同じ出版物のAHSは10種類の異なる影響を調査しており、結果は複数 の試験のための調整が必要であることを繰り返している。

#### 8.1.4. 多発性骨髄腫

また、11件の研究では、農薬と多発性骨髄腫、骨髄異形成症候群及び意義不明の単クローン性免疫グ ロブリン血症との関連が調査された。これらの研究は全般的に異質であり、定量的な統合は示唆され なかった。全体的に、いくつかの分析は統計的に有意であったが、それらは主にフランスの症例対照研 究(ID CAN\_049)からのものであり、147の個別の分析が行われており、結果にバイアスがかかりやす い。AHSはまた、ペルメトリン、ディルドリン、四塩化炭素/二硫化炭素の混合物、クロルタロニルとの 間の有意な関連を報告しているが、これらは他の52分析の中に含まれており、慎重な解釈が必要であ る。ある研究では、骨髄異形成症候群(ID CAN\_070)に対して7.3という非常に高い有意な効果量が報 告されているが、研究の質が悪く、共変量の調整が非常に限られており、同じ表現型の他の研究で結果 が再現されていなかった。

#### 8.2. 前立腺がん

全体では、39件の研究(260件の分析)で農薬ばく露が前立腺がんに及ぼす影響が調査された。その うちの1件は学会発表の要約であり、その結果を評価するための方法論に関するデータがほとんど提供 されていなかった(ID CAN\_107)。また、これら39件の研究のうち25件はAHS集団を対象とした研究で あり、いくつかの結果が重複していた。例えば、2つの研究(ID CAN\_022、ID CAN\_106)では、農薬ば く露と前立腺がんとの関連における遺伝子変異との相互作用が調査されていた。これらのAHS研究では、 農薬ばく露の主な効果は同じであったが、効果はほとんど無効であり、もしあるとすれば、カルバリ ル、クロルデン、メタクロルなどでは有意な逆効果が認められた。残りのAHS研究では、特定の農薬間

の関連を調査したが、いずれの農薬と前立腺がんとの間にも統計的に有意な関連は認められなかった が、ブチル酸塩へのばく露と前立腺がんとの間には弱い有意な影響が認められた。残りのエビデンス は、小規模で質の低い後ろ向き研究から得られたものである。ほとんどの研究(ID CAN\_103、ID CAN\_101、 ID CAN\_100、ID CAN\_094、ID CAN\_143、ID CAN\_142)では、有機塩素の影響を調査したが、ほとんどが 小規模で有意ではない結果であった。2件の研究(ID CAN\_099、ID CAN\_095)では、農薬ばく露と前立 腺がんに関連した高く有意に増加したリスクが示されたが、いずれの研究も質が低く、ばく露の定義 が非常に広く、結果は慎重な解釈が必要であり、適切に実施された大規模な前向き研究(AHSなど)で 報告されたものとは一致しない。特筆すべきは、ばく露量の多い地域で行われた1件の集団ベースの症 例対照研究(ID CAN 104)では、臭化メチルへのばく露と前立腺がんリスクとの間に強い関連が認めら れたが、この研究ではばく露反応関係を示すエビデンスは観察されなかった。まとめると、農薬ばく露 に関連した前立腺がんリスクに関するほとんどのエビデンスは有機塩素の影響に関係しており、研究 では有意ではない弱い影響が示されている。農薬への職業ばく露と前立腺がんに関するメタアナリシ ス (Maele-Fabry 2003) も発表されている。22の疫学研究に基づく統合効果推定値は1.13 (95%CI 1.04 ~1.22)であり、研究間でかなりの不均一性があった。さらに、レビューされた研究には、農薬の影響 を他の職業的、環境的、生活習慣的要因と区別するためのばく露に関する質的及び量的情報が不十分 であった(Maele-Fabry 2003)。全体として、農薬ばく露と前立腺がんとの関連を支持するエビデンス はない。

### 8.3. 肺がん

30件の研究が45の分析を行っており、農薬ばく露と肺がんとの関連を調べているが、これまでに発 表されたメタアナリシスは確認されなかった。ここでも、公表された30件の研究のうち23件、45件の分 析のうち30件がAHSの分析であった。AHSの50種類の分析のうち、統計的に有意な結果が観察されたの は1件のみであった。3つの研究では、ばく露としての農薬の定義が広く(ID CAN\_080、ID CAN\_082、ID CAN\_083)、1つの研究では蚊取り線香の火傷を調査し(ID CAN\_081)、残りの研究では有機塩素系殺虫 剤に重点を置いて様々な農薬を調査していた。殺虫剤のカテゴリーが多様であること、半数以上の研 究で同じコホート集団(AHS)を繰り返し使用していることから、定量的な統合はできなかった。特筆 すべきは、蚊取り線香の火傷と肺がんとの関連は、大きな効果量(3.78(1.55、6.90);使用あり vs 使用なし)で統計的に有意であったが、この研究は相対的に小規模であり、交絡因子の調査が限られた 後ろ向きであり、全体的に質は中等度であった。2件の症例対照研究(ID CAN\_082、ID CAN\_082)では、 農薬への職業ばく露による肺がんリスクの2倍以上の増加が報告されているが、個々の農薬については 調査されていない。別の症例対照研究(ID CAN\_080)では、農薬ばく露と肺がん死亡率との間のこれら の観察を再現することはできなかった。全体として、農薬ばく露と肺がんに関するエビデンスは限ら れており、結論は出ていない。

### 8.4. 小児がん

### 8.4.1. 小児の造血器新生物

全体として、農薬ばく露に関連して小児の造血器新生物を調査した17件の研究(及び1件の要約)が 同定された。17件の研究はすべて小児白血病を対象としており、そのうち4件には他の造血器新生物も 含まれていた。

小児白血病に関するこれまでのメタアナリシスは、農薬への住居内ばく露のみを対象とした研究に 集中していた。メタアナリシスに含まれ、2006年以降に発表されたすべての研究が我々の検索で同定 され、利用可能なすべてのエビデンスが同定されたことを確認した。特定された研究は、国の登録ベー スの症例対照研究ESCALE (Etude sur les cancers de l'enfant) (ID CAN\_073) とNorthern Region Young Persons' Malignant Disease Registry (ID CAN\_120) の2つの研究を除いて、一般的に小規模な ものであった。これらの研究の結果は、サンプルサイズが大きいにもかかわらず、調査された仮説の数 が多い(偽陽性率が高い)ため、慎重に解釈されるべきである。各研究はそれぞれ42件、64件の個別の 分析結果を報告している。すべての研究は症例対照研究であり、大多数は住居内ばく露を調査してお り、職業ばく露に関する研究はほとんど見出されなかった。ほとんどの研究では、農薬または農薬のサ ブグループ(殺虫剤、除草剤、殺菌剤)の使用またはばく露を評価していたが、一部の研究では特定の 農薬に関する情報の収集を試みており(ID CAN\_031、ID CAN\_032)、1つの研究(ID CAN\_032)ではバ イオマーカーレベルを評価していた。農薬の使用頻度や使用期間に関するデータはほとんどなく、ほ とんどの研究では、対象となる農薬の「今までに使用したことがあるかないか」「使用したことがある かないか」のみを報告している。小児の造血器新生物のリスク因子がほとんど確立されていないため、 交絡因子の評価は困難であるが、ほとんどの研究では、少なくとも様々な社会人口統計学的及び母性 の特性を調査または調整している。ほとんどすべての研究では、農薬ばく露を妊娠前、妊娠期、小児期 に分けて評価している。非常に質が低く、統計分析の結果が不完全であった1件の研究では、すべての ばく露期間を調査し、他の2件(ID CAN\_073, ID CAN\_044)では、妊娠前と妊娠期を合わせて調査している。

研究 ID CAN\_040 はCI が不足していたため除外され、研究 ID CAN\_030 はNorthern California Childhood Leukemia Studyのデータが重複していたため(ID CAN\_031と重複)、研究 ID CAN\_037 は研 究集団が特殊であったため(ダウン症患者のみ)、3つの研究が定量分析から除外された。結果の定量 的統合を期間(ばく露の時間域)ごとに分けた。

#### 8.4.1.1 妊娠期のばく露

7件の研究では妊娠期の農薬ばく露に関する情報が得られた。86件中11件の分析で統計的に有意な結 果が得られたのは5件であり、すべての研究で急性白血病を対象とした結果であった。最大の効果推定 値は、国の登録ベースの症例対照研究ESCALE (Etude sur les cancers de l'enfant) から報告されて いる。本研究では、妊娠期の殺虫剤使用は小児急性白血病と有意に関連し(OR = 2.1;95%CI, 1.7-2.5)、父方の住居での殺虫剤使用も急性白血病と関連し(OR = 1.5; 95%CI, 1.2-1.8)、また、父方 の住居での殺虫剤使用も急性白血病と関連した(OR = 1.5; 95%CI, 1.2-1.8)。結果の一連の定量的 統合を行った。まず、各発表報告書の中で最大のサンプルサイズを持つ分析を選択し、結果を統合した (図 10)。この分析は、各研究でばく露評価(農薬の種類と親のばく露経路)が異なり、影響評価に ばらつきがあったため、大きな不均一性(I2>80%)と関連していた。図10の残りのメタアナリシスは、 「住居用農薬と小児白血病」に関する以前に発表されたメタアナリシス(Turner 2010)と結果を調和 させるために、調査した農薬のクラスに基づいた統合または結果を示している。我々は、この系統的レ ビューで確認された殺虫剤及び農薬に関するすべての研究を定量的に統合し、その後、住居ばく露を 評価した研究のみを残して、以前に発表されたメタアナリシスを更新した。全体的に、結果は研究間で 中等度の不均一性を示しており、これは農薬ばく露の定義、影響の定義、ばく露時間域の定義などにば らつきがあることに起因していると考えられる。しかし、メタアナリシスでは、特定されていない農薬 及び殺虫剤へのばく露に関連した小児白血病のリスクの一貫した増加を示した(要約0R=1.69; 96% CI=1.35, 2.11)。我々の更新されたメタアナリシスでは、2010年に発表されたメタアナリシスと比較 して、より保守的な結果となったが、妊娠期の農薬へのばく露と小児白血病との関連は依然として支 持されている。しかし、元のメタアナリシスでは出版バイアスの懸念があったこと、研究の規模が一般 的に小さいこと、ばく露は誤分類されやすい検証されていない自記式質問紙で測定されていることな どから、エビデンスは慎重に解釈する必要がある。ファンネルプロットは、小規模な研究を中心とした 相対的な対称性を示している。住居用殺虫剤への出生前ばく露を減らすことに公衆衛生上のメリット があるかどうかを確認するためには、過去のばく露の有効なバイオマーカーを用いた大規模研究から のさらなるエビデンスが必要である。

## 8.4.1.2. 妊娠前

4つの研究では、ばく露の時期として妊娠前を調査したが(ID CAN\_032、ID CAN\_043、ID CAN\_073、 ID CAN\_120)、いずれも統計的に有意な結果の報告ではなかった。

## 8.4.1.3. 小児期

小児期のばく露に関する情報を有する7件の研究が確認された(ID CAN\_031、ID CAN\_032、ID CAN\_035、 ID CAN\_036、ID CAN\_041、ID CAN\_043、ID CAN\_133)。1件の研究では、現在使用されていないエンド スルファンを調査したが、その研究は非常に質が低く、これ以上の調査は行われなかった。これらの研 究のメタアナリシスを以下の図14に示す。2つの分析が提示されており、A)各報告書の最大サンプルサ イズの分析に基づいて2006年以降に同定された研究(任意の農薬)についての分析と、B)小児期及び 小児白血病における農薬ばく露に関する2010年のメタアナリシスについての更新である。あらゆる農 薬に関するメタアナリシスでは中等度の不均一性があったのに対し、更新されたメタアナリシスでは、 住居ばく露と殺虫剤/特定されていない農薬のみに限定されており、結果に不均一性は見られなかった。 更新されたメタアナリシスの結果は、元のメタアナリシスよりも保守的であるが、それでも2010年に 報告された統合推定値に非常に近いものであった(図14)。ファンネルプロットでは、結果にかなりの 対称性があることが示されている。全体的には、小児期の農薬ばく露と小児白血病との間には関連を 示すいくつかのエビデンスがあるが、これは妊娠期のばく露よりも弱く、確固とした結論を出すため には、適切に実施された大規模な出生コホートからのより多くのエビデンスが必要である。

#### 8.4.2. リンパ腫

白血病以外の小児の造血器新生物に関するエビデンスは3件の研究から得られているのみであり、その中には非ホジキンリンパ腫とホジキンリンパ腫に関する分析を含む多くの分析が報告されている(ID: CAN\_073、ID: CAN\_120、ID: CAN\_133)。すべての分析は統計的に有意ではなく、効果推定値も弱いものであった。

## 8.4.3. その他の小児がん

その他の小児がんに関する研究が7件同定された。4件の研究では脳腫瘍(ID CAN\_006、ID CAN\_011、 ID CAN\_089、ID CAN\_133)、1件の小児生殖細胞腫瘍(ID CAN\_114)、2件の研究では様々な小児がん (ID CAN\_120、ID CAN\_133)が調査された。有意な関連が観察されたのは脳腫瘍のみであったが、これ らは多くの分析のごく一部のサブセットに関連しており、現段階では情報を得ることはできない。

#### 8.5. 大腸がん

全体では、26件の研究で農薬ばく露と大腸がんとの関連性が 207 件の分析で調査された。大腸がん と直腸がんについては、それぞれ24件と11件の研究で別々の分析が行われた。非常に多くのエビデン スがAHS研究から得られている。この研究では、50種類の農薬を用いた大腸がんに関する207の分析の うち194の分析で、これら 3 つの影響発現事象すべてとの関連性が調査されており、多重試験の調整 は行われていない。これら194の分析のうち、影響発現事象と統計的に有意に関連したのは7つの分析 のみであった(カルバリル、アルジカルブ、トキサフェン、ペンディメタリン、ジプロピルチオカルバ ミン酸 S-エチル (EPTC)、イマゼタピル、フォノフォス)が、偽陽性確率が高いため、解釈には注意 が必要である。27の研究が発表されているにもかかわらず、全体的には7つの異なる集団からのエビデ ンスしか得られていない。この事実は、大腸がんに関連してこれらの研究のそれぞれで分析された異 なる農薬の範囲とともに、結果の意味のある定量的な統合を可能にするものではない。表8は、複数の 論文における1つのコホートからの重複データの公表の程度を示しており、結果の一貫性が良好である ことを示している。大腸がんと農薬ばく露に関するこれまでのメタアナリシスは確認されていない。 全体的に、農薬と大腸がんに関するエビデンスは非常に限られており、現在の文献の状態では、農薬と 大腸がんの関連性を支持するものではない。

## 8.6. 皮膚がん

17件の研究では、メラノーマと農薬ばく露との関連が調査された。大半の研究は有機塩素系農薬を 対象としたものであった。ここでも、メラノーマに関する17件の研究のうち14件がAHSの結果であり、 各論文では異なる農薬のカテゴリーやばく露の定義が異なっており、いくつかの結果が提示されてい た。AHSの26の異なる分析のうち、8つは統計的に有意であり、すべて同じ出版物(ID CAN\_085)に由来 するもので、50種類の農薬と皮膚メラノーマとの量反応関係が報告されている。この研究では、皮膚メ ラノーマとmaneb/mancozeb(63日以上のばく露日数:OR = 2.4;95%CI、1.2-4.9;トレンドp = 0.006)、 parathion(56日以上のばく露日数:OR = 2.4;95%CI、1.3-4.4;トレンドp = 0.003)及びcarbary1 (56日以上のばく露日数:OR = 1.7;95%CI、1.1-2.5;トレンドp = 0.013)との間の有意な関連が報 告されている(155)。他の研究では、これらの農薬に関して再現性を調査できるような結果を報告し ていない。1件の症例対照研究では、屋内での農薬ばく露とメラノーマとの間に統計的に有意なリスク の増加が示されたが、同じ研究では屋外での農薬ばく露はメラノーマとは関連していなかった(106)。 有機塩素系農薬に関する残りの研究では、統計的に有意な結果がほとんど得られない不均質な結果が 得られており(ヘキサクロロベンゼン(HCB)、マイレックス)、これらの農薬とメラノーマとの関連 を示すエビデンスは得られてない。

## 8.7. 乳がん

全体では、2006年以降の14件の研究(うち3件は要約)が農薬ばく露と乳がんとの関係を調査した。 大半の研究と分析は有機塩素系農薬に焦点を当てており、バイオマーカー分析によって評価されてい る。乳がんとDDTばく露に関する2つの過去のメタアナリシスが発表されている(Khanjani 2007, López-Cervantes 2004)。全体的に、以前のメタアナリシスでは、ヘプタクロルを除き、シクロジエン系化学 物質と乳がんとの間に有意な関連は示されなかったが、それはわずか2件の研究に基づくものであった。 ジクロロジフェニルジクロロエチレン(DDE)と乳がんに関するシステマティックレビューで同定され た研究(5研究)のメタアナリシスでも、関連を示すエビデンスは示されなかった。我々はまた、乳が んに関する同定されたすべての研究でメタアナリシスを実施し、サンプルサイズが最も大きい各研究 内の分析を毎回選択した。研究ID CAN\_019とID CAN\_023は、統合を可能にする効果量と信頼区間が提 供されていなかったため、統合から除外された。また、研究ID CAN\_022は、誤って報告されたとは想定 されていない非常に狭い信頼区間を報告していたため除外された。ここでの統合には、多くの異なる 農薬の定義とバイオマーカー(DDE、リンデン、広義の農薬定義)の統合効果が含まれており、解釈が 難しい。統合効果は統計的に有意な乳がんリスクの増加(1.07(0.87~1.31))を示したが、この結果 は慎重な解釈が必要である。メタアナリシスでは、非常に異なるカテゴリーの農薬を組み合わせてお り、主に1つの研究(ID CAN\_022)が占めているが、これは住居用農薬の自己申告による農薬ばく露を 評価したもので、バイオマーカーを介して農薬を評価した他の研究に比べて質が低いものである。

## 8.8. 膀胱がん

農薬ばく露に関連して膀胱がんを調査した16件の研究が同定されたが、13件は他の発がん影響について以前に観察されたのと同様にAHSの同じ集団からの研究であった。25の異なる分析結果のうち、AHSにおけるイマゼタピルへの職業ばく露について統計的に有意な結果を示したのは1件のみであった。しかし、複数の試験を行っているため、結果は慎重に解釈する必要があり、報告書で調査されたエビデンスに基づいても、農薬ばく露と膀胱がんとの関連は示唆されていない。

### 8.9. 腎臓がん

10件の研究が農薬ばく露に関連して腎臓がんを調査したが、データはAHSとBC(ブリティッシュコロ ンビア州)製材所労働者コホート研究の2つの集団からのみであった。BC州製材所労働者コホート研究 (ID CAN\_129及びID CAN\_125)の結果は、いずれもペンタクロロフェノール及びテトラクロロフェノ ールへの職業ばく露に関するものであったが、統計分析のアプローチが異なっていた。AHSの結果は、 異なる農薬クラスに関するものであった。全体的に、統計的に有意な結果は観察されず、寄与した集団 の数が限られているため(n=2)、これ以上の定量的な統合はできなかった。

#### 8.10. 膵臓がん

農薬ばく露に関連して膵臓がんを調査した研究は7件、AHSからの報告は4件であった。分析の圧倒的 多数は有機塩素系農薬を対象としたものであった。質の低い小規模な症例対照研究では、ヘキサクロ ロベンゼン(HCB)、クロルデンの和及びポリ臭化ジフェニルエーテル(PBDE)の濃度が健常対照者と 比較して膵臓がん症例で有意に上昇していた(ID CAN\_090)。AHSでは、46種類の分析のうち、 PendimethalinとS-Ethyl dipropylthiocarbamate (EPTC)について有意な関連が報告されている。ペン ディメタリンの生涯使用量の上位半分の散布者は、非使用者と比較して3.0倍(95%CI 1.3-7.2、pトレ ンド5 0.01)のリスクを有し、EPTCの生涯使用量の上位半分の散布者は、非使用者と比較して2.56倍 (95%CI 5 1.1-5.4、pトレンド=0.01)のリスクを有していた。有機塩素系薬剤は、AHSにおける膵臓 がんの過剰リスクとは関連していなかった。これらの知見は、除草剤が膵臓がんと関連している可能 性を示唆しているが、これらはすべて単一の集団から得られたものであり、多重検定調整を行ってい ないため、今後の研究で反復する必要がある。

#### 8.11. 精巣がん

全体では8件の研究で精巣がんが調査された。2件の研究では精上皮腫への影響も報告された。1件を 除くすべての研究がバイオマーカーレベルを評価し、有機塩素系殺虫剤に集中しており、さまざまな バイオマーカーが評価され、精巣がんとの関連には弱い影響を示した研究もあった。しかし、p-p'DDE のみについては4件以上の研究の情報が得られ、定量的統合では有意ではない効果と適度な不均一性が 示された(図20)。各研究で評価されたバイオマーカーの不均一性のため、どの農薬についても定量的 統合は行われなかった。全体的に、ここで調査されたエビデンスに基づいて、農薬ばく露と精巣がんと の関連を支持するエビデンスはない。

#### 8.12. 胃がん

6件の研究で、農薬ばく露と胃がんとの関連が調査された。すべての研究は農薬への職業ばく露を調 査したもので、農薬のクラスは多岐にわたっていた;2件の研究は前向きデザインであったが、すべて の研究でサンプルサイズは中等度から低度であった。農家に関する以前のメタアナリシス(Saphir 1998)と一致するように、研究は不十分で、主に有意でない結果を報告していた。United Farm Workers of America (UFW) コホートにおける胃がんのコホート内症例対照研究(ID CAN\_028)では、有意な関 連が報告された。フェノキシ酢酸除草剤2,4-ジクロロフェノキシ酢酸(2,4-D)の使用量が多い地域で 働くことは、胃がんと関連していた(OR 1.85;95%CI 1.05-3.25)。有機塩素系殺虫剤クロルデンの 使用もこの疾患と関連していた(OR 2.96;95%CI 1.48-5.94)。胃がんは、殺ダニ剤プロパルギトの 使用と関連していた(OR 2.86;95%CI 1.56-5.23)。にもかかわらず、評価が生態学的なものであっ たため、この研究は症例数と対照数が比較的少ないこと、多重検定及びばく露の誤分類によって制限 されている。AHSでは、15例のばく露例に基づいて、胃がんリスクは臭化メチルの使用量の増加ととも に単調に増加した(RR = 3.13;95%CI、非使用と比較して高頻度使用では1.25-7.80;トレンドのp = 0.02)。しかし、この研究では他のすべてのがんのサブタイプが臭化メチル使用と関連していたため、 やはり多重検定の問題がある(ID CAN\_147)。最大のサンプルサイズで分析を選択したメタアナリシス は図21に示されているが、結果は慎重に調査する必要がある。統計的に有意な統合された大きな効果 量にもかかわらず、この結果は、欧州連合で承認されていない2つの化合物であるペンタクロロフェノ ールと臭化メチルを調査している2つの研究(ID CAN\_125, ID CAN\_147)の影響が大きい。

## 8.13. 肝臓がん

5件の研究(11件の個別の分析を含む)と1件の学会発表の要約で、農薬ばく露と肝臓がんとの関連が 調査された。分析の大部分は有機塩素系農薬へのばく露を対象としており、すべての研究は農薬への 職業ばく露を対象としていた。DDTに関する両研究(ID: CAN\_076及びID: CAN\_079)では、肝がんとの 統計的に有意な関連が報告されたが、残りの分析では統計的に有意ではなかった。これら2つの研究が 肝臓がんに関するメタアナリシスの大部分を占めており、統合された結果は統計学的に有意であり、 主にDDT研究に牽引されている。

## 8.14. 研究数が少ないがんサブグループ

表6に示されているように、多数のがんについては、各がんサブグループのエビデンスを総合的に判 断できる研究は非常に限られている。我々のシステマティックレビューでは、過去に発表されたエビ デンス(2006年以前)との比較を可能にするために、これらのがんサブタイプに関する過去に発表され たメタアナリシスを確認しなかった。一般的に、これらのがんサブタイプに関する結果では影響は小 さく、職業ばく露のみに関する少数の例外を除いて統計的に有意ではなかった。各研究内の分析数が 多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農薬ば く露とこれらのがんサブタイプとの間の関連を示唆するエビデンスはない。

また、農薬に関連してすべてのがん(複合発がん影響)を調査した研究も多数あった。がんは非常に 異質な疾患群であり、すべてのがんサブタイプを同時に調査すると、関連にバイアスがかかる可能性 がある。全体では、「すべてのがん」を対象とした30の分析が同定されたが、そのうち28の分析は同じ コホート(AHS)を対象としたものであり、結果をさらに統合することはできなかった。31件のうち統 計的に有意な結果が得られたのは4件のみで、質の低い研究と関連していたため、現段階では解釈に値 しない。

# 表 6: がんサブグループごとに同定された調査対象研究の要約

がん	研究数	メタアナリシス推奨	以前のメタアナリシス
造血器新生物	88	Yes	Yes
前立腺がん	39	No	Yes
肺がん	30	Yes	No
すべてのがん	30	No	No
小児がん	45	Yes	Yes
大腸がん	26	No	No
皮膚がん	17	Yes	No
膀胱がん	16	Yes	No
乳がん	14	Yes	Yes
腎臓がん	10	No	No
膵臓がん	7	No	No
精巣がん	8	No	No
口唇・口腔・咽頭のがん	5	No	No
胃がん	6	No	No
肝臓がん	5	No	No
脳腫瘍	6	No	No
骨がん	5	No	No
食道がん	5	No	No
喉頭がん	3	No	No
胆道がん	2	No	No
軟組織	2	No	No
女性生殖器系がん	2	No	No
その他	9	No	No

## 表7:同一農薬クラスと白血病リスク(DDE:ジクロロジフェニルジクロロエチレ ン)についての情報を報告した調査対象試験全体の要約結果

Study ID	<b>揭載</b> 日	殺虫剤クラス	殺虫剤の種類	影響	N	OR	95% LCI	95% UCI	Level of Adjustment
DDE									
CAN_064	2010	p,p'-DDE	バイオマーカー	慢性リンパ性白血病	210	0.78	0.28	2.21	+++
CAN_063	2010	p,p'-DDE	質問紙	慢性リンパ性白血病	148	0.62	0.29	1.3	++
CAN_056	2008	p,p'-DDE	バイオマーカー	慢性リンパ性白血病	71	1	0.4	2.5	+
Insectic	ides								
CAN_072	2006	Insecticides	質問紙	すべての白血病	1304	1	0.7	1.4	+
CAN_049	2009	Insecticides	質問紙	慢性リンパ性白血病	37	0.8	0.3	2.1	+
CAN_024	2010	Insecticides	質問紙	急性骨髓性白血病	158	1.52	0.16	2.04	+++
Herbicid	es								
CAN_072	2006	Herbicides	質問紙	すべての白血病	1260	1.4	0.8	2.3	++
CAN_049	2009	Herbicides	質問紙	慢性リンパ性白血病	39	0.5	0.2	1.3	+
CAN_024	2010	Herbicides	質問紙	急性骨髓性白血病	45	1.83	0.99	3.38	+++
CAN_058	2008	Herbicides	質問紙	慢性リンパ性白血病	523	1.15	0.76	1.74	++

表8:大腸がん(DDVP:2,2-ジクロロビニルジメチルリン酸塩)に関連して農薬ば く露の同じバイオマーカーを評価した農業健康調査 (Agricultural Health Study: AHS) から同定された研究の例

					効果推定		上位	
Study ID	殺虫剤	影響	比較	<u>サイズ</u>		95% CI	95% CI	調整
			Highest tertile					
	ジクロロボス		of exposure vs					
CAN_122	/DDVP	大腸がん	no	202	1.48	0.78	2.8	+
	ジクロロボス							
CAN_024	/DDVP	大腸がん	Ever vs. never	56813	1.5	0.9	2.4	++
CAN_024	フォノフォス	大腸がん	Ever vs. never	56813	1.5	1	2.2	++
			Highest tertile					
			of exposure vs					
CAN_119	フォノフォス	大腸がん	no	126	1.66	0.92	3.03	++
CAN_024	マラチオン	直腸がん	Ever vs. never	56813	0.8	0.6	1.1	++
			Highest tertile					
			of exposure vs					
CAN_121	マラチオン	直腸がん	no	58	0.84	0.48	1.48	++
CAN_118	トキサフェン	直腸がん	Yes vs. no	75	2	1.1	3.5	+++
CAN_024	トキサフェン	直腸がん	Ever vs. never	56813	2.1	1.2	3.6	++

## 表9:農薬ばく露のバイオマーカーと精巣がんについて、1 つのバイオマーカーに つき 2 件以上の研究を行った研究 (DDE:ジクロロジフェニルジクロロエチレ ン、HCB:ヘキサクロロベンゼン)

Study ID	殺虫剤	効果推定型	比較レベル	合計数	効果の 推定	95% LCI	95% UCI	調整
CAN_111	ディルドリン	OR	high tertile vs low	418	0.79	0.44	1.41	++
CAN_115	ディルドリン	OR	high tertile vs low	60	2.1	0.5	9.5	+
CAN_113	HCB	OR	yes/no	57	4.4	1.7	12	+
CAN_115	HCB	OR	high tertile vs low	70	2.9	0.5	15.2	+
CAN_111	ヘプタクロルエ ポキシド	OR	high tertile vs low	407	0.67	0.35	1.29	++
CAN_115	ヘプタクロルエ ポキシド	OR	high tertile vs low	68	2.4	0.6	9.1	
CAN_111	ミレックス	OR	high tertile vs low	557	0.87	0.5	1.53	
CAN_112	ミレックス	RR	high tertile vs low	1333	0.24	0.9	1.74	
CAN_115	ミレックス	OR	high tertile vs low	66	1.2	0.4	3	
CAN_111	o,p-DDT	OR	high tertile vs low	514	1.3	0.67	2.53	++
CAN_115	o,p'-DDT	OR	high tertile vs low	71	1.4	0.4	4.5	+
CAN_116	o,p'-DDT	Mean difference	unit increase	60	0.46	n/a	n/a	n/a
CAN_111	p, p'-DDT	OR	high tertile vs low	533	1.17	0.68	2	++
CAN_112	p,p'-DDT	RR	high tertile vs low	1493	1.13	0.71	1.82	
CAN_115	p,p'-DDT	OR	high tertile vs low	63	2.1	0.6	7.2	+
CAN_116	p,p'-DDT	Mean difference	unit increase	60	-1.2	n/a	n/a	n/a
CAN_111	p,p'-DDE	OR	high tertile vs low	554	0.61	0.32	1.14	++
CAN_112	p,p'-DDE	RR	high tertile vs low	884	1.71	1.23	2.38	+++
CAN_113	p,p'-DDE	OR	yes/no	44	1.3	0.5	3	+
CAN_115	p,p'-DDE	OR	high tertile vs low	65	2.2	0.7	6.5	+
CAN_116	p,p'-DDE	Mean difference	unit increase	60	-15.29	n/a	n/a	
CAN_117	p,p'-DDE	OR	high tertile vs low	98	3.21	0.77	13.3	+
CAN_111	オキシクロルデ ン	OR	high tertile vs low	538	0.93	0.5	1.73	
CAN_112	オキシクロルデ ン	RR	high tertile vs low	841	1.27	0.92	1.76	
CAN_115	オキシクロルデ ン	OR	high tertile vs low	68	3.2	0.6	16.8	+
CAN_111	全クロルデン	OR	high tertile vs low	562	0.93	0.51	1.68	
CAN_112	全クロルデン	RR	high tertile vs low	842	1.51	1.09	2.1	
CAN_113			yes/no	49	1.9	0.7	5	
CAN_115	全クロルデン	OR	high tertile vs low	70	2.3	0.6	7.2	+
CAN_111	トランス-ノナ クロル	OR	high tertile vs low	564	0.89	0.49	1.61	
CAN_112	トランス-ノナ クロル	RR	high tertile vs low	875	1.46	1.07		+++
CAN_115	トランス-ノナ クロル	OR	high tertile vs low	62	2.6	0.7	8.9	+

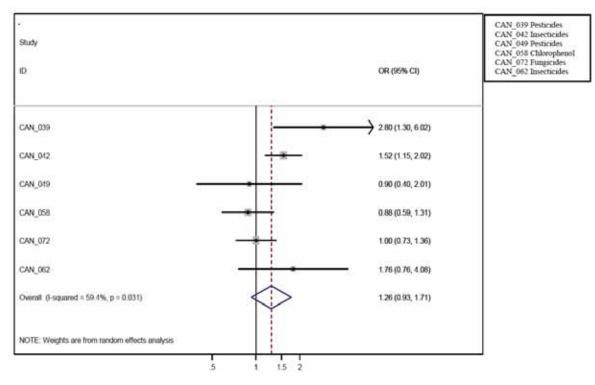


図8: 農薬ばく露と白血病との関連のランダム効果メタアナリシス

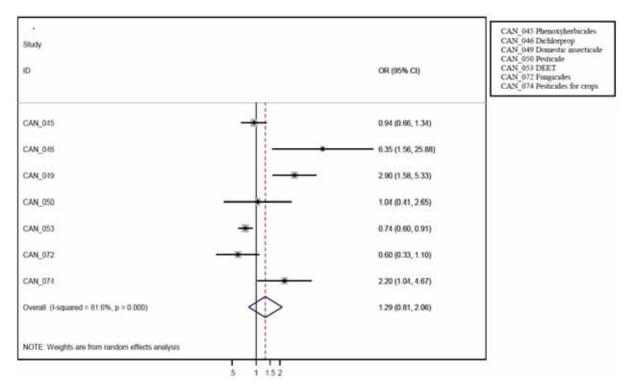


図9:農薬ばく露とホジキンリンパ腫との関連のランダム効果メタアナリシス

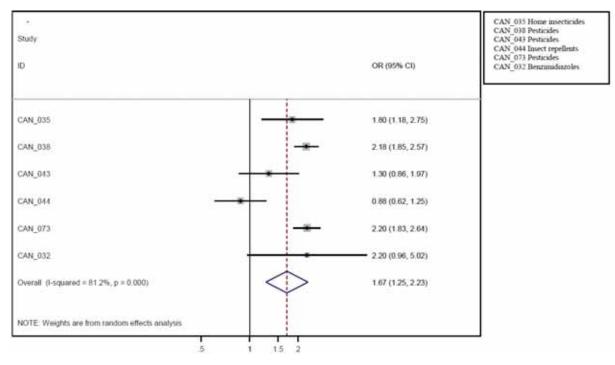


図10:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期のあらゆる農薬ばく露と小児白血病)

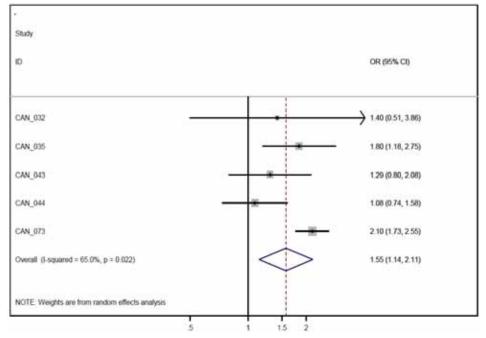
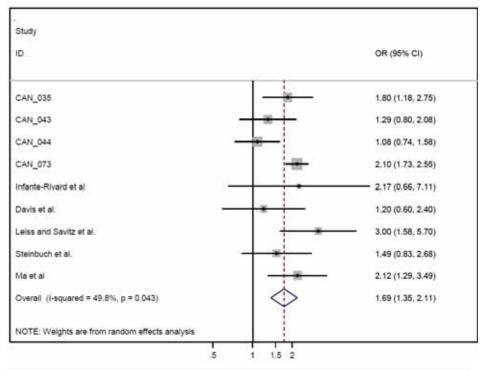


図11:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期の殺虫剤ばく露と小児白血病)



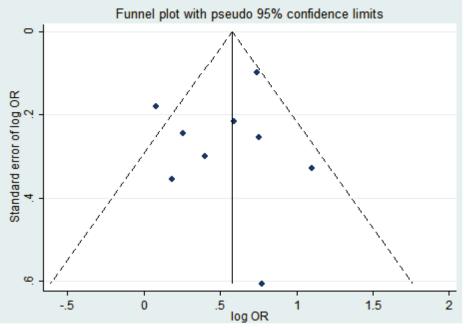


図12:妊娠期の農薬ばく露と小児白血病との関連のランダム効果メタアナリシス (妊娠期の殺虫剤への住居内ばく露と小児白血病(公表されている効果量を用 いたメタアナリシス2010の更新;Turner 2010)、ならびに関連するファンネル プロット)

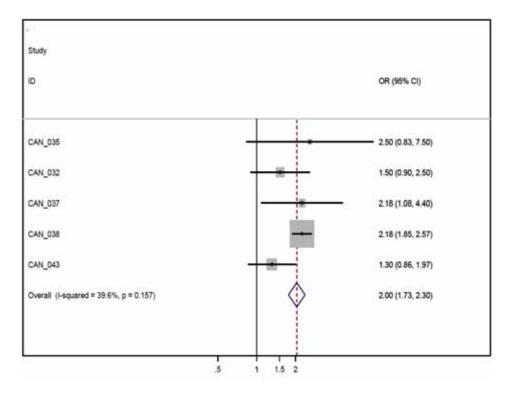
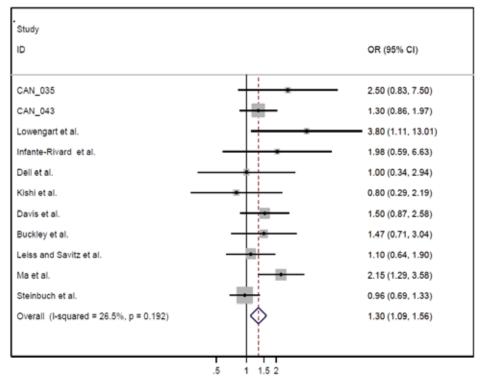


図13:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期の不特定の農薬ばく露と小児白血病)



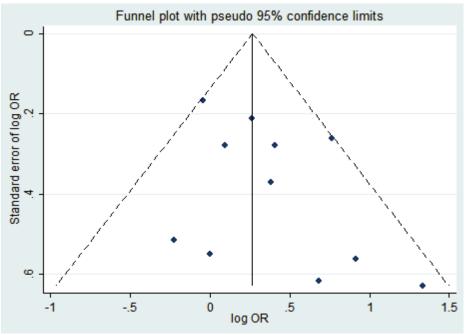


図14:妊娠期の農薬ばく露と小児白血病との関連のランダム効果メタアナリシス (妊娠期の不特定の農薬への住居内ばく露と小児白血病(メタアナリシス2010、 Turner 2010への更新)、ならびに関連するファンネルプロット)

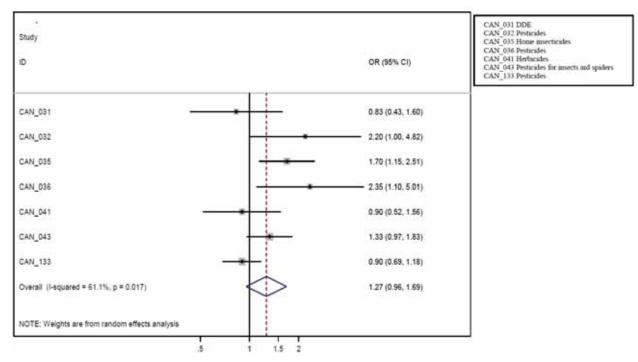
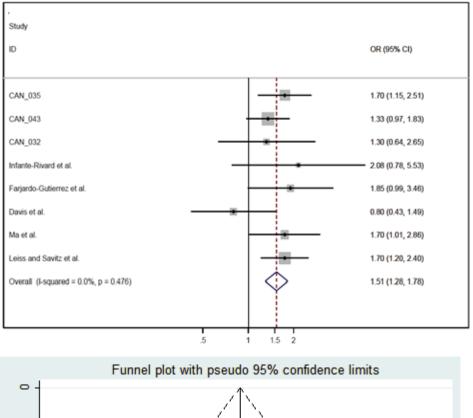


図15:小児白血病と小児期の農薬ばく露との関連のランダム効果メタアナリシス (小児期のあらゆる農薬ばく露と小児白血病)



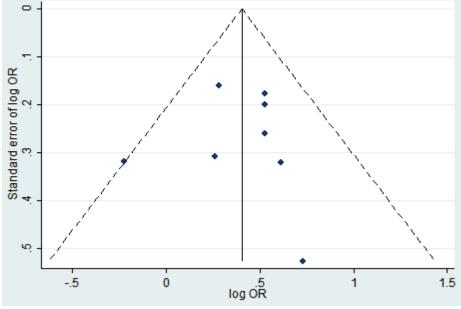
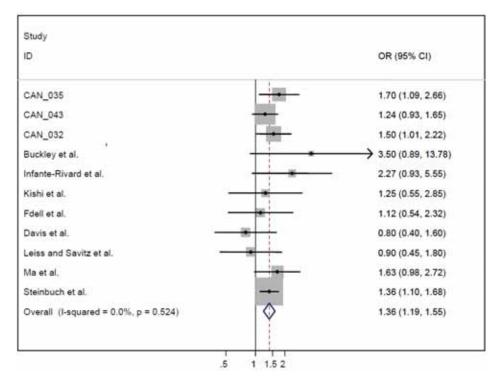


図16:小児白血病と小児期の農薬ばく露との関連のランダム効果メタアナリシス (小児期の殺虫剤への住居内ばく露と小児白血病(公表されている効果量を用 いたメタアナリシス2010の更新、Turner 2010)、ならびに関連するファンネル プロット)



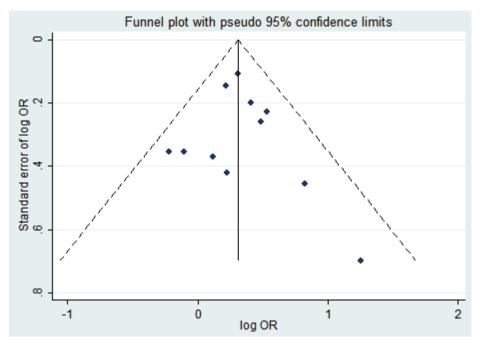


図17:小児白血病と小児期の農薬ばく露との関連のランダム効果メタアナリシス (小児期の不特定の農薬への住居内ばく露と小児白血病(公表されている効果 量を用いたメタアナリシス2010の更新、Turner 2010)、ならびに関連するファ ンネルプロット)

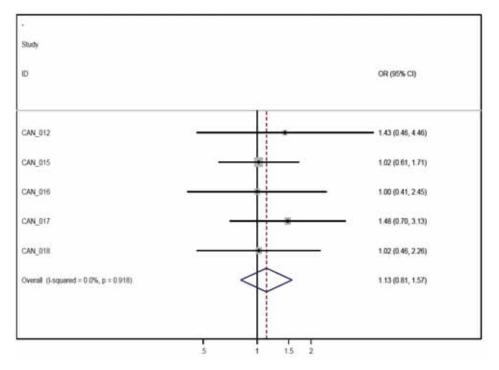


図18: 農薬のジクロロジフェニルジクロロエチレン(DDE) ばく露と乳がんを調査した研究でのDDEの情報と乳がんのランダム効果メタアナリシス

Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Pesticide epidemiology

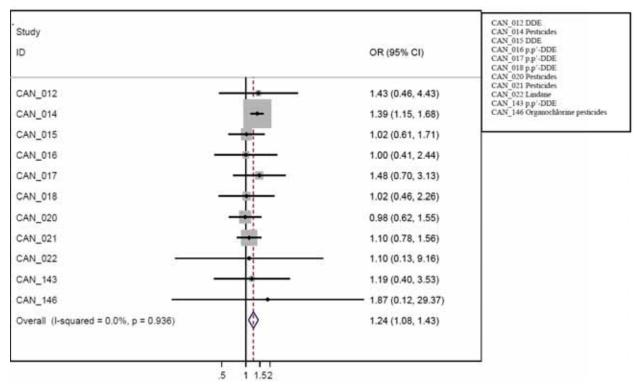


図19:各研究内で最大のサンプルサイズでの分析を選択したジクロロジフェニルジ クロロエチレン(DDE)の情報と乳がんのランダム効果メタアナリシス(各研究で 評価された農薬は右に示されている)

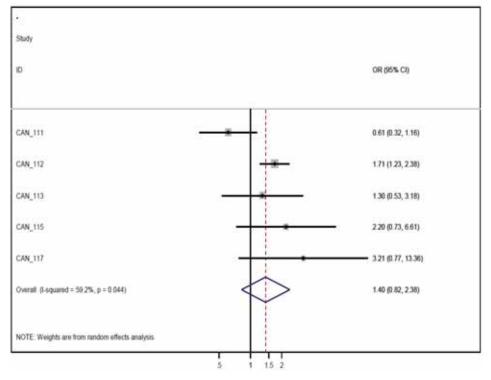


図20:ジクロロジフェニルジクロロエチレン(DDE)に関する情報と精巣がんのラン ダム効果メタアナリシス

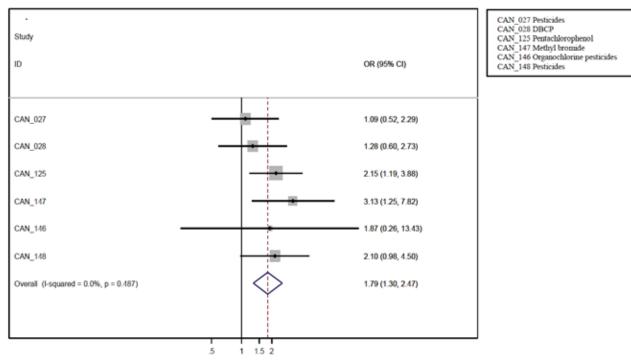


図21:あらゆる農薬ばく露と胃がんを調査した研究のうち、各研究内で最大のサンプ ルサイズでの分析を用いた研究のランダム効果メタアナリシス

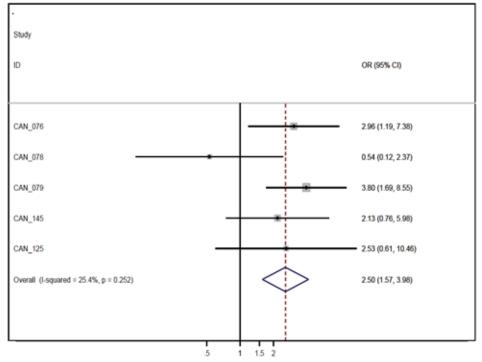


図22:あらゆる農薬ばく露と肝臓がんを調査した研究のうち、各研究内で最大のサ ンプルサイズの分析を用いた研究のランダム効果メタアナリシス(各研究で評 価された農薬は右に示されている)

#### **9.** 小児の健康

全体では 84 件の個別研究が農薬ばく露の小児への健康影響を調査し(サンプルサイズ中央値:267、 IQR 119-811)、データ抽出データベースでは 821 件の分析が行われた。120 以上の健康関連影響が 評価されており、その大部分は先天性奇形と身体測定を含むがそれに限定されない発生パラメータに 焦点を当てている(表 10)。他の影響に見られるように、ばく露の定義の多様性には目を見張るもの があり、データ統合に特別な問題を与えている。84 件のうち 38 件のみが前向きコホート研究であり、 他の 5 件はコホート内症例対照研究であった。エビデンスの大部分は後ろ向き症例対照分析に由来し ており、これはばく露量測定においてリコールバイアスがかかりやすい。報告された分析におけるサ ンプルサイズはしばしば小さく、23~183,313人(中央値267人)であり、この領域の最大の研究は、が ん分野で評価された最大の研究よりも小さい。ここでは、農業健康調査(Agricultural Health Study: AHS)のような、この分野の大規模でよく知られた研究に由来する多数の出版物まとまっては観察され なかったが、26件の研究が職業ばく露を評価していた。さらに、ばく露のバイオマーカーに関する情報 を有する研究の存在は、ここではより多かった(n=49,58%)が、3つの研究はJEMによる職業ばく露を 評価した。調査した異なるカテゴリーに属する影響を、各カテゴリーに寄与した研究数が少なかったため、デ ータの統計的統合(メタアナリシス)は泌尿器の奇形のみを対象に実施した。

## 9.1. 未熟児

妊娠期の農薬ばく露と未熟児との関連を評価した研究は 15 件あり、サンプルサイズの中央値は 193 件(IQR 87-469)で、データベースには 54 件の比較が抽出されている。半数以上の研究が後ろ 向きで、4分の3以上の研究ではバイオマーカーを用いてばく露が評価されていた。個々の農薬の評価 は多種多様で、DDT代謝物の評価がより頻繁に行われていた(8件の研究)。それにもかかわらず、同じ 比較単位を使用した 4 件以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われず、 定量的な統合は行われなかった。最大の前向き研究(ID CH 091)では、オランダの温室労働者を対象 とし、温室労働者の男性における早産リスクの減少(OR= 0.47; 95%CI= 0.35-0.65)が報告されたが、 女性におけるリスクの増加は統計的に有意ではなかった(OR= 1.14, 95%CI= 0.57-2.31)。残りの研 究では、正の関連を示す推定効果が得られたが、統計的に有意な結果はなかった。さらに、発表された 研究のメタアナリシスは確認されなかった。これらのデータに基づいて、農薬ばく露と未熟児との間 に臨床的に有意な関連を示唆する最近のエビデンスはない。

## 9.2. 胎児の発育制限

12の研究では、妊娠期の農薬ばく露と胎児の発育制限及び/または在胎不当過小児との関連を評価し ており、サンプルサイズの中央値は422(IQR 178-1,630)で、データベースには44件の個別の比較が抽 出されていた。研究の60%は前向きで、3つの研究では職業ばく露を評価し、3分の2以上の研究ではバ イオマーカーを用いてばく露を評価している。個々の農薬の評価は多種多様で、DDT代謝物の評価がよ り頻繁に行われていた(4件の研究)。それにもかかわらず、同じ比較単位を使用した 4 件以上の研 究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は行われなかった。最 大の研究(ID RPD 26)では、後ろ向きコホートを対象に、飲料水中のアトラジンが在胎不当過小(SGA) 及び早産の罹患率の増加と関連しているかどうかを評価した。著者らの報告によると、妊娠第3 期及 び全妊娠期間の飲料水中のアトラジンは、SGA(Small for Gestational Age)の罹患率の有意な増加 と関連しており、妊娠第3期に飲料水中のアトラジンが0.1 µg/Lを超えると、対照群(0.1 µg/L 未満)と比較して SGA の罹患率が17~19%増加した。残りのすべての研究では、効果量の方向性に 関する一貫したパターンがなく、統計的に有意ではない結果が報告された。さらに、発表された研究の メタアナリシスは確認されなかった。これらのデータに基づいて、農薬ばく露と未熟児との間に臨床 的に有意な関連を示唆する最近のエビデンスはない。

## 9.3. 身体測定(体格計測)

農薬ばく露と発育の関連を調査した研究は数多くある。

## 9.3.1. 出生時の体長/身長

出生時の体長と身長はそれぞれ13件と8件の研究で評価され、データベースでは78件の比較が行われた。大半の研究では、バイオマーカーを用いてばく露が評価されている。個々の農薬の評価は多種多様で、DDT代謝物の評価がより頻繁に行われていたが、4件以上の研究では単一の農薬と関連するバイオマーカーの評価は行われておらず、定量的な統合は行われていない。

1980 年以前に生まれた北米の集団を対象とした最大の前向き研究(ID CH 073)では、出生前の p,p'-DDE の最高濃度(>60 mg/1)のみが、最低濃度(<15 mg/1)と比較して、7 歳時の身長低下と統 計的に有意に関連していることが報告された[調整係数(SE) -2.21 cm (0.67)]。残りの研究では、 効果の方向性または効果量に一貫したパターンがなく、相反する結果が報告された。さらに、メタアナ リシスは確認されなかった。多数の分析を考慮すると、これらの結果は慎重に解釈する必要があり、こ れらのデータに基づいて、農薬ばく露と出生時の体長または身長の間に、妥当で臨床的に有意な関連 を示唆する最近のエビデンスはない。

#### 9.3.2. 体重

妊娠期の農薬ばく露と出生時体重との関連を評価した研究は 26 件あり、データベースには 134 件 の個別抽出比較が掲載されている。他の 5 件の研究では、農薬とポンデラル指数との関連を評価した。 多くの比較では、バイオマーカーを用いてばく露が評価された。個々の農薬の評価は多種多様で、DDT の代謝物の評価がより頻繁に行われた(11 研究)。しかし、同じ比較単位を使用した 4 件以上の研 究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は行われなかった。最 大の前向き研究(ID CH 014)は農業健康研究(Agricultural Health Study: AHS)の論文で、妊娠3ヶ 月までの農薬関連作業は出生時体重とは関連しておらず、複数回の分析の結果、カルバリル農薬の使 用があった場合は出生時体重の減少と関連していた(-82 g、95% CI = -132, -31)と報告された。残 りの研究では、効果の方向性や効果量に一貫したパターンがなく、相反する結果が報告された。さら に、発表された研究のメタアナリシスは確認されなかった。しかし、我々はp,p<sup>-</sup>-DDEの1µg/Lの増加は 出生時体重の7gの減少(95% CI = -18, 4g)と関連していた(Govarts E 2012)ことを報告したヨーロ ッパのコホートの個々の参加者のデータのメタアナリシスを同定した。分析の数が多いことを考える と、これらの結果は慎重な解釈が必要であり、これらのデータに基づくと、農薬ばく露と出生体重との 間の妥当で臨床的に有意な関連を示唆する最近のエビデンスはない。 26件の研究が、出生後の様々な時点での農薬ばく露と体重との関連を評価しており、データベース には68件の個別の比較が抽出された。評価された比較のほぼ85%では、バイオマーカーを介してばく 露が評価された。個々の農薬の評価は多岐にわたり、DDT代謝物の評価がより頻繁に行われた(10研究)。 しかし、同じ定義の影響、同じタイムポイントでの影響評価、同じ農薬、同じ比較単位を使用した4件 以上の研究において評価された単一の農薬とそれに関連するバイオマーカーはなく、定量的な統合は 行われなかった。2002年と2003年に生まれたメキシコの男子集団におけるDDTばく露を評価した 最大の研究(ID CH 074)では、全体的に、出生前のDDEレベルと任意の年齢におけるBMI (Body Mass Index)との間の関連は観察されず、予測値は、最も高いばく露量(DDE:49.00 mg/g)の小児と最も低 いばく露量(DDE:<3.01 mg/g)の小児とでは発育が似通っており、BMI は参照グループと同程度であ ったことを示した。残りの研究では、効果の方向性や効果量に一貫したパターンがなく、相反する結果 が報告された。さらに、メタアナリシスは確認されなかった。分析数が多いことから、これらの結果は 慎重な解釈が必要であり、これらのデータに基づいて、農薬ばく露と体重との間に臨床的に有意な関 連を示唆する最近のエビデンスはない。

#### 9.3.3. 頭囲

妊娠期の農薬ばく露と出生時及び出生後の頭囲との関連を評価した研究はそれぞれ 14 件と 3 件 あり、データベースには 85 件の個別比較が抽出されている。3 分の 2 以上の比較では、バイオマー カーを用いてばく露が評価されていた。出生時の頭囲については、多種多様な農薬が評価されており、 中でも DDT 代謝物がより頻繁に評価されていた(7 研究)。それにもかかわらず、同じ比較単位を用 いた 4 件以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は 行われていない。最大の前向き研究(ID CH 026)は、オランダの集団ベースの前向きコホート研究 (2002-2006)に参加した 4,680 人の妊婦を対象に、様々な化学物質への母親の職業ばく露と胎児の 発育との関連を調査したジェネレーション R 研究の出版物である。胎児頭囲については、アルキルフ ェノール化合物への母親の職業ばく露のみが、潜在的な交絡因子を調整した上でばく露していない母 親と比較して統計的に有意に低い成長率(妊娠週あたり-0.01752 SD)を示した。残りの研究では、効 果の方向性や効果量に一貫したパターンがなく、相反する結果が報告されている。さらに、発表された 研究のメタアナリシスは確認されなかった。分析数が多いことを考えると、報告された研究結果は慎 重に解釈する必要があり、これらのデータに基づいて、農薬ばく露と頭囲との間に臨床的に有意な関 連を示唆する最近のエビデンスはない。

## 9.3.4. 先天性奇形

5件の研究では、農薬ばく露と先天性奇形全般との関連が調査された。最大の研究(ID CH 002)はカ ナダの農場の集団を評価し、146の潜在的な関連を報告したが、主要分析では統計的に有意な結果は得 られず、シアナジン(OR = 4.99、95%CI:1.63-15.27)及びジカンバ(OR = 2.42、95%CI:1.06-5.53) への妊娠前のばく露が、男児の先天性奇形のリスクの増加と関連していることが提案された。それに もかかわらず、利用可能な比較研究の数及び本研究におけるばく露と影響が自己報告であることを考 慮すると、今回の知見は慎重に調査されるべきである。残りの4件の後ろ向き研究では、相反する結果 が報告された(ID CH 043、職業ばく露(父親)、OR: 3.42、95% CI: 1.97-5.92; ID CH 035、少な くとも片方の親の職業ばく露、OR = 1.3、95%CI = 0.4-3.9; ID CH 008、母体の尿中メトラクロルの HR、95% 0.4-1.4)。)多数の分析を考慮すると、これらの結果は慎重な解釈が必要であり、これらの データに基づいて、農薬ばく露と先天性奇形との関連を示唆する最近のエビデンスはない。

### 9.3.5. 神経管閉鎖障害

同定された研究では、農薬ばく露と無脳症や二分脊椎を含む神経管閉鎖障害との関連を調査してお り(N=4研究)、種々の農薬と神経管閉鎖障害、無脳症、二分脊椎との間の分析が非常に多く報告され ており、多重検定の調整は行われていない(1論文あたり平均27分析)。抽出された134の分析のうち、 43の分析は結果と統計的に有意に関連していたが(うち14の分析は境界線上で有意であった)、偽陽性 の確率が高いので注意して解釈する必要がある。5つの研究のそれぞれで分析された農薬の種類の多さ と、農薬ばく露の定義の違いにより、ばく露期間や対象とした親に関しても研究間でかなりの不均一 性があるため、「何らかの農薬」ばく露の定義を用いても、結果の意味のある定量的な統合を行うこと はできない。神経管閉鎖障害と農薬ばく露に関するこれまでのメタアナリシスは確認されていない。 全体的に、農薬と神経管閉鎖障害に関するエビデンスは限られており、最新の文献の現状では、妥当な 関連を支持するものではない。注目すべきは、この分野で最大の研究(ID CH 044)では、妊娠初期に 特定の農薬または農薬の物理化学的グループの使用に母親が居住している場所に近いことが、これら の奇形のリスクを増加させるかどうかが調査され、731例と940例の対照が含まれ、個々の農薬、農薬の 物理化学的カテゴリー、任意のばく露、ばく露なし、多重ばく露の定義について107の異なる分析を報 告した後、多重試験の補正なしで、農薬のカテゴリーまたは相加効果に関して特定のパターンを持た ない15の統計的に有意な結果が得られたことである。

#### 9.3.6. 泌尿生殖器の奇形

全体では、19件の研究で泌尿生殖器の奇形、すなわち停留精巣(n=9)と尿道下裂(n=9)が調査された。

停留精巣は9件の研究で評価されたが、そのうちの9件はほとんどが後ろ向き研究であり、サンプルサ イズの中央値は199件(IQR 136-710)であった。4件の研究ではDDTレベルが評価され、ヘキサクロロベ ンゼン(HCB)とクロルデンはそれぞれ1件ずつ評価され、一般的な農薬ばく露は2件の研究で評価され た。評価したすべての研究で、いずれかの農薬へのばく露と停留精巣との関連を調査しようとしたと ころ、観察された影響は統計的に有意ではなかった(OR 1.19、95%CI 0.96~1.49、I2 24%)(図 23)。 さらに、DDTばく露と停留精巣との間の潜在的な関連を評価したところ、再び統計的に有意ではない関 連が観察された(OR 1.47、95% CI 0.98-2.2、I2 51%)(図 24)。多数の分析を考慮すると、これら の結果は慎重に解釈する必要があり、これらのデータに基づいて、あらゆる農薬ばく露と停留精巣と の間に、臨床的に有意な関連を示唆する最近のエビデンスはない。

尿道下裂は主に9件の後ろ向き研究で評価され、サンプルサイズの中央値は784人(IQR 200 - 861) であった。2件の研究ではDDTレベルが評価され、6件の研究では農薬ばく露一般が評価された。評価さ れたすべての研究において、何らかの農薬への母親のばく露(妊娠前及び妊娠期)と尿道下裂との関連 を調査しようとしたところ、観察された影響は統計的に有意ではなかった(OR 1.02、95%CI 0.74-1.39、I2 72%)(図25)。特定の農薬を評価した 3 つの研究(DDT、n=2; クロルデン、n=1)を分析 に含めると、再び統計的に有意でない関連が観察された(OR 1.00、95% CI 0.84-1.16、I2 66%)(図 26)。我々のシステマティックレビューでは、1966年1月から2008年3月までに英語で出版され、PubMed に索引付けされたオリジナルの研究を含む1つのメタアナリシスを検索した(Rocheleau CM, 2009)。 2007年以前に発表された9件の研究がすべての研究の包含基準を満たしており、著者らは、尿道下裂の リスクの上昇は、母親の職業ばく露 (PRR1.36、CI=1.04-1.77)及び父親の職業ばく露 (PRR1.19、CI=1.00-1.41)と関連していたが、わずかに有意であったと報告している。異なる期間に文献評価した結果とし て、我々のレビューと発表されたメタアナリシスとの重複が最小限であったために、我々は2つの成果 を統合することができ、再び我々は統計的に有意ではない結果を取得した (OR 1.14、95%CI 0.84 -1.55、I2 73%)。このように、いかなる農薬ばく露と停留精巣との間に、妥当で臨床的に有意な関連 を示唆する最近のエビデンスはない。

## 9.4. 研究が少ない小児健康影響

Table 10にはない健康影響には、個々の健康影響のエビデンスの統合には研究数が少なすぎるもの がある。すなわち、これらの健康影響には、腹壁裂、心臓先天異常、横隔膜ヘルニア、食道閉鎖症など のような明確に定義されてはいるが研究数が少ない臨床所見から、広範な臨床所見に関連する多数の 指標からなるが健康影響の定義の一致と標準化が著しく欠如しているものまで、多種多様な情報が収 集されている。例えば、神経発達に関連した健康影響は広範囲に評価されているが、IQ測定から知覚推 論に至るまで使用されている指標は、定量的な統合を目指したさらなる試みは不可能であると考えら れた。我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を 可能にするために、これらの健康影響に関する以前に発表されたメタアナリシスは確認されなかった。 一般的に、これらの健康影響に関する結果は効果量が小さく、統計的に有意ではなかった(少数の例外 を除く)。分析数が多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータ に基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

## 表10:対象となる研究で定義された小児健康分野で評価された影響

健康影響		
尿路性器距離の異常	体格指数 (BMI) Z スコア	血清プロラクチン値の上昇
異常なボディマス指数 (BMI)	体脂肪率(対数変換)	血清トータルテストステロン値の上
		昇
骨年齢の異常	尿道索	IQ
胸の大きさの異常	大動脈の動脈硬化	LH 調節障害
肥満度指数の異常変化	先天性横隔膜ヘルニア (CDH)	年間の高さ速度が低い
身長の異常な変化	先天性心疾患	重篤な先天性異常
胸囲の異常	先天性奇形	男性性器奇形
在胎月齢の異常	クレチン症	母体年齢
年齢に応じた頭囲の異常	頭踵長	母体の体重増加
異常な高さ	停留精巣	流産・死産
股関節周囲の異常	インヒビン B 値の低下	筋骨格系の欠陥
身長の異常	血清 FSH 値の低下	神経管障害
卵巣の異常測定	血清インヒビン B 値の低下	肥満
ペニスの長さの異常(伸びる)	血清 SHBG 値の低下	エストラジオールの調節障害
ペニスの幅の異常	精巣容積の減少	知覚推論
血清 DHT 値の異常	テストステロン値の低下	パフォーマンス IQ
異常な座高	血清 LH 値の低下	心室中隔欠損
異常な立位の高さ	授乳期間	胎盤重量
異常なタナー段階	食道閉鎖症	胎盤重量
上腕囲の異常	胎児の死亡	ポンデラルインデックス
屈曲上腕囲の異常	胎児頭囲	ポンデラルインデックス
子宮の測定値の異常	胎児の長さ	思春期早発
ウエスト周りの異常	胎児の体重	子癇前症
異常な体重	FGR	早熟な乳房の発達
長さに対する重量の異常	注意散漫でない	早期エストラジオール分泌
乳房の発達への影響	FSHの調節障害	思春期早発症(陰毛)
Anal position index	腹壁裂	未熟児
アンドロステンジオンの調節障害	在胎月齡	処理速度
無脳症	女性化乳房	乳幼児の急激な体重増加
抗ミュラーホルモンの調節障害	頭囲	SGA
APGAR 1分間のスコア	尿道下裂	SHC
APGAR 5分間のスコア	特発性早熟性思春期	二分脊椎
房室中隔欠損症	FSH 値の上昇	皮下脂肪計測
出生時の頭囲	SHBG の増加	テストステロンの調節障害
出生時の体長	LH/テストステロン比率の増加	ファロー四徴症

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出生時体重	血清 AMH 値の上昇	大動脈の転換
出生時体重、在胎月齢で調整	血清アンドロステンジオン値	言語理解
	の上昇	
BMI	血清 DHEAS 値の上昇	言語性 IQ
出産時の BMI	血清遊離テストステロン値の	ワーキングメモリ
	上昇	
妊娠前の BMI		

# 表 11:4 件以上の研究がある影響のサブグループごとに同定された研究の要約

<b>X</b> = = = = <b>1 47 1</b>			
影響	研究数	メタアナリシス実施	前回のメタアナリシス結果
先天性奇形			
全身	¥ 5	No	NA
神経管閉鎖障害	<b>=</b> 4	No	NA
泌尿生殖器の奇刑	¢ 19	Yes	尿道下裂:母方の職業ばく露(RR 1.36; 95%CI 1.04-1.77)及び父方の職業ばく露 (RR 1.19;95%CI 1.00-1.41)
発生	40	No	NA
発育			
身長/出産時長	ŧ 21	No	NA
体重	<u>ā</u> 26	No	<u>出生時体重(ヨーロッパの12のコホートを</u> <u>対象とした個々の参加者のデータメタアナリ</u> <u>シス)</u> : p,p <sup>-</sup> -DDEの1µg/L増加は、出生 時体重の7g減少と関連していた(95%CI:- 18.4g)
頭囲	E 17	No	NA
性的成素	<u>ң</u> 9	No	NA

(NA:利用できない)

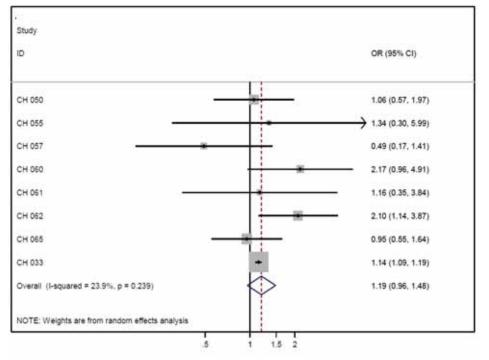


図23: 農薬ばく露に関する情報と停留精巣のリスクのランダム効果メタアナリシス

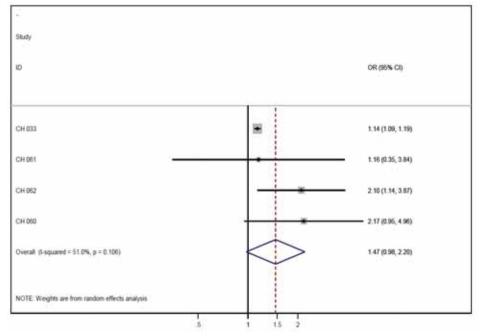


図24:DDTばく露に関する情報と停留精巣のリスクのランダム効果メタアナリシス

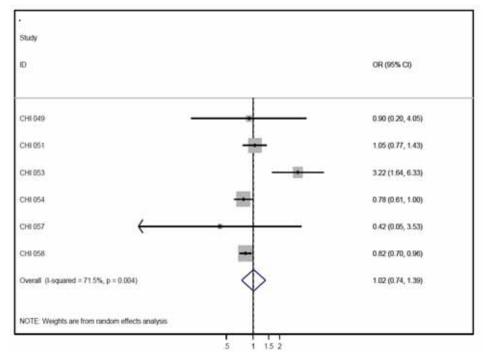


図25: 農薬ばく露一般に関する情報と尿道下裂のリスクのランダム効果メタアナリ シス

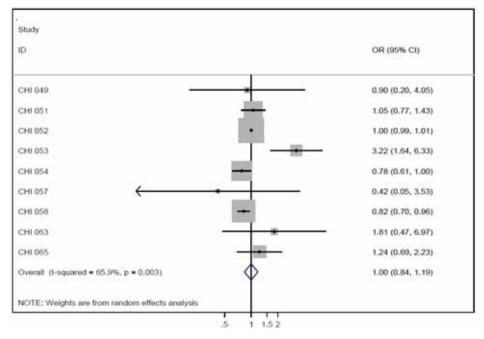


図26:特定の農薬に関する研究を含む農薬ばく露一般に関する情報と尿道下裂のリ スクのランダム効果メタアナリシス

## 10. 生殖疾患

全体では、63の論文が農薬ばく露の小児健康影響を調査しており(サンプルサイズ中央値:299、IQR 111-544)、データ抽出データベースには578の個別の分析が含まれていた。分析の3分の1以上(n=217、 38%)が精子/精液の質を評価しているのに対し、他の研究/分析では生殖関連ホルモン、不妊、自然流産などを評価している。他の影響に見られるように、ばく露の定義の多様性は顕著であり、データ統合に特別な問題を与えている。64件のうち4件のみが前向きコホート研究であり、大多数の研究は横断研究であった(n=45、70%)。報告されている分析のサンプルサイズはかなり小さく、41人から29,649人の範囲であった(中央値161)。この領域での最大の研究は、がん分野で評価された最大の研究よりも小規模である。ここでは、グリーンランドのイヌイットの集団とともにヨーロッパ3カ国で設立されたプロジェクトであるINUEND0(INUit-ENDOcrine)研究グループ(n=8)からの出版物を調査した。研究のほぼ3分の2がヨーロッパとアメリカで実施された(それぞれn=22と20)。22件の研究が職業ばく露を 評価し、さらに半数以上の研究がばく露のバイオマーカーに関する情報を有し(n=38, 59%)、3件の研究が職業ばく露を職業ばく露マトリックス(JEM)で評価し、2件の研究が質問紙とバイオマーカーの両方を使用した。調査した異なる影響カテゴリーを、各カテゴリーに寄与した研究の数と定量的統合の決定とともに表12に示した。データの不均一性のため、データの統計的統合(メタアナリシス)は流産についてのみ実施された。

## 10.1 障害のある精子パラメータ

25件の研究(中央値 189件: IQR 87-336)が、様々な影響を用いて農薬と精子/精液の質との関連を 評価した。これらの結果について実施された分析の総数は217件で、実施された分析のサンプルサイズ は41~763件と小規模である。最大の研究はINDUENDO研究グループによるヨーロッパの横断研究(ID RPD 009)で、p,p'-DDEの精子濃度、精子運動性、精子形態への影響を評価し、精子運動性は研究対象 の4つの集団においてp,p'-DDEと負の関係があることが示された。同じグループによる別の大規模研 究(ID RPD 012)では、残留性有機汚染物質(POPs)が男性の生殖機能を阻害する可能性のエビデンス は示されていない。多数の分析が行われたにもかかわらず、同じ比較単位と分析を用いた 4 件以上の 研究では、単一の農薬と関連するバイオマーカーは評価されていないため、定量的な統合は行われな かった。

## 10.2 生殖能障害

30種類の分析を含む 8件の研究が、低生殖能に対する農薬の影響を評価している。サンプルサイズ は 41 から2,365 人とかなり小さい。潜在的な関連の評価には異なる効果量と分析が使用されている ため、メタアナリシスによる結果の統合は不可能である。温室栽培農場の女性労働者の農薬ばく露を 調査した最大規模の研究(ID: RPD 038)では、生殖能の低下が報告された(OR=0.68、95% CI=0.49-0.94)。しかし、温室栽培農場の女性労働者を対象とした2番目に大きなフィールド研究(ID RPD 034) では、有意な関連は示されなかった(OR=1.11、95%CI=0.96-1.29)。14の追加分析では有意な結果は報 告されなかったため、フィールド調査ではエビデンスが矛盾している。

## 10.3. 自然流産

自然流産に関する10件の研究では、職業ばく露に焦点が当てられていた。効果推定値とその変動の 指標を提供した6件の研究からデータを統合することができた。ランダム効果モデルを用いた要約0Rは 1.52 (95%:1.09-2.13)であり、大きな不均一性が観察された(I2=63%)(図27)。しかし、INUENDO 研究グループが実施したこの影響に関する最大の横断研究(ID RPD 003)では、p,p'-DDEの農業労働 と流産との間に統計的効果(OR=1)は示されなかった。さらに1つの研究では、フルタイムとパート タイムを比較したが、有意な関連は報告されていない(p値=0.99)。他の3つの研究では、メタア ナリシスに含めるのに十分な情報が得られなかった。

## 10.4 生殖ホルモン

このシステマティックレビューでは、様々な生殖ホルモンについて250の分析を行った19の研究(サ ンプルサイズ中央値257:IQR 97-322)が同定された。これらの研究は、精子障害パラメータに関する 他の大規模なグループのサンプルサイズに匹敵するものであり、その範囲は62から887までであった。 最大の研究は、テストステロンとエストラジオールのレベルに対するヘキサクロロベンゼンの影響を 評価したヨーロッパの横断研究である。14~15歳の男性青年のホルモン状態は、汚染物質への内部ば く露に関連して研究された。この研究では、ばく露はホルモン濃度の大きな差異と関連していること が示された。INUENDO研究グループが行った研究では、DDEと生殖関連ホルモンの全体的な分析では有 意な結果は得られなかったが、異なるパターンが観察された。しかし、センターごとの分析では、ゴナ ドトロピン濃度と性ホルモン結合グロブリンはp,p'-DDEばく露の影響を受けているようであり、母集 団間に大きなばらつきがあることが示唆された。評価された結果と農薬の種類が多いため、データの 定量的な統合はできなかった。

## 10.5. 研究数が少ない生殖影響

表12に含まれていないすべての影響については、月経周期の評価では利用可能なエビデンスを統合 することができない。我々のシステマティックレビューでは、過去に発表されたエビデンス(2006年以 前)との比較を可能にするために、これらの影響に関する過去に発表されたメタアナリシスは確認し なかった。異なる月経影響に関する結果から、DDEへのばく露が月経障害の主な原因であるとは考えに くいことが示された。 表 12:4 件以上の研究がある影響サブグループごとに同定された研究の要約(NA: 利用不可)

影響	研究数	メタアナリシス実施	前回のメタアナリシス結果
精子障害パラメータ	25	No	NA
生殖能障害	8	No	NA
流産	10	Yes	NA
生殖ホルモン	19	No	NA

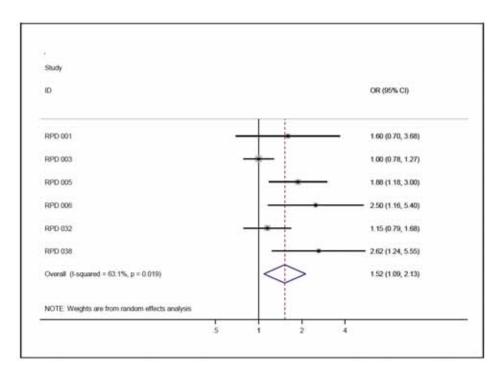


図27:農薬ばく露に関する情報と流産リスクのランダム効果メタアナリシス

### 11. 神経疾患

全体では 60 の論文が農薬ばく露の神経学的影響に及ぼす効果を調査しており(サンプルサイズ中央 値:390、IQR 246-781)、データ抽出データベースでは 573 件の個別分析が行われた。30 以上の健康 関連影響が評価されており、パーキンソン病に焦点を当てた研究が最も多く 32 件あった(表 13)。他 の影響に見られるように、ばく露の定義の多様性には目を見張るものがあり、データ統合に特別な問 題を与えている。60 件のうち 8 件のみが前向きコホート研究で、残りの 2 件はコホート内症例対照研 究であった。報告された分析におけるサンプルサイズはしばしば小さく、参加者数は 46~143,325 人 (中央値 390 人)であり、この領域における最大の研究は、がん分野で評価された最大の研究よりも 小規模であった。ここでは、農業健康調査(Agricultural Health Study: AHS)のようなこの分野の大 規模でよく知られた研究からの多くの出版物のまとまりも観察されたが、一方で 43 件の研究が職業ば く露を評価していた。さらに、ばく露のバイオマーカーに関する情報を持つ研究の存在は、ここではあ まり目立たなかった(n=7、12%)。評価された影響カテゴリーを表 13 に示す。評価された影響ごとに 同定された研究の数が少ないため、データの統計的統合(メタアナリシス)はパーキンソン病と筋萎縮 性側索硬化症についてのみ実施された。

## 11.1 パーキンソン病

農薬ばく露とパーキンソン病との関連を調査した研究は32件あり、サンプルサイズの中央値は399件 (IQR 286-711)で、データベースには266件の比較が抽出されている。検索された研究の80%は職業ば く露を評価していたが、前向き研究はわずか10%で、ばく露をバイオマーカーを介して評価した研究 は少数であった(10%)。個々の農薬の評価は多岐にわたったが、一般的な農薬(28件)、DDT(5件)、 パラコート(9件)などの農薬がより頻繁に評価されていた。

まず、一般的な農薬の使用とパーキンソン病との関連を評価した。観察された効果は、かなりの不均 一性が存在し、統計的に有意な関連を示した(ランダム効果OR 1.58、95%CI 1.35~2.85、I2 61%) (図 28)。特定の農薬を評価した 4 つの研究(例:パラコート)を除いて、他のすべての研究では、 主に質問紙による後ろ向きな方法で、主に職業上の一般的な農薬使用を評価している。メタアナリシ スの結果は、この研究の課題に関する最大の研究と一致している。

次に、DDTばく露とパーキンソン病との関連を評価した。観察された効果は、不均一性の存在なしに 統計学的に有意ではないことが示された(ランダム効果OR=1.01、95%CI=0.78-1.30、I2=0%)(図29)。 最後に、パラコートばく露とパーキンソン病との関連を評価した。観察された効果は、中等度の不均一 性の存在下で統計的に有意な関連を示した(ランダム効果OR=1.32、95%CI=1.10-1.60、I2=34%)(図 30)。メタアナリシスの結果は、これらの研究課題に関する最大規模の研究と一致している。

我々の文献検索では、2000年から2013年までに発表された農薬ばく露とパーキンソン病との関連に 関する7つのシステマティックレビュー及び/またはメタアナリシスが得られた (Pezzoli 2013, Van-Maele Fabry 2012, van der Mark 2012, Dick 2006, Priyadarshi 2001, Priyadarshi 2000, Allen 2013)。研究統合の最も古い取り組みと最新の取り組みとのかなりの時間的間隔、また、方法論(前向 き研究のみの評価、対象研究の方法論的評価など)の違いにもかかわらず、結果はメタアナリシス全体 で一貫しており、2006年からの現在の取り組みとも一致している(表14)。

## 11.2. 筋萎縮性側索硬化症(ALS)

7件の研究が農薬ばく露と筋萎縮性側索硬化症との関連を評価しており、サンプルサイズの中央値は 356 (IQR 201-1156) で、データベースには11件の個別の比較が抽出されている。検索されたすべての 研究は職業ばく露を評価しており、4つの研究は住居ばく露も評価していた。前向き研究は1件のみで、 ほとんどの研究では質問紙を用いてばく露を評価していた(n=6)。

我々は一般的な農薬使用とALSとの関連を評価した。観察された効果は、小さな不均一性(固定効果 OR=1.58、95%CI=1.31~1.90、I2 10%)の存在とともに統計的に有意な関連を示し(図31)、メタア ナリシスの結果は、その研究課題に関する最大の研究に沿ったものであった。

文献検索を行った結果、2012 年に発表された農薬ばく露と ALS の関連に関する 2 つのシステマティックレビュー及び/またはメタアナリシスが得られた(Kamel 2012, Malek 2012)。これらの結果によれば、大多数の研究では農薬の化学物質やクラスが特定されていなかったが、我々の知見や男性で対照と比較して農薬へのばく露とALSの関連を示すエビデンス(OR=1.88、95% CI: 1.36-2.61)についての著者らの報告と一致していた。

## 11.3. 研究数の少ない神経学的影響

パーキンソン病と筋萎縮性側索硬化症を除いて、残りのすべての神経学的影響については、2006年 以降の研究が少なすぎて、それぞれの影響だけでエビデンスを統合することができない。我々のシス テマティックレビューでは、2006年以前に発表されたエビデンスとの比較を可能にするために、これ らの影響に関する以前に発表されたメタアナリシスは確認されなかった。一般的に、これらの影響に 関する結果は効果が小さく、統計的に有意ではなかったが、少数の例外を除いては有意であった。分析 数が多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農 薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

# 表13:神経学分野で評価された影響

健康影響		
手の交代運動の異常	アルツハイマー病	カタプレキシーを伴うナルコレプシー
足首反射の異常	筋萎縮性側索硬化症	神経症状
遠位運動振幅の異常	原因不明の多発神経障害	パーキンソン病
遠位運動潜時の異常	握力の低下	パーキンソン症候群
表情の異常	遅延記憶障害	末梢神経障害
神経伝導速度異常	認知症	進行性核上性麻痺
異常な姿勢振戦	本態性振戦	レストレスレッグス症候群
異常な姿勢	歩行障害	ロンベルグ徴候
短 F 波潜時の異常	難聴	散発性運動ニューロン病
足尖の固有知覚の異常	多系統萎縮症	潜在的神経障害
足尖の振動知覚の異常	ナルコレプシー (カタプレキシー	つぎ足歩行異常
	の有無にかかわらず)	

# 表14: 農薬ばく露とパーキンソン病リスクを評価した研究の特徴(n/a:なし)

ID	年	場所	研究	ばく	<b>扅 ばく露評価</b>	比較単位	調整	サンプル
			デザイン	タイプ	,			サイズ
DDT								
NRD 027	2007	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	8899
					の質問紙			
NRD 025	2011	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	808
					の質問紙			
NRD 032	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	578
					の質問紙	never		
NRD 033	2010	ヨーロッパ	Nested case-	環境	バイオマーカー	per IQR	yes	292
			control			increase		
NRD 019	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	no	184
					の質問紙			
パラコー	ŀ							
NRD 019	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	no	184
					の質問紙			
NRD 027	2007	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	7393
					の質問紙			
NRD 023	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
NRD 030	2009	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	1030
					の質問紙			
NRD 037	2011	アメリカ	Nested case-	職業	直接ばく露について	ever/never	yes	468
			control		の質問紙			
NRD 020	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
NRD 022	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	578
					の質問紙	never		
NRD 038	2010	アメリカ	Case-control	職業	職歴	yes/no	yes	58
NRD 020	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
農薬								
NRD 033	2010	ヨーロッパ	Nested case-	環境	バイオマーカー	per IQR	yes	292
			control.		(HCB)	increase		
NRD 058	2010	ヨーロッパ	Case-control	混合	直接ばく露について	yes/no	no	330
	•						-	-

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Pesticide epidemiology

ID	年	場所	研究		ばく露評価	比較単位	調整	サンプル
			デザイン	タイプ				サイズ
					の質問紙(殺虫剤)			
NRD 034	2010	アジア	Case-control	職業	直接ばく露について	ever/never	n/a	608
					の質問紙			
NRD 018	2008	ヨーロッパ	Case-control	職業	直接ばく露について	ever/never	yes	233
					の質問紙			
NRD 017	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	yes	1666
					の質問紙			
NRD 014	2006	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	143325
					の質問紙			
NRD 029	2009	ヨーロッパ	Case-control	混合	直接ばく露について	ever/never	no	388
					の質問紙とJEM			
NRD 036	2011	ヨーロッパ	Cohort	職業	JEM	JEM class		
NRD 015	2007	アジア	Case-control	職業	直接ばく露について	yes/no	yes	308
					の質問紙			
NRD 020	2009	アメリカ	Case-control	職業	職歴	yes/no	yes	709
NRD 028	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	yes	615
					の質問紙			
NRD 023	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
NRD 016	2007	ヨーロッパ	Case-control	混合	職歴・直接ばく露に	high vs. no	yes	2756
					ついての質問紙	exposure		
NRD 024	2010	ヨーロッパ	Case-control	職業	職歴	yes/no	no	387
NRD 025	2011	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	808
					の質問紙			
NRD 058	2006	アメリカ	Case-control	混合	直接ばく露について	ever/never	no	278
					の質問紙			
NRD 027	2007	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	65183
					の質問紙			
NRD 035	2010	アジア	Case-control	職業	職歴	yes/no	no	525
NRD 030	2006	アメリカ	Case-control	職業	直接ばく露について		yes	430
					の質問紙		-	
NRD 030	2009	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	1030
11110 000	2000	1111			下した らう 第一 しょう	0 Y OI / 110 Y CI	,00	1000

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ID	年	場所	研究	ばく露	ばく露評価	比較単位	調整	サンプル
			デザイン	タイプ				サイズ
					の質問紙			
NRD 022	2009	ヨーロッパ	Case-control	職業	直接ばく露について の質問紙	ever/never	yes	781
NRD 019	2008	アメリカ	Case-control	職業	直接ばく露について の質問紙	yes/no	no	184
NRD 032	2010	アメリカ	Case-control	職業	直接ばく露について の質問紙	ever vs. never	yes	352
NRD 032	2010	アメリカ	Case-control	職業	直接ばく露について の質問紙	ever vs. never	yes	578
NRD 003	2010	ヨーロッパ	Case-control	n/a	n/a	yes/no	yes	264

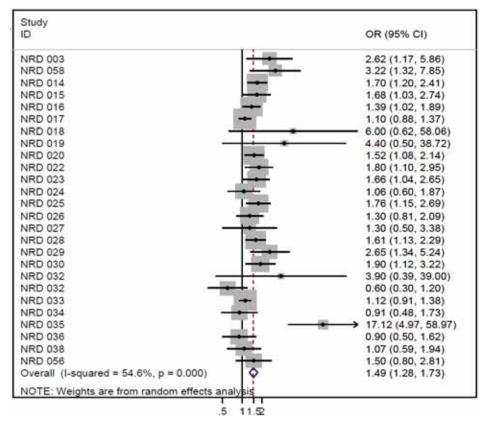


図28: 農薬ばく露に関する情報とパーキンソン病のリスクのランダム効果メタアナ リシス (ID NRD 033の研究、特にヘキサクロロベンゼンを評価)

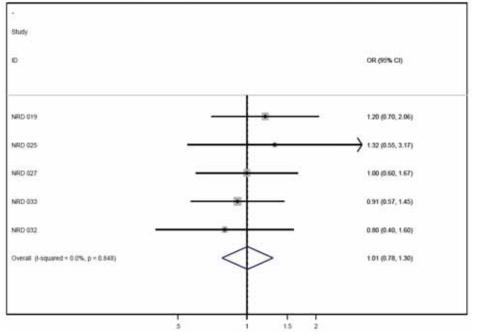


図29:パーキンソン病のリスクとばく露に関する情報の固定効果メタアナリシス

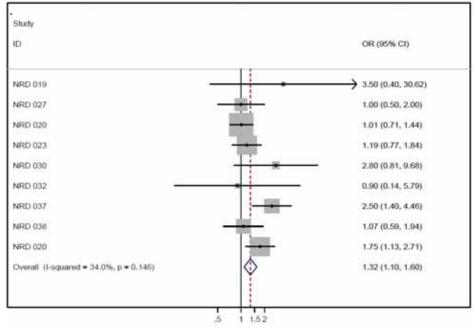


図30:パラコートばく露に関する情報とパーキンソン病リスクの固定効果メタアナ リシス

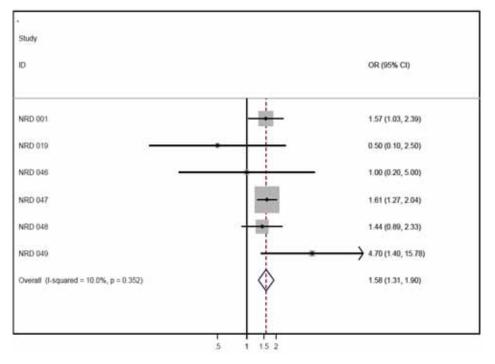


図31: 農薬ばく露に関する情報と筋萎縮性側索硬化症のリスクの固定効果メタアナ リシス

## 12. 内分泌疾患

全体では、35の出版物で農薬ばく露が甲状腺ホルモン調節障害に及ぼす影響が調査され(中央値サ ンプルサイズ226; IQR 130-453)、データ抽出データベースでは343の個別の分析が行われた。評価さ れた主な影響は、サイロキシン(T4)、トリヨードサイロニン(T3)、甲状腺刺激ホルモン(TSH)レ ベルであった。この分野で実施された前向きコホート研究は3件のみであり、エビデンスの大部分は後 ろ向き症例対照または横断分析によるものであるが、これらはばく露測定においてリコールバイアス がかかりやすい。報告されている分析におけるサンプルサイズはしばしば小さく、参加者数は27~ 16,529人(中央値341人)であった。ここでは、この分野でよく知られた大規模な研究からの出版物の 大きなクラスタは観察されず、大多数の研究は環境ばく露を評価していた(n=28、80%)。しかし、ば く露のバイオマーカーに関する情報を有する研究の存在は、ここではより多数であった(n=29、83%)。 甲状腺機能低下症、甲状腺機能亢進症、その他の甲状腺疾患が分析全体の1/3以上を占めているにもか かわらず(n=123)、利用可能なエビデンスは、この分野では明らかに最大規模で農薬使用と女性の甲 状腺疾患との関連を調査している農業健康調査(Agricultural Health Study: AHS)に由来している。 この研究では、甲状腺機能低下症と有機塩素系殺虫剤(0R=1.2、95% CI=1.0-16)及び殺菌剤(0R=1.4、 95% CI=1.1-1.8)の使用歴との間に関連があることが明らかになった。しかし、この結果は、有意水準 の境界線上であったことと多重比較によるタイプIの誤差補正がないため、注意して解釈されるべきで ある。この分野でいくつかの甲状腺ホルモンレベルを評価している他の研究は非常に小規模であり、 矛盾する結果を示している。他の影響に見られるように、まちまちなばく露の定義に注目すべきであ り、データ統合に特別な問題を及ぼしている。データの不均一性と異なる分析、効果量及び指標の使用 のために、データの統計的統合(メタアナリシス)は行われていない。

### 13. 精神及び精神運動発達影響

全体では、32の出版物が小児集団における精神及び精神運動発達影響に対する農薬ばく露の影響を調 査しており(サンプルサイズ中央値:238、IQR 109-305)、データ抽出データベースでは462の個別の 分析が行われた。非ヨーロッパ系(アジア系)の集団を対象とした研究は1件のみであり、注意欠陥多 動性障害(ADHD、6件、102件の分析)を中心に17件の健康関連影響が評価されていた。他の影響に見ら れるように、ばく露の定義はかなりまちまちであり、データ統合に特別な問題をもたらしている。大多 数の研究(23の論文、72%)は前向きコホート研究を参考にしているが、報告されている分析における サンプルサイズはしばしば小さく、25~7,440人の範囲で、最大のものはカリフォルニア・セントラル バレーの小児における農薬散布付近の母親の居住と自閉症スペクトラム障害の後ろ向き研究であった。 ここで、我々はまた、CHAMACOS(サリナスの母と小児の健康評価センター)(5出版物)などの大規模 な、この分野でよく知られた研究から来ている出版物のクラスタを観察したが、研究の84%が環境ば く露を評価していた。さらに、ばく露のバイオマーカーに関する情報を有する研究の存在がここでは 多数であった(n=28、88%)。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究の 数と定量的統合の決定とともに表15に示す。データの不均一性と同定された研究数が少なかったため、 どの影響についてもデータの統計的統合(メタアナリシス)は行われなかった。

### 13.1. 研究が少ない精神及び精神運動発達影響

精神及び精神運動発達影響と注意欠陥多動性障害(ADHD)を除いて、表15に記載の影響はすべて、そ れぞれの影響だけでエビデンスを総合的に判断するには、利用可能な研究が少なすぎる。これらの影 響は、自閉症や広汎性発達障害のように研究数が少ないが適切に定義された臨床所見から、コミュニ ケーション、微細及び粗大な運動発達、または表現的言語発達などの神経発達の中間形質を表す多数 の影響まで収集されたさまざまな情報で構成されている。我々のシステマティックレビューでは、2006 年以前に発表されたエビデンスとの比較を可能にするために、これらの影響に関する以前に発表され たメタアナリシスは確認しなかった。一般的に、これらの影響に関する結果は影響が小さく、統計的に 有意ではなかったが、少数の例外はあった。分析数が多く、研究数やサンプル数が少ないことを考える と、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農薬ばく露とこれらの影 響との間に妥当な関連を示唆するエビデンスはない。

### 13.2. 注意欠陥多動性障害(ADHD)

6件の研究では、278人から2,539人の参加者のサンプルサイズで農薬ばく露とADHDとの関連を評価し、 データベースに102件の別個に抽出された比較を提供した。3つの研究はコホートであり、すべての研 究は環境ばく露を評価し、すべての研究でばく露はバイオマーカーを介して評価された。一般的な有 機リン剤へのばく露は3つの研究で、DDTへのばく露は2つの研究で評価され、trans-ノナクロル、ヘキ サクロロベンゼン、2,4,6-トリクロロフェノール(TCP)はそれぞれ1つの研究で評価された。このよう に、同等の影響の定義または同一の比較単位を用いた4つ以上の研究では、単一の農薬と関連するバ イオマーカーは評価されておらず、定量的な統合は行われなかった。この分野で最大の研究は、国民健 康・栄養調査(NHANES)の報告書(ID 17)で、1999年から2004年のNHANESのデータを使用して、6歳か ら15歳の小児2546人の間で尿中トリクロロフェノール(TCPs)と親が報告したADHDとの関連を評価し ている。著者らの報告によると、尿中の2,4,6-トリクロロフェノール(TCP)の濃度が低値(3.58 mg/g 未満)及び高値(3.58 mg/g以上)の小児は、検出限界値未満の小児に比べて、親に報告されたADHDの リスクが高かった(OR 1.54、95%CI 0.97~2.43、OR 1.77、95%CI 1.18~2.66、それぞれpはトレン ド=0.006)。

我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を可能 にするために、ADHDに関する以前に発表されたメタアナリシスは確認しなかった。一般的にADHDに関 する結果は効果が小さく、少数の例外を除いて統計的に有意ではなかった。したがって、多数の分析を 考慮すると、これらの結果は慎重な解釈が必要であり、これらのデータに基づいて、農薬ばく露とADHD との関連を示唆するエビデンスはない。

### 13.3. 神経発達

31件の研究が農薬ばく露と神経発達の関連を評価しており、サンプルサイズは25~1,041で、データ ベースには325件の個別比較が掲載されている。アジア系の小児の神経発達を評価した研究は1件のみ であり、その他の研究はすべてヨーロッパ系の集団を対象としたものであった。研究の74%はコホー ト研究で、27の研究ではバイオマーカーを用いてばく露が評価されていた。個々の農薬の評価は多種 多様で、有機リン系農薬一般というカテゴリーがより頻繁に評価されている(表16)。比較可能な影響 の定義または同一の比較単位を用いた 4 件以上の研究では、単一の農薬と関連するバイオマーカーの 評価は行われていないため、定量的な統合は行われていない。実際、神経発達の評価は、認知機能に見 られるように一般的な影響カテゴリーのもう一つの典型的な例であり、使用されている 35 のツール とサブツールの多様性と複雑性(表 17)は、公表されている文献の結果を体系的かつ定量的に統合し ようとする試みを無意味なものにしている。

この分野で最大の研究は、Collaborative Perinatal Projectの報告書(ID MPD 029)であり、ジク ロロジフェニルトリクロロエタンへの胎内ばく露と乳児及び学童期の小児の認知発達を評価している。 著者らの報告によると、この集団では DDT と DDE の濃度は比較的高かったが(DDT 濃度中央値 8.9 g/L、DDE 24.5 g/L)、7歳時のベイリー尺度の精神・精神運動発達スコアやフルスケール知能指数のい ずれにも関連していなかった。

我々のシステマティックレビューでは、2006年以前に発表されたエビデンスとの比較を可能にする ために、これらの結果に関する過去に発表されたメタアナリシスは確認しなかった。一般的に、神経発 達の影響に関する結果は、ほとんどの例外を除いて効果が小さく、統計的に有意ではなかった。したが って、多くの分析が行われたことを考えると、これらの結果は慎重に解釈する必要があり、これらのデ ータに基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

# 表15:研究の概要と精神及び精神運動発達影響

影響	分析数
注意欠陥多動性障害(ADHD)	102
自閉症	2
知能指数	13
学習障害	4
認知障害	20
精神・精神運動発達	318
広汎性発達障害	3

# 表16:神経発達の観点で評価された農薬

評価した農薬	分析数
DDT	81
クロルデコン	5
クロルピリホス	8
ヘキサクロロベンゼン (HCB)	5
殺虫剤	6
マラチオン	8
マイレックス	13
有機塩素系農薬	2
有機リン系・カーバメート系殺虫剤	7
有機リン系農薬	115
農薬	80
ピペロニルブトキシド	1

# 表 17:神経発達を評価する 31の研究で使用された影響の定義とツール

影響の定義・使用ツール
正確性、衝動制御 [Accuracy, impulse control]
年齢・段階別の質問紙 [Ages and Stages Questionnaire]
行動評価研究システム [Behavioral Assessment and Research System (BARS)]
乳幼児のためのベイリー精神運動発達指数尺度 [Bayley Psychomotor Development Index Scales for
Infants]
乳幼児のためのベイリー精神発達指数尺度 [Bayley Mental Development Index Scales for Infants]
ベアリーブクテニカ VMI 発達検査 [Beery-Buktenica VMI developmental test]
ベントン視覚記銘検査 [Benton Visual Retention Test (BVRT)]
ボックステスト [Box test]
ブラゼルトン新生児行動評価 [Brazelton neonatal behavioral assessmen]
ブルネ・レジン精神運動発達尺度 [Brunet-Lezine scale of psychomotor development]
小児の記憶力尺度 [Children's Memory Scale]
イメージ連結 [Combining the Picture]
完成、Codin [Completion, Codin]
持続的パフォーマンステスト [Continuous Performance Test (CPT)]
数列暗唱 [Digit Span]
ファーガンテスト(乳児知能) [Fagan test of infant intelligence (FTII)]
フィンガータッピングタスク [Finger Tapping Task]
ゲゼル発達スケジュール [Gesell Developmental Schedules]
グラハムローゼンブリット検査 [Graham-Rosenblith test]
グリフィス精神発達尺度 [Griffiths Mental Developmental Scale]
ヒット反応時間 [Hit reaction time]
大型ペレット検査 [Large-pellet test]
マッカーシーの小児能力尺度 [McCarthy Scales of Children's Abilities]
早期学習の Mullen スケール:AGS Ed [Mullen Scales of Early Learning: AGS Ed]
持続的パフォーマンス検査 (CPT) のパフォーマンス [Performance on Continuous Performance Test]
レーヴン検査 [Raven Test]
Santa Ana Form Board
Lincoln-Oseretsky Motor スコア [Score in Lincoln-Oseretsky Motor]
小型ペレット検査 [Small-pellet test]
スタンフォードビネーコピー検査 [Stanford-Binet Copying Test]
テラー視力カード(TAC)検査 [Teller visual Acuity Card(TAC)test]
トレイルメイキング [Trail Making]
カリフォルニア大学バークレー校の選好注視テスト [University of California Berkeley Preferential
Looking Test]
ウェクスラー小児知能検査 [Wechsler Intelligence Scale for children]
ウィスコンシンカード分類テスト [Wisconsin Card Sorting Test]

### 14. 呼吸器疾患

全体では、29の出版物が呼吸器影響に対する農薬ばく露の効果を調査しており(サンプルサイズ中 央値:249、IQR 126-1728)、データ抽出データベースでは399の個別の分析が行われた。そのうち67% はヨーロッパとアメリカからのもので、10の健康関連影響が評価されており、その中でも特に喘息に 焦点を当てた影響が多かった(N=9)。他の影響に見られるように、ばく露の定義はかなりまちまちで あり、データ統合に特別な問題をもたらしている。29の出版物のうち、前向きコホート研究に言及して いるのは6件のみで、12件は横断研究であった。報告された分析におけるサンプルサイズはしばしば小 さく、35~47,756人の範囲であり、最大の研究はSingapore Chinese Health Studyであった。ここで は、AHS (6件)のようなこの分野でよく知られた大規模な研究からの出版物の大規模なクラスタも観察 され、17件(68%)の研究が職業的ばく露を評価していた。さらに、ばく露のバイオマーカーに関する 情報を持つ研究の存在は、ここではあまり目立たなかった(N=8、34%)が、1件の研究ではJEMによる 職業的ばく露を評価していた。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究 の数と定量的統合の決定とともに表 18 に示した。データの不均一性と同定された研究数が少ないた め、データの統計的統合(メタアナリシス)は喘息についてのみ実施した。

### 14.1. 研究数が少ない呼吸器影響

これらの影響は、特発性肺線維症やサルコイドーシス、努力性呼気量(FEV)などの多数のバイオマ ーカーに加えて、研究数が少なすぎるが、明確に定義された臨床所見まで、様々な情報を収集したもの で構成されている。我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以 前)との比較を可能にするために、これらの影響に関する以前に発表されたメタアナリシスは確認し なかった。一般的に、これらの影響に関する結果は効果が小さく、統計的に有意ではなかったが、少数 の例外を除いては有意であった。分析数が多く、結果のほとんどが農業健康調査(Agricultural Health Study: AHS)からのものであることを考えると、これらの結果は慎重に解釈する必要があり、これらの データに基づいて、農薬ばく露とこれらの結果との間に妥当な関連を示唆するエビデンスはない。

#### 14.2. 喘息

9件の研究が農薬ばく露と喘息との関連を評価しており、サンプルサイズの中央値は402(IQR 127-724)で、データベースには196件の比較が抽出されている。半数以上の研究が横断的に行われ、3分の 2以上の研究ではばく露は質問紙で評価されていた。個々の農薬の評価は多岐にわたり、DDT、パラコー ト、クロルピリホスがより頻繁に評価されている。DDT、クロルピリホス、パラコートを除いて(表 19)、 同じ比較単位を用いた 4 件以上の研究では、他の単一の農薬と関連するバイオマーカーは評価されて おらず、定量的な統合は行われていない。

利用可能な 5 つの研究で DDT へのばく露と喘息との関連を調査しようとしたところ、観察された 影響は不均一性を示すことなく統計的に有意であった (OR 1.29、95%CI 1.14~1.45、I2 0%) (図 32)。 次に、利用可能な6つの研究について、パラコートへのばく露と喘息との関連を調査しようとしたが、 観察された効果は、不均一性を示すもので統計的に有意ではなかった (OR=1.40, 95%CI=0.95-2.06, I2=53%) (図33)。最後に、利用可能な5つの研究でクロルピリホスへのばく露と喘息との関連を調査 しようとしたが、観察された効果は、不均一性を示し統計的に有意ではなかった (OR=1.03、95% CI=0.82-1.28、I2=0%) (図34)。メタアナリシスの結果は主にAHSによるものであること、また、男 女別、アレルギー性・非アレルギー性喘息別に報告されているため、4つがAHSに属していることに注 意が必要である。また、メタアナリシスの結果は2006年以降に発表されたデータに限定されているこ とも認めている。したがって、DDTについては、これらの農薬へのばく露と喘息との間に統計的に有意 な中等度の関連を示唆する最近のエビデンスがあると結論付けたが、クロルピリホスとパラコートに ついてはそうではない。

# 表18:呼吸器医学の研究と影響のまとめ(N/A:なし)

影響	研究数	メタアナリシス実施	以前に発表されたメタア ナリシス
咳	2	No	N/A
息苦しさ	1	No	N/A
咳・痰	2	No	N/A
1 秒量(FEV1)	1	No	N/A
FEV1 / 努力性肺活量 (FVC)	2	No	N/A
喘息	9	Yes	N/A
慢性気管支炎	5	No	N/A
過敏性肺炎	2	No	N/A
下気道感染症	2	No	N/A
サルコイドーシス	1	No	N/A
異常呼吸音	2	No	N/A

# 表19:メタアナリシスの対象となる関連付けの特徴

ID	年	場所	研究 デザイン	ばく露 タイプ	ばく露評価	比較	調整	サンプル サイズ
DDT								
RESP_002	2006	ヨーロッパ	コホート	環境	バイオマーカー	Yes/no	+++	402
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	936
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	946
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	4391
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	4468
パラコート								
RESP_019	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	134
RESP_022	2012	アジア	横断	職業	質問紙	Yes/no	+++	125
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	292
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	294
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	3096
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	3108
クロルピリ	ホス							
RESP_019	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	134
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	1017
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	1019
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	2174
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	2199

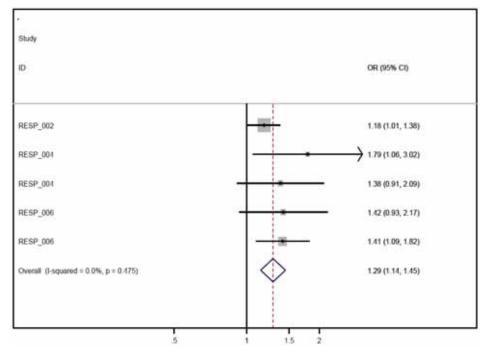


図 32: DDT ばく露に関する情報と喘息のリスクの固定効果メタアナリシス(研究 6 と 10 は農業健康研究の出版物を参照)

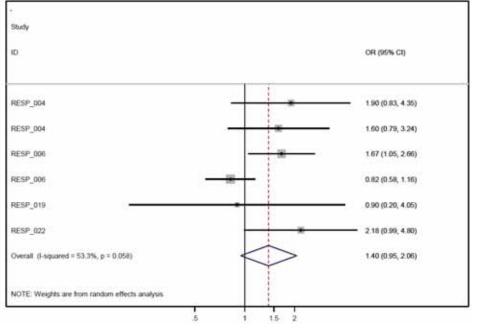


図 33:パラコートばく露に関する情報と喘息のリスクのランダム効果メタアナリシ ス(研究6と10は農業健康研究の出版物を参照)

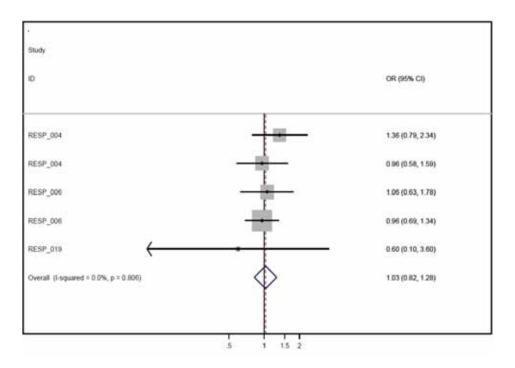


図34: クロルピリホスばく露に関する情報と喘息のリスクの固定効果メタアナリシ ス(研究6と10は農業健康研究の出版物を参照)

#### 15. 神経精神疾患

全体では、15の出版物が成人集団の神経精神影響に対する農薬ばく露の影響を調査しており(サン プルサイズ中央値:596、IQR158-12263)、データ抽出データベースでは358の個別の分析が行われてい る。4分の3はヨーロッパとアメリカの研究者であり、17の健康関連影響が評価され、大部分が認知機能 に焦点を当てていた(9研究、246の分析)。他の影響に見られるように、ばく露の定義はかなりまちま ちであり、データ統合に特別な問題を与えている。15の出版物のうち、前向きコホート研究に言及して いるのは2件のみで、出版物の60%は横断研究であった。報告された分析におけるサンプルサイズはし ばしば小さく、66~112,683人の範囲であり、最大の研究は米国の後ろ向き研究であった。ここでは、 農業健康調査(Agricultural Health Study、AHS)(4件)のようなこの分野でよく知られた大規模な 研究からの出版物のクラスタも観察されたが、1件を除いてすべての研究が職業ばく露を評価していた。 さらに、ばく露のバイオマーカーに関する情報を有する研究の存在は、ここではあまり目立たなかっ た(n=2、13%)。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究の数と定量的 統合の決定とともに表20に示した。データの不均一性と同定された研究数が少ないため、どの影響に ついてもデータの統計的統合(メタアナリシス)は行われていない。

#### 15.1. 認知機能

9件の研究が農薬ばく露と認知機能との関連を評価しており、サンプルサイズの中央値は80(IQR 141-205)で、データベースには246件の比較が抽出されている。1件を除くすべての研究が横断的で、 7件の研究では質問紙でばく露が評価されていた。個々の農薬の評価は多種多様で、有機リン系農薬の 一般的なカテゴリーがより頻繁に評価されていた。比較可能な影響の定義や同じ比較単位を用いた4件 以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われておらず、定量的な統合は行 われていない。実際、認知機能の評価は一般的な影響カテゴリーの典型的な例であり、利用可能な 15 の研究(表21)で使用された62のツールとサブツールの多様性と複雑性のため、公表されている文献の 結果を体系的かつ定量的に統合する試みは実りのないものとなっている。

この分野で最大の研究は、農薬の長期使用と神経行動機能との間の潜在的な関連を評価したAHSの報告書(ID NPD 014)であり、農薬散布者にこれらに関連する検査を行っている。著者らは、「検査結果は一部の農薬の生涯使用日数と関連していた」と報告している。エトプロプは、運動速度と視覚走査のテストのパフォーマンス低下と有意に関連していた。マラチオンは、視覚的走査と処理のテストのパフォーマンス低下と有意に関連していた。逆に、5種類の有機リン系農薬では、検査結果の有意な改善が観察された。具体的には、クロルピリホス、クマホス、パラチオン、ホレート、テトラクロルビンホスは言語学習と記憶力の向上と関連しており、クマホスは運動速度と視覚走査のテストの成績向上と関連しており、パラチオンは持続注意力のテストの成績向上と関連していました。全体的に、有機リン系農薬の使用とテスト成績低下との間には、この高齢の農薬使用者のサンプルでは一貫した関連のエビデンスは見られなかった。これらのほとんどが無効な結果となった理由としては、真の効果がないことや、より健康的な農薬使用者が選択的に参加している可能性が考えられる。

我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を可能 にするために、これらの結果に関する以前に発表されたメタアナリシスは確認しなかった。一般的に、 神経精神影響に関する結果は効果が小さく、統計的に有意ではなかったが、いくつかの例外を除いて は有意であった。

したがって、多数の分析を考慮すると、これらの結果は慎重に解釈する必要があり、これらのデータ に基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

# 15.2. 研究数が少ない神経精神影響

これらの影響は、うつや強迫性障害のような明確に定義されているが研究数が少なすぎる臨床所見 から、また敵意や見当識障害のような神経精神医学的な中間形質を表す影響も多数含まれている。我々 のシステマティックレビューでは、以前に発表されたエビデンス(2006 年以前)との比較を可能にす るために、これらの影響に関する以前に発表されたメタアナリシスは確認しなかった。一般的に、これ らの影響に関する結果は効果が小さく、統計的に有意ではなかった(少数の例外を除く)。分析の数が 多く、結果の多くが AHS からのものであることを考えると、これらの結果は慎重に解釈する必要があ り、これらのデータに基づいて、農薬ばく露とこれらの結果との間の妥当な関連を示唆するエビデン スはない。

影響	研究数
不安	3
注意力・計算障害	1
認知機能	9
うつ	4
脳波 (EEG) の状態	1
敵意	1
対人感受性障害	1
学習障害	1
吐き気	1
神経精神症状	3
強迫性障害	1
見当識障害	1
被害妄想的なイデオロギー	1
精神病傾向	1
REM 睡眠行動障害(RBD)	1
身体化	1
自殺	3

# 表 20:研究と神経精神影響のまとめ

表 21:認知機能を評価する 15 の研究で使用された影響の定義とツール (BARS. 行 動評価研究システム、AVLT:Auditory Verbal Learning Test、BVFT:Benton Visual Form Discrimination Test CALCALP:California Computerised Assessment Package Manual、WAIS:Wechsler Adult Intelligence Scale、 WMS:Wechsler Memory Scale)

影響の定義・使用ツール				
正解率 (BARS)[% Correct rejects]	選択的注意潜時(BARS)[Selective attention latency]			
ヒット数 (BARS) [% Hits]	選択的注意試験(BARS)[Selective attention trials]			
リコール (AVLT)	シーケンス A テスト性能 (秒)			
[Recal1]	[Sequences A test performance]			
認識(AVLT)	シーケンス B テスト性能(秒)			
[Recognition]	[Sequences B test performance]			
トータルリコール (AVLT)	シリアルディジット学習タスク (BARS)			
[Total recall]	[Serial digit learning task]			
ベントン視覚形態判別テスト (BVFT)	シリアルデジット学習テスト			
[Benton Visual Form Discrimination Test]	[Serial Digit Learning Test]			
ブロックデザイン(積み木問題) [Block design test]	単純反応時間試験(ms)[Simple Reaction Time Test]			
CALCAP選択テスト [CALCAP choice test]	空間スパンテスト [Spatial span test]			
連続性能試験スコア (m/s)	ストループテスト			
[Continuous Performance Test Score]	[Stroop test]			
カウントエラー [Counting errors]	サマリーインデックス(BARS)[Summary index]			
数列暗唱逆唱 (BARS) [Digit span backward task]	記号桁置換試験 [Symbol Digit Substitution Test]			
数列暗唱順唱 (BARS) [Digit span forward task]	記号桁遅延タスク(BARS)[Symbol-digit latency task]			
符号問題のスコア (秒)	症状チェックリスト 90 改訂版(SCL-90-R)			
[Digit-Symbol test score]	[Symptom Checklist 90 revised]			
誤報待ち時間 (BARS) [False alarm latency]	トレイルズBテスト [Trails B test]			
微細運動制御試験 [Fine motor control test]	言語流暢性テスト [Verbal fluency test]			
フィンガータッピング (利き手) (BARS) [Finger tapping]	WAIS-Ⅲ絵画配列 [WAIS-III picture arrangement test]			
フィンガータッピング、利き手 (BARS)	WAIS-Ⅲ計算問題			
[Finger tapping, dominant hand]	[WAIS-III arithmetic test]			
フィンガータッピング、(非利き手) (BARS) [Finger	WAIS-Ⅲ理解力 [WAIS-III comprehension test]			
tapping]				
フィンガータッピング、交互に手を動かす (BARS)	WAIS-Ⅲ数列暗唱			
[Finger tapping, alternating hand]	[WAIS-III digit span test]			
グレーデッドネーミングテスト [Graded naming test]	WAIS-Ⅲ符号問題 [WAIS-III digit symbol test]			
溝付きのペグボード、支配的な手のスコア	WAIS-Ⅲフルスケール IQ			
[Grooved pegboard, dominant hand score]	[WAIS-III full scale IQ]			
ヒット潜時 (BARS) [Hit latency]	WAIS-Ⅲ段階的命名試験[WAIS-III graded-naming test]			
マッチサンプル (BARS) [Match-Sample]	WAIS-Ⅲ類似 [WAIS-III similarities test]			
N100 潜時(ms)[N100 latency]	WAIS-Ⅲ語彙 [WAIS-III vocabulary test]			
N200 潜時(ms)[N200 latency]	WMS-Ⅲ 聴覚遅延記憶 [WMS-III auditory delayed memory test			
P200 潜時 (ms)	WMS-Ⅲ聴覚即時記憶			
[P200 latency]	[WMS-III auditory immediate memory test]			
P300 振幅 (μv), Cz [P300 amplitude]	WMS-Ⅲ聴覚認識 [WMS-III auditory recognition test]			
P300の潜時 (ms) [P300 latency]	WMS-Ⅲ文字・数字配列決定 [WMS-III letter-number test]			
進歩率 (BARS) [Progressive ratio]	WMS-Ⅲ視覚的遅延記憶 [WMS-III visual delayed memory test]			
反応時間潜時 a (BARS) [Reaction time latency a]	WMS-Ⅲ視覚即時試験 [WMS-III visual immediate test]			
反応時間潜時 a (BARS)	選択的注意間刺激間隔 (BARS)			
[Reaction time latency a]	[Selective attention interstimulus interval]			

#### 16. 糖尿病

全体として、糖尿病関連影響に対する農薬ばく露の効果を調査した論文は23編(サンプルサイズ中 央値:430、IQR 192-1721)で、データ抽出データベースには125の個別の分析結果が掲載されていた。 4つの健康関連影響が評価されており、1型糖尿病(n=93、74%)に大きな割合を占めていたのに対し、 18の分析では2型糖尿病に焦点が当てられていた。評価されたその他の影響は、前糖尿病(n=10)、妊 娠糖尿病(n=2)、その他のグルコース及びインスリン関連影響(n=2)であった。前向きコホート研究 は1件のみで、大多数は横断的デザイン(n=15)であったが、3件は症例対照、4件はコホート内症例対 照を使用した研究であった。大多数の研究はアメリカで行われており(n=15、65%)、7研究はヨーロ ッパ人であり、アジア人は1研究のみであった。ここでは、AHSのような分野でよく知られた大規模な研 究からの出版物の大規模なクラスターは観察されなかった。3つの研究のみが職業ばく露を評価し、残 りは環境ばく露(n=19)またはその両方(n=1)を調査した。さらに、ばく露のバイオマーカーに関す る情報がある研究は9件に限られていたが、10件の研究では質問紙とバイオマーカーの両方の情報が含 まれていた。調査した異なる影響カテゴリーを、各カテゴリーに寄与した研究の数とともに表 22 に 示す。DDE と DDT ばく露と 1 型糖尿病、DDE ばく露と 2 型糖尿病についてはメタアナリシスが可能 であった。

### 16.1.1型糖尿病

1型糖尿病に対する農薬の影響を評価した研究は 3 件あり(サンプルサイズ中央値 309、IQR:159-398)、DDE と DDT ばく露については OR のメタアナリシスが可能であった。DDEについては、9件の 研究がサンプルサイズ中央値202、IQR=142-334であった。我々は、(有意な)罹患率比(IRR)7.1を示 し、DDE被曝の最上位層と最下位層を比較した前向き研究を含めることができなかった。計算された要 約ORは、ランダム効果モデルを用いたDDEばく露で1.90(95%CI:1.25-2.86)であった。中等度の不均 一性が観察された(I2=49%)。DDTに関しては、6件の研究が統合に利用可能なデータを持っていた(サ ンプルサイズ中央値:577、IQR:27-2-2163)ため、1.76(95%CI:1.20-2.59)の要約効果が得られ、 非常に大きな不均一性が観察された(I2=76%)。不均一性の主な原因は、効果推定値の計算に使用さ れた異なるばく露レベルである。ランダム効果メタアナリシスでは、1型糖尿病リスクの増加が存在す るというエビデンスがあるとはいえ、観察された不均一性のため、この結果は慎重に解釈されるべき である。

### 16.2.2型糖尿病

DDEばく露と2型糖尿病リスクを評価するために4件の研究が対象となった(サンプルサイズ中央値: 471、IQR=292-642)。これらの研究から得られた要約0Rは1.30(95%CI:1.13-1.48)であった。不均 一性は観察されなかったが、要約結果は、効果量0R=1.30(95%CI=1.11-1.52)を報告した症例対照研 究に牽引されている。DDEばく露が2型糖尿病発症のリスク因子であることを示唆するエビデンスはあ るが、これは小規模な研究に基づくものである。

# 表 22:4 研究以上のサブグループごとに確認された研究の概要(NA:利用不可)

影響	研究数	メタアナリシス実施	前回のメタアナリシス結果
1型糖尿病	13	Yes	NA
2型糖尿病	6	Yes	NA
妊娠糖尿病	2	No	NA
インスリン・グルコース耐性	2	No	NA

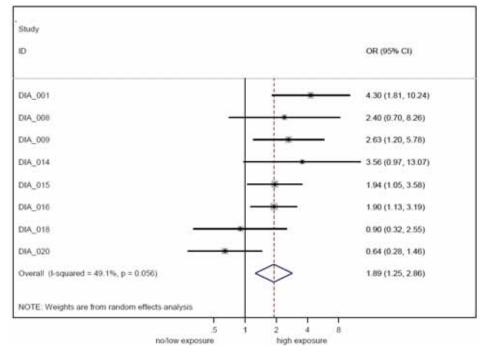


図35:DDEばく露と1型糖尿病との関連のサマリーオッズ比(OR)

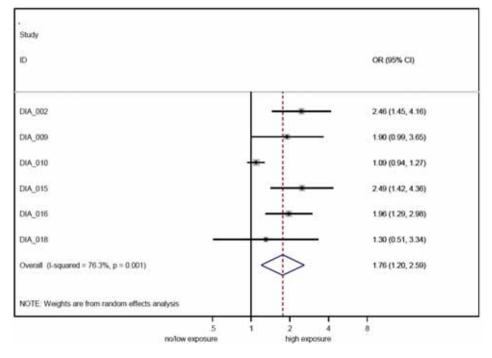


図36:DDTばく露と1型糖尿病との関連のサマリーオッズ比(OR)

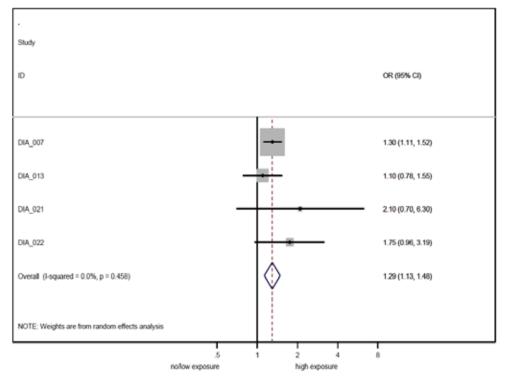


図37:DDEばく露と2型糖尿病との関連のサマリーオッズ比(OR)

### 17. 循環器疾患

重篤な心血管影響(心筋梗塞、脳卒中など)、心血管リスク因子(脂質、血圧)、その他の心血管影響(メタボリックシンドローム、肥満)を含む。これらのいずれについても、これまでのメタアナリシスは同定されていない。この系統的レビューで収集されたエビデンスは、特に心血管リスク因子と有機塩素に関する関連を弱く示唆しているが、他のクラスの農薬は研究されておらず、有機塩素に関する結果でさえ限られており、前向き研究の反復が必要である。

## 17.1. 重篤な心血管影響

心筋梗塞(ID CVD 005、ID CVD 006)、末梢動脈疾患(PAD)(ID CVD 007)、脳卒中(ID CVD 008)、 複合心血管疾患(ID CVD 009)を含む5つの研究が重篤な心血管影響を調査した。Agricultural Health Study(AHS)は2つの前向き分析(ID CVD 005、ID CVD 006)、National Health and Nutrition Examination Survey(NHANES)は他の2つの横断分析(ID CVD 007、ID CVD 009)を提供している。心筋梗塞に関す る研究(ID CVD 005、ID CVD 006)では、AHSの男性の心筋梗塞死亡率と農薬使用(個人またはクラス 別)との関連を示すエビデンスは示されなかった。同様に、AHSの女性では、農薬の使用と心筋梗塞と の全体的な関連は認められなかった。評価された27種類の農薬のうち、クロルピリホス、クマホス、カ ルボフラン、メタラキシル、ペンディメタリン、トリフラリンを含む6種類の農薬が女性の非致死的心 筋梗塞と有意に関連し(ID CVD 006)、いずれも比較的高いオッズ比(1.7以上)を示したが、複数回 の検査による偽陽性の確率も高かった。

別の前向き研究(8)では、脳卒中との関連で21種類の残留性有機汚染物質(POPs)を調査した。既 知の脳卒中リスク因子を調整した後、塩素原子が4、5、または6個のポリ塩化ビフェニル(PCB)、p、 p'-DDE、トランスノナクロル及びオクタクロロジベンゾ-p-ジオキシンのほとんどが脳卒中のリスク を有意に予測した。にもかかわらず、結果は今後の研究で再現する必要がある。末梢動脈疾患(PAD) 及び複合心血管疾患が、横断的なNHANESコホートでPOPsとの関連で研究された。PADのない被験者と比 較して、PADのある被験者では有機塩素系殺虫剤の濃度が有意に高かったが、肥満でない被験者では関 連は認められなかった。複合心血管疾患については、クロルデンのみに有意な関連が観察された。横断 的なデザインと自己申告による心血管疾患のため、これらの所見は慎重に解釈する必要がある。

全体的に、農薬ばく露と心血管影響との間の関連エビデンスは弱く、主に有機塩素系農薬に集中している。

### 17.2. 心血管リスク因子

## 17.2.1. 血圧

5件の研究で農薬と血圧の関連が調査された(ID CVD 002、ID CVD 003、ID CVD 004、ID CVD 010、 ID CVD 011)。1件の研究(ID CVD 011)では、横断的な研究が行われていた。 すべての効果量は非常に小さく、農薬ばく露と血圧との関連を示唆するものではなかった。

### 17.2.2. メタボリックシンドロームの構成要素

9件の研究では、脂質レベル、グルコース、インスリンレベルを含む農薬ばく露に関連したメタボリ ックシンドロームの構成要素が調査された。1件を除くすべての研究で有機塩素系農薬へのばく露が調 査され、いくつかのクラスと脂質レベルまたはグルコースレベルとの有意な関連が観察された。最も 質の高いエビデンスは、前向きCARDIA(Coronary Artery Risk Development in Young Adults)研究 (ID CVD 016)から得られている。CARDIAでは、様々な交絡因子を調整した後、20年目にp,p'-DDEが 最も一貫してトリグリセリド、インスリン抵抗性のホメオスタシスモデル評価値(HOMA-IR)及び高密 度リポ蛋白(HDL)コレステロールの低下を予測していた。また、オキシクロルデン、トランスノナク ロル、ヘキサクロロベンゼンもトリグリセリドの上昇を有意に予測した。最後に、中国で行われた症例 対照研究では、ピレスロイド(職業ばく露)に高度にばく露された参加者のグルコース調節の違いが調 査された。その結果、ピレスロイドにばく露されると異常なグルコース調節のリスクが高まることが 示された(OR = 1.48、95%CI = 1.24-1.77) (ID CVD 021)。しかし、この研究は後ろ向きであり、 残留交絡因子を除外できないため、これらの結果は他の集団で外部で再現の必要がある。

#### 17.2.3. 無症候性アテローム性動脈硬化症

集団ベースの Prospective Investigation of the Vasculature in Uppsala Seniors (ウプサラ高 齢者の血管系に関する前向き調査)では、POPのレベルが無症候性アテローム性動脈硬化症と関連して いるかどうかを横断研究で調査した。循環中のPCBレベルは、心血管リスク因子とは無関係に、アテロ ーム性動脈硬化性斑点と内膜複合体の超音波反射性と関連していたが、関連は前向き研究で確認する 必要がある。

#### 17.3. メタボリックシンドロームと肥満

3件の研究(ID CVD 010、ID CVD 011)で有機塩素ばく露とメタボリックシンドローム罹患率との関 連を調査した。国民健康・栄養調査(NHANES)(ID CVD 010)では、有機塩素系農薬の四分位間のORが 1.0、1.5、2.3、5.3であり、有機塩素ばく露とメタボリックシンドロームの罹患率との間に有意な関連 が報告された(傾向<0.01の場合はp)。他の症例対照研究(ID CVD 011)では、ヘプタクロルのみで 有意な関連が認められた。

全体として、12件の横断研究で農薬ばく露と体脂肪率または肥満度の測定値との関連が調査された。 また、12件中10件の研究では有機塩素系農薬と肥満または体脂肪率との関連が調査されていたが、他 のクラスの農薬についてはエビデンスが乏しかった。3件の研究(ID CVD 012、ID CVD 013、ID CVD 014)では、体脂肪率の測定値との相関分析のみが示された。残りの研究では、ウエスト周囲長、体格 指数(BMI)、有機塩素系農薬(DDTとクロルデン)との間に何らかの有意な関連が示されているが、エ ビデンスは横断的な分析に限られており、結果は関連を示唆するものに過ぎない。

## 18. 死亡率

全体では 11 の論文が農薬ばく露の死亡率への影響を調査し(サンプルサイズ中央値:1,986)、デ ータ抽出データベースでは 318 の個別分析が行われた。このセクションは、農薬と主要な死因との関 連を評価した異種の出版物群で構成されている。これらの研究は大規模なものであったにもかかわら ず、質は中等度であり、多重検定の補正を行わずに種々の疾患を同時に検定しているため、あまり有益 ではなかった。結果は、農薬ばく露と死亡との明らかな傾向を示していない。

## 19. 免疫疾患/自己免疫疾患

全体では、農薬ばく露が免疫障害に及ぼす影響を調査した論文は 10 (サンプルサイズ中央値 196、 IQR 81-476) で、データ抽出データベースでは 67 の個別の分析が行われた。60の研究はアメリカで 実施され、3つの研究はヨーロッパで実施され、1つの研究はアジアで実施された。関節炎、変形性関節 炎、関節リウマチ、様々な抗体、サイトカインなどの広範なリストを含む様々な健康関連影響が表 23 にまとめられている。10の出版物のうち7つは前向きコホート研究に言及していたが、2つの研究は横 断的であり、1つだけが症例対照であった。報告されている分析のサンプル数はかなり少なく、19~532 人で、最大の研究はCarolina Lupus Studyであった。研究の半分は職業ばく露を評価しており、ばく 露のバイオマーカーに関する情報は2つの研究で得られたが、4つの研究ではバイオマーカーと質問紙 の両方を使用していた。他の影響に見られるように、ばく露の定義と評価された影響はまちまちであ り、データ統合に特別な問題をもたらしている。2件以上の研究で単一の影響が評価されたものはなく、 免疫障害の分野ではデータの統合は不可能であった。

健康影響	
抗核抗体	インターロイキン-4(IL-4
関節炎	インターロイキン13(IL-13
補体成分 C3、C4	免疫学的効果
好酸球	白血球数
赤血球数	リンパ球レベル
糖タンパク質	好中球
ヘマトクリット/ヘモグロビン	ナチュラルキラー (NK) 細胞
インターフェロン γ	変形性関節症
免疫グロブリン1(IgG1)	関節リウマチ
免疫グロブリン4(IgG4)	全身性エリテマトーデス
免疫グロブリン M (IgM)	

## 表23:免疫障害の分野で評価された健康影響

## 20. アレルギー疾患

8つの異なる集団における9つの研究が、農薬ばく露とアレルギー性障害との関連を報告した。7件の 研究では職業ばく露が調査され、2件の研究では環境ばく露が調査された。8件の研究は横断的な調査 であったため、結論は逆因果関係やその他のバイアスがかかりやすい。結果については、5件の研究が 自己申告によるアレルギー性鼻炎、1件の研究が自己申告による喘息、残りの3件の研究が自己申告に よる皮膚刺激、接触性皮膚炎、食物アレルギー、花粉症、香料アレルギーを調査した。アレルギー性鼻 炎に関する4件の研究 (ID ALL\_003、ID ALL\_004、ID ALL\_005、ID ALL\_006) で統計学的に有意な結果 が報告された。これらの研究では、様々な農薬クラスとアレルギー性鼻炎との間に有意な関連が報告 されている。特に、Agricultural Health Study (AHS) では、アレルギー性鼻炎と除草剤である2,4-ジ クロロフェノキシ酢酸(2,4-D)グリホサート及び石油油、殺虫剤であるジアジノン及び殺菌剤である ベノミルへのばく露との間に有意な関連が報告されている。しかし、この研究には多くの限界があり、 結果は慎重な解釈が必要であり、将来の前向き研究での再現が必要である。この研究は、アレルギー性 鼻炎と非アレルギー性鼻炎の症状を区別できているか、また、横断的なデザインのためにばく露と症 状の間の時系列を示せるかという点で制限されている。全体的に質の低い1件の研究では、温室内の花 卉及び観賞用植物の栽培者におけるアレルギー性鼻炎について、手押しポンプによる農薬散布と農薬 散布なしの比較で高い効果量(OR、12.50;95%CI、2.00-78.05)が報告されている(ID ALL\_006)。 ここでも、この研究は全体的に質が低く、化学物質の種類ではなく散布方法に関連して定義された高 濃度ばく露集団に関係している。全体的に、アレルギー性障害と農薬ばく露に関するエビデンスは弱 1

## 21. 血液疾患

## 21.1. 再生不良性貧血

3件の研究では、農薬ばく露と再生不良性貧血(まれな血液疾患)との関連が調査された。すべての 研究は症例対照デザインであり、サンプルサイズは小さい(9~310の範囲)。2件の研究では、大きな 効果量で有意な関連が報告されたが、利用可能な研究の数が少ないことと、これらの研究の限界があ るため、確実な結論を出すことは難しい(表24)。もう1件の症例対照研究(ID APL\_002)では、効果 量は報告されていないが、関連のp値のみが報告されており、有意ではなかった。これらの示唆に富む 結果を明らかにするためには、さらなるエビデンスが必要である。

# 表 24: 効果量を報告した 2 つの症例対照研究における農薬ばく露と再生不良性貧 血の結果のまとめ

Study ID	  調査した農薬	比較	OR	下位 95% CI	上位 95% CI	ケース数	コントロ ール数
APL_001	有機リン剤	yes/no	2.1	1.1	4.2	21	32
APL_001	DDT	yes/no	6.7	1.5	30	5	4
APL_001	カーバメート	yes/no	7.4	1.7	31	8	3
APL_001	パラコート	yes/no	2.3	1	5.1	12	24
APL_001	職業的ばく露の農薬	yes/no	1	0.4	2.2	11	32
APL_001	住居用農薬	yes/no	1.3	0.9	1.9	64	238
APL_001	有機リン剤	yes/no	2.1	1	4.4	17	26
APL_001	パラコート	yes/no	1.9	0.7	4.9	7	20
APL_001	職業的ばく露の農薬	yes/no	1.1	0.4	2.7	9	24
APL_003	農業用農薬	yes/no	2.2	1.1	4.7	12	23
APL_003	住居用農薬	yes/no	1.3	0.9	1.9	70	240
APL_003	有機リン剤	高濃度ばく露 /ばく露なし	3	0.9	10.1	5	7
APL_003	ピレスロイド	高濃度ばく露 /ばく露なし	1.8	1	3.1	23	57
APL_003	除草剤	yes/no	2.4	0.9	6	8	15

# 21.2. 血液学的及び生化学的変化

14の研究では、農薬ばく露に関連した様々な血液学的及び生化学的変化を調べた。主に一般血液検 査とビタミンレベルであった。サンプル数は51~1,275人であった。これらの研究の質は中等度から低 度であった。ほとんどの研究では、血液学的パラメータと農薬ばく露との間の無補正相関統計値また は平均値が報告されており、p値以外に効果量は報告されていない。すべての研究は横断的なエビデン スを提供している。報告された分析の多くが統計的に有意であったにもかかわらず、これらのデータ に関連するエビデンスが限られており、質も中等度であるため、現段階では結果を解釈すべきではな い。

#### 22. その他の影響

全体では 30 の論文がその他の影響に対する農薬ばく露の効果を調査した。データ統合のための 我々の基準に基づき、これらの影響に対するメタアナリシスは実施されなかった。

### 21.1. 骨疾患

農薬ばく露が骨粗鬆症に及ぼす影響を調査した研究は3件あり、13の異なる分析を行った。我々は2 つのヨーロッパの横断研究と1つのアジアのコホート研究を同定した(サンプルサイズ中央値:176、 IQR:153-908)。すべての研究は、ばく露のバイオマーカーに関する情報とともに環境ばく露を評価し ており、すべての研究は有機塩素へのばく露のみを調査している。骨粗鬆症は超音波測定と骨密度に よって評価された。908名の女性を対象とした最大の研究では、p,p'-DDEが骨密度と明確な関連を示 し、交絡因子を調整した後も関連は維持されたが、効果は弱いことが示された。

#### 22.2. 皮膚疾患

11件の分析のうち農薬ばく露が皮膚病変に及ぼす影響を調査した研究は6件(サンプルサイズ中央値 356、IQR 26-2203)であった。4件の研究では横断的デザインが用いられていた。環境ばく露は3件の研 究で評価された。影響の定義は多くの場合、発疹や湿疹であった。結果はほとんど統計的に有意ではな かった。1つの前向き研究(ID SKD 004)では、男性5,042人を対象としたAraihazarのヒ素の健康影響 縦断研究から、皮膚病変と農薬使用について非常に有意な効果量が報告されているが、この研究では ヒ素ばく露も評価されており、それぞれのばく露の効果を区別することは困難であった。

#### 22.3. 歯科疾患

アメリカで行われた1つの横断研究では、496人の参加者が2つの影響で評価された。この研究では、 バイオマーカー(ID PER 001)の情報を用いて環境ばく露を評価した。この研究では、有機塩素系(0C) 農薬は歯周病と強く関連していた。

#### 22.4. 代謝性疾患

ヨーロッパで行われた横断研究では、農薬が代謝性疾患、特に様々なprorfyrins(誤植?)のレベル に及ぼす影響を評価し、8つの分析を行ったが、有意な結果は報告されていない。環境ばく露は、ばく 露評価のためのバイオマーカーを用いて研究された。

#### 22.5. 男性機能疾患

1件の症例対照研究では、農薬ばく露と勃起不全との関連が報告されている。この研究では有機塩素 系農薬に焦点を当て、勃起不全の症例101例を234例の同等の対照群と比較した。結果は統計的に有意 ではなく、関連を示すエビデンスとはならなかった。

## 22.6. 婦人科疾患

このグループには、上述の影響カテゴリーには含まれていない婦人科学的影響が含まれている。こ のグループには4件の研究が含まれており、3件は子宮内膜症、1件は閉経のタイミングを調査した。子 宮内膜症に関する3件の研究(ID GYN 001、ID GYN 002、ID GYN 003)はすべて横断的であり、すべて 有機塩素を調査していた。子宮内膜症と有機塩素に関する12の個別分析のうち1つは統計学的に有意で あった;芳香族系殺菌剤の上位3位までは、下位3位までと比較して子宮内膜症のリスクが5倍(OR = 5.3;95%CI、1.2-23.6)であった。この効果量は大きく、他の前向き研究で独立した再現が必要であ る。

農業健康調査(Agricultural Health Study: AHS)のデータを用いて、閉経前の女性を対象とした前 向き調査において、農薬へのばく露と閉経時年齢との関連を調査した。年齢、喫煙状況、経口避妊薬の 過去の使用状況をコントロールした後、閉経までの期間の中央値は、農薬を使用した女性では約3ヵ月

(HR 0.87、95%CI: 0.78、0.97)、ホルモン活性農薬を使用した女性では約5ヵ月(HR 0.77、95%CI: 0.65、0.92) 増加した。農薬の使用は、これらの結果に基づいて、閉経年齢の遅延と関連している可能性がある。しかしながら、結果は偽陽性バイアスがかかりやすく、独立した再現が必要である。

#### 22.7. 症状及び一般的な疾患

吐き気、倦怠感、めまい、息切れなどの一般的な疾患を5つの研究で調査した。これらの結果の定義 は非常に難しく、大きな測定誤差と関連している。研究の質は中等度から低度で、すべての研究が職業 ばく露に関係していた。いくつかの統計的に有意な結果が観察されたが、報告されたデータの不均一 性とこれらの研究に関連する限界のため、現段階では決定的な結論には程遠い。

#### 22.8. 腎臟疾患

3つの研究では、慢性腎臓病や結石疾患などの腎臓病を調査した。1件の研究では、DDE及びDDT残留農薬と結石疾患との間に統計的に有意な結果が報告された。

#### 22.9. 良性腫瘍

聴神経腫瘍に関する集団ベースの症例対照研究では、農薬ばく露と聴神経腫瘍との関連は認められ なかった。

#### 22.10. 消化器疾患

農薬ばく露と肝臓酵素との関連を調査した 7 件の研究が同定された。すべての研究は横断的または 症例対照であった。1件の研究、国民健康・栄養調査(NHANES)では有機塩素について、もう1件の研究 では2,4-ジクロロフェノキシ酢酸(2,4-D)とパラコートへのばく露について、残りの研究では広く定 義された農薬のカテゴリーについて調査が行われた。これらの研究は限られた規模で質が低く、多く の場合調整なしで、被ばく露者と非ばく露者の酵素の平均値のみが提示されていた。ほとんどすべて の研究で、農薬にばく露された参加者の肝臓酵素(例:γ-グルタミルトランスフェラーゼ(GGT)、ア ラニンアミノトランスフェラーゼ(ALT)、アスパラギン酸アミノトランスフェラーゼ(AST))のレベ ルが高いほど、統計的に有意な結果が報告されている。しかし、データの質の低さと研究の数が限られ ているため、しっかりとした結論を出すことはできず、データは現段階では関連をほのめかすだけで ある。

#### 結論

約46,000の科学出版物を網羅的かつ包括的に検索した結果、602の出版物を確認した。農薬ばく露に 関連した健康影響の全領域については、これまでにも研究されたことがなかった。我々の結果は、24の 主要な疾患カテゴリーを含む非常に幅広いスペクトルを示している。これほど広範囲の影響と関連し た環境ばく露はこれまでほとんどなかった。最も一般的な影響は、がんと母子の健康影響である。しか し、神経疾患や生殖器疾患など、他の疾患カテゴリーにも注目が集まっている。利用可能なデータが大 量にあり、利用可能な分析の数が多い(6,000件以上)にもかかわらず、研究された影響の大部分につ いて確固たる結論を出すことはできない。この調査は、この分野の研究量の多さを考慮すると、特に残 念な結果となった。しかし、この調査は環境疫学、特に農薬に関するこれまでの研究と一致しており、 疫学研究には多くの限界があり、データの不均一性から確固たる結論を出すことができないことを認 めている。

研究されている農薬のカテゴリーの範囲は広いが、研究は多くの場合、広く定義された農薬のカテ ゴリーに集中しており、集団がどの農薬にばく露されているかを理解するのは難しい。研究では、欧米 の集団や欧州連合ですでに禁止されている農薬を調査することが多い。ばく露評価の手段としてバイ オマーカーを使用することはほとんどないが、ほぼ半数の研究ではまだ使用可能である。さらに、コホ ート研究はこの文献の中では少数派であり、症例対照研究と横断研究が対象となる論文のほぼ同じ割 合を占めている。症例対照研究や横断研究では、時間的関係を研究することができないため、関連の因 果関係に関する裏付けを提供することができない。ばく露の評価は、おそらくこれらの研究の最も重 要な方法論的限界である。研究では、ばく露の評価と割り付けに異なる方法が用いられていた。ほとん どの研究では、農薬へのばく露を、使用したことがあるかないか、あるいは定期的に使用したことがあ るかないかという自己申告に基づいていた。このような方法は、高い誤分類率に悩まされ、特に後ろ向 き研究の場合には、病気のある参加者で報告されたばく露量が多いほど誤分類に差が出てしまう(リ コールバイアス)。とりわけ、このような質問紙は、非常に高いばく露量の被験者と非常に低いばく露 量の被験者を区別することができるかもしれないが、ばく露の段階にわたって有効なばく露分類がで きないため、用量反応関係の研究を行うことができない。また、幅広いカテゴリーの農薬や一般的に使 用されている農薬ではばく露の精度が高いかもしれないが、特定の農薬ではそうではない。ばく露評 価に使用される質問紙の妥当性が確認されていることが重要である。しかし、研究では、ほとんどの場 合、質問紙の「自己流」バージョンが使用されており、ばく露評価に使用された実際の質問紙に関する 情報が得られないことがある。さらに、複数の物質への同時ばく露は一般的であり、結果にさらなる偏 りが生じる可能性がある。例えば、農薬への職業ばく露は、ベンゼン、重金属、溶剤、浮遊粒子状物質 などへのばく露と共存している可能性が高い。真の関連を明らかにするためには、複数の物質へのば く露による交絡を考慮することが不可欠であるが、ここで評価されたエビデンスの圧倒的多数では、 これは不可能であった。

さらに、ここで収集されて評価されたエビデンスは、選択的な報告と多重検定に悩まされている可 能性が高い。研究は非常に広範囲の分析を報告しており、602件の論文で6000件の分析が行われていた。 多重仮説検定の量は膨大である。これらの分析は多重仮説検定のために調整する必要があり、そうし ないと結果は高い偽陽性率に悩まされることになる。研究が1つの分析しか行われていない場合でも、 他の疫学分野でも示されているように、選択的な報告が行われる可能性は常にある。さらに、結果を解 釈する際には、特に特定の影響(がんなど)については、エビデンスの大部分が単一の研究集団と特に AHSから得られていることも考慮に入れるべきである。

ばく露の定義以外に、臨床所見の定義は妥当な疫学研究においても大きなばらつきを示しており、 これが結果のばらつきの原因となっている。おそらく、このような状況で最も重要なのは、調査された 代替健康影響の使用であろう。ここでは非常に多くの代替健康影響が観察された。代替健康影響とは、 特定の臨床所見の代用または予測因子として一般的に受け入れられているバイオマーカーまたは身体 測定値である。しかし、多くの場合、これらの代替健康影響は検証されておらず、代替健康影響の厳密 な定義を満たしていない。このような影響は、臨床所見の予測因子として定義される可能性はあるが、 代替健康影響の基準を満たしていない。検証されていない代替健康影響に関するエビデンスは、検証 されていない代替健康影響の暗黙の想定を考慮に入れて評価されることが不可欠である。

これらの限界を認識した上で、我々は本報告書で検索されたエビデンスを要約することを試みた。 ここで追加された重要な制限は、このレビューが2006年以降の出版物に限定されているという事実で ある。これにより、最近のエビデンスのみをレビューすることが可能となり、メタアナリシスは利用可 能なすべてのエビデンスを含んでいるわけではないため、非常に慎重な解釈が必要となる。2006年以 降に発表されたデータがそれ以前のエビデンスと異なる場合、結果に偏りが生じる可能性がある。こ の目的のために、主要な影響及び2006年以降に発表された関連するメタアナリシスが確認されたもの については、更新されたメタアナリシスも提供した。これは小児白血病とパーキンソン病についての み可能である。これらの影響については、以前のエビデンスに沿って、農薬ばく露と疾患との間に有意 な関連があることがわかった。有意な要約推定値は他の影響についても報告されており、以下の表25 にまとめられている。しかし、これらは2006年以降の研究であるため、結果はあくまでも関連を示唆す るものであり、特にばく露の不均一性に関する限界を常に考慮に入れるべきである。

# 表 25:本報告書で実施されたメタアナリシスの要約

健康影響	研究数	メタアナリシス結果	$I^2$
白血病	6	1.26 (0.93, 1.71)	59.40%
ホジキンリンパ腫	7	1.29 (0.81, 2.06)	81.60%
小児白血病(妊娠期の農薬ばく露)	6	1.67 (1.25, 2.23)	81.20%
小児白血病(妊娠期の殺虫剤ばく露)	5	1.55 (1.14, 2.11)	65%
小児白血病(妊娠期の殺虫剤ばく露-ターナー2010 年に更 新)	9	1.69 (1.35, 2.11)	49.80%
小児白血病(妊娠期の不特定農薬ばく露)	5	2.00 (1.73, 2.30)	39.60%
小児白血病(妊娠期の不特定殺虫剤ばく露-ターナー2010 年に更新)	11	1.30 (1.09, 1.56)	26.50%
小児白血病(小児期の農薬ばく露)	7	1.27 (0.96, 1.69)	61.10%
小児白血病(小児期の殺虫剤ばく露-ターナー2010 年に更 新)	8	1.51 (1.28, 1.78)	0%
小児白血病(小児期の不特定殺虫剤ばく露-ターナー2010 年に更新)	11	1.36 (1.19, 1.55)	0%
乳がん(DDE ばく露)	5	1.13 (0.81, 1.57)	0%
乳がん	11	1.24 (1.08, 1.43)	0%
精巣がん(DDE ばく露)	5	1.40 (0.82, 2.39)	59.50%
胃がん	6	1.79 (1.30, 2.47)	0%
肝臓がん	5	2.50 (1.57, 3.98)	25.40%
停留精巣	8	1.19 (0.96, 1.49)	23.90%
停留精巣(DDT ばく露)	4	1.47 (0.98, 2.20)	51%
尿道下裂(一般的な農薬ばく露)	6	1.01 (0.74, 1.39)	71.50%
尿道下裂(特定の農薬ばく露)	9	1 (0.84, 1.18)	65.90%
流産	6	1.52 (1.09, 2.13)	63.10%
パーキンソン病	26	1.49 (1.28, 1.73)	54.60%
パーキンソン病(DDT ばく露)	5	1.01 (0.78, 1.30)	0%
パーキンソン病(パラコートばく露)	9	1.32 (1.09, 1.60)	34.10%
筋萎縮性側索硬化症	6	1.58 (1.31, 1.90)	10%
喘息 (DDT ばく露)	5	1.29 (1.14, 1.45)	0%
喘息(パラコートばく露)	6	1.40 (0.95, 2.06)	53.30%
喘息(クロルピリホスばく露)	5	1.03 (0.82, 1.28)	0%
1 型糖尿病 (DDE ばく露)	8	1.89 (1.25, 2.86)	49%
1 型糖尿病 (DDT ばく露)	6	1.76 (1.20, 2.59)	76.30%
2 型糖尿病(DDE ばく露)	4	1.29 (1.13, 1.48)	0%

## 推奨事項

上述したように、本報告書のために収集された広範なエビデンスは、疫学研究から得られる農薬ば く露と健康影響に関する膨大な量の情報があることを浮き彫りにしている。しかし、これらのエビデ ンスの質は通常低く、多くのバイアスが結果に影響を与え、確固とした結論を出すことができない可 能性が高い。小児がんとパーキンソン病は、2006年以降に対応するメタアナリシスが行われた2つの 影響であり、農薬ばく露に関連したリスクの増加を示すデータが一貫している。しかし、特定の農薬ク ラスや個々の農薬の影響を切り離すためには、ばく露の研究をさらに進める必要がある。内分泌疾患、 喘息、アレルギー、糖尿病、肥満などの他の影響への影響はリスクの増加を示しており、さらに調査が 必要である。本報告書では、健康影響を個別に調査することに集中した。別のアプローチとしては、農 薬のクラス、サブクラス、あるいは個々の農薬でさえも、さまざまな影響にわたって調べることであろ う。これらのアプローチにより、ある農薬クラスが様々な疾患エンドポイントにおいて特定の有害な 影響を及ぼすかどうかを明らかにすることができる。最後に、ばく露疫学は長い間、ばく露の測定と定 義に悩まされてきたが、特に農薬については、これは常に評価と定義が非常に困難であった。技術的な 進歩により、オミクスのハイスループット技術を用いて、大規模かつ断定的でない方法でばく露のバ イオマーカーを測定することが可能になった。例えば、メタボローム分析は、最小限の測定誤差とばく 露を特定する能力で、環境ばく露の全範囲を捕捉する方法を提供している。これらのアプローチは現 在開発が進められており、食事ばく露を含む環境ばく露と健康影響との関連について、より明確な見 解を提供してくれる可能性が高い。

## 参考文献

- ALLEN, M. T. & LEVY, L. S. 2013. Parkinson's disease and pesticide exposure--a new assessment. Crit Rev Toxicol, 43, 515-34.
- BAILEY, H. D., ARMSTRONG, B. K., DE KLERK, N. H., FRITSCHI, L., ATTIA, J., SCOTT, R. J., SMIBERT, E. & MILNE, E. 2011. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer, 129, 1678-88.
- BUDNIK, L. T., KLOTH, S., VELASCO-GARRIDO, M. & BAUR, X. 2012. Prostate cancer and toxicity from critical use exemptions of methyl bromide: environmental protection helps protect against human health risks. Environ Health, 11, 5.
- COOPER, G. S. & JONES, S. 2008. Pentachlorophenol and cancer risk: focusing the lens on specific chlorophenols and contaminants. Environ Health Perspect, 116, 1001-8.
- DICK, F. D. 2006. Parkinson's disease and pesticide exposures. Br Med Bull, 79-80, 219-31.
- GOVARTS, E., NIEUWENHUIJSEN, M., SCHOETERS, G., BALLESTER, F., BLOEMEN, K., DE BOER, M., CHEVRIER, C., EGGESBO, M., GUXENS, M., KRAMER, U., LEGLER, J., MARTINEZ, D., PALKOVICOVA, L., PATELAROU, E., RANFT, U., RAUTIO, A., PETERSEN, M. S., SLAMA, R., STIGUM, H., TOFT, G., TRNOVEC, T., VANDENTORREN, S., WEIHE, P., KUPERUS, N. W., WILHELM, M., WITTSIEPE, J. & BONDE, J. P. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect, 120, 162-70.
- IOANNIDIS, J. P., PATSOPOULOS, N. A. & EVANGELOU, E. 2007. Uncertainty in heterogeneity estimates in meta-analyses. BMJ, 335, 914-6.
- ISMAIL, A. A., BODNER, T. E. & ROHLMAN, D. S. 2012. Neurobehavioral performance among agricultural workers and pesticide applicators: a meta-analytic study. Occup Environ Med, 69, 457-64.
- KAMEL, F., UMBACH, D. M., BEDLACK, R. S., RICHARDS, M., WATSON, M., ALAVANJA, M. C., BLAIR, A., HOPPIN, J. A., SCHMIDT, S. & SANDLER, D. P. 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology, 33, 457-62.
- KHANJANI, N., HOVING, J. L., FORBES, A. B. & SIM, M. R. 2007. Systematic review and metaanalysis of cyclodiene insecticides and breast cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 25, 23-52.
- KOUREAS, M., TSAKALOF, A., TSATSAKIS, A. & HADJICHRISTODOULOU, C. 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett, 210, 155-68.
- LAU, J., IOANNIDIS, J. P. & SCHMID, C. H. 1997. Quantitative synthesis in systematic reviews. Ann Intern Med, 127, 820-6.
- LI, A. A., LOWE, K. A., MCINTOSH, L. J. & MINK, P. J. 2012. Evaluation of epidemiology and animal data for risk assessment: chlorpyrifos developmental neurobehavioral outcomes. J Toxicol Environ Health B Crit Rev, 15, 109-84.
- LOPEZ-CERVANTES, M., TORRES-SANCHEZ, L., TOBIAS, A. & LOPEZ-CARRILLO, L. 2004. Dichlorodiphenyldichloroethane burden and breast cancer risk: a meta-analysis of the epidemiologic evidence. Environ Health Perspect, 112, 207-14.

- MALEK, A. M., BARCHOWSKY, A., BOWSER, R., YOUK, A. & TALBOTT, E. O. 2012. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS. Environ Res, 117, 112-9.
- MERHI, M., RAYNAL, H., CAHUZAC, E., VINSON, F., CRAVEDI, J. P. & GAMET-PAYRASTRE, L. 2007. Occupational exposure to pesticides and risk of hematopoietic cancers: meta-analysis of case-control studies. Cancer Causes Control, 18, 1209-26.
- PEZZOLI, G. & CEREDA, E. 2013. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology, 80, 2035-41.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & PRIYADARSHI, S. S. 2001. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res, 86, 122-7.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & SHRIVASTAVA, S. 2000. A meta-analysis of Parkinson's disease and exposure to pesticides. Neurotoxicology, 21, 435-40.
- SAPHIR, A. 1998. Farmers and cancer: old crop of data gets new scrutiny. J Natl Cancer Inst, 90, 651-3.
- SHIRANGI, A., NIEUWENHUIJSEN, M., VIENNEAU, D. & HOLMAN, C. D. 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. Paediatr Perinat Epidemiol, 25, 172-91.
- SNIJDER, C. A., TE VELDE, E., ROELEVELD, N. & BURDORF, A. 2012. Occupational exposure to chemical substances and time to pregnancy: a systematic review. Hum Reprod Update, 18, 284-300.
- SUTEDJA, N. A., VELDINK, J. H., FISCHER, K., KROMHOUT, H., HEEDERIK, D., HUISMAN, M. H., WOKKE, J. H. & VAN DEN BERG, L. H. 2009. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph Lateral Scler, 10, 302-9.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Environ Health Perspect, 118, 33-41.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Cien Saude Colet, 16, 1915-31.
- VAN DER MARK, M., BROUWER, M., KROMHOUT, H., NIJSSEN, P., HUSS, A. & VERMEULEN, R. 2012. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect, 120, 340-7.
- VAN MAELE-FABRY, G., DUHAYON, S., MERTENS, C. & LISON, D. 2008. Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. Environ Res, 106, 121-37.
- VAN MAELE-FABRY, G., HOET, P., VILAIN, F. & LISON, D. 2012. Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. Environ Int, 46, 30-43.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2010. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control, 21, 787-809.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2011. Residential exposure to pesticides and childhood leukaemia: a systematic review and meta-analysis. Environ Int, 37, 280-91.
- VINSON, F., MERHI, M., BALDI, I., RAYNAL, H. & GAMET-PAYRASTRE, L. 2011. Exposure to

pesticides and risk of childhood cancer: a meta-analysis of recent epidemiological studies. Occup Environ Med, 68, 694-702.

WIGLE, D. T., TURNER, M. C. & KREWSKI, D. 2009. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ Health Perspect, 117, 1505-13.

## 付録

## 付録 I. MEDLINE における拡張検索アルゴリズム

Pesticid\* OR Pesticide OR pest control OR "pest control" OR (Chemosteril\* OR Chemosterilant OR Fungicid\* OR fungicide OR Fungicide, Industrial OR Herbicid\* OR Herbicide OR Defoliant\* OR Defoliant, Chemical OR Insect Repellent\*OR Insect Repellent OR Insecticid\* OR Insecticide OR Molluscacid\* OR Molluscacide OR Pesticide Synergist\* OR Pesticide Synergist OR Rodenticid\* OR Rodenticide OR organochlor\* OR organochloride OR organochlorine OR chlorocarbon OR chlorinated hydrocarbon OR chlorinated solvent OR organophosphat\* OR organophosphate OR carbamat\* OR carbamate OR pyrethroid\* OR pyrethroid) OR (1, 2-dibromo-3-chloropropane OR 1, 3- dichloro-1-propene OR 1-(4-ethynylphenyl)-4propyl-2, 6, 7-trioxabicyclo(2.2.2) octane OR 1-Methyl- 4-phenylpyridiniumOR 2, 4, 5-Trichlorophenoxyacetic Acid OR 2, 4-Dichlorophenoxyacetic AcidOR 2- dichlorobenzeneOR 2-Methyl-4-chlorophenoxyacetic Acid OR 2-methyl-4-chlorophenoxyacetic acid dicamba herbicide solution OR 2-phenylphenol OR 3, 5, 6-trichloro-2-pyridinolOR 4''-epiacetylamino- 4''deoxyavermectin B1 OR 4-dichlorobenzeneOR abamectin OR acephate OR acetochlor OR acifluorfen ORAgent OrangeOR alachlor OR Aldicarb OR Aldrin OR Allethrin OR allosamidin OR alpha-Chlorohydrin OR alpha-naphthyl thiourea OR alpha-naphthylphthalamic acid OR aluminum phosphide OR aminocarb OR amitrazOR AnabasineOR arsenic acidOR Atrazine OR avermectinOR azadirachtin OR AzinphosmethylOR Bacillus thuringiensis protoxinOR bendiocarbOR BenomylOR bentazoneOR benthiocarbOR benzyl benzoate OR bialaphos OR binB protein Bacillus sphaericus OR bioallethrinOR bioresmethrin OR bis(tri-n-butyltin)oxideOR boric acid OR bromacil OR bromadiolone OR bromfenacoumOR bullatacinOR butachlorOR butyl phosphorotrithioate OR Cacodylic Acid OR captafol OR CaptanOR Carbaryl OR Carbofuran OR CarboxinOR Chloranil OR ChlordanOR ChlordeconeOR Chlorfenvinphos OR chlorocresol OR chlorophacinoneOR ChlorphenamidineOR Chlorpropham OR Chlorpyrifos OR chlorsulfuronOR chlortoluronOR cismethrinOR closantel OR CoumaphosOR crotamiton OR cyanazine OR cyclonite OR cyfluthrinOR cyhalothrinOR cyhexatinOR cypermethrinOR cyromazineOR cythioateOR daminozideOR decamethrinOR DEETOR dexon (fungicide)OR diallyl trisulfideOR Diazinon OR Dicamba OR dichlobanilOR Dichlorodiphenyl DichloroethyleneOR DichlorodiphenyldichloroethaneOR dichlorodiphenyltrichloroethane OR DDT OR Dichlorvos OR Dicofol OR dieldrin OR difenacoumOR DimethoateOR dimethyl 4,4'-o-phenylene bis (3-thioallophanate) with carbamic acid ethylene bis (dithio)-mangenese zinc complexOR dimethyl 4-phthalateOR dimethyl phthalateOR Dinitrophenols OR dinosebOR diphenylOR DiquatOR DisulfotonOR DiuronOR doramectin OR EndosulfanOR EndrinOR ethionOR Ethylmercuric Chloride OR Ethylmercury Compounds OR famophos OR fenarimol OR FenitrothionOR fenoxycarb OR fenpropimorphOR Fenthion OR fenvalerate OR fipronil OR fluazifop OR fluazifop-butyl OR fluoroacetic acid OR fluphenacur OR fluridoneOR fluvalinate OR folpet OR FonofosOR glyphosateOR hedolit OR Hempa OR HeptachlorOR Heptachlor Epoxide OR heptenophosOR HexachlorobenzeneOR hexachlorobutadiene OR hexazinoneOR hydramethylnonOR imazalilOR imidaclopridOR insecticidal crystal protein Bacillus ThuringiensisOR iprodioneOR isofenphosOR isoproturonOR IvermectinOR jasplakinolideOR LeptophosOR linaloolOR LindaneOR Linuron ORmalachite greenOR malaoxonOR MalathionOR Maleic HydrazideOR mancozebOR ManebOR mecarzoleOR mecopropOR metalaxy1OR metaldehydeOR methamidophosOR methidathionOR MethiocarbOR MethomylOR MethoxychlorOR methyl demetonOR methyl isothiocyanateOR Methyl ParathionOR methylbromfenvinphosOR methyldithiocarbamateOR methyllycaconitineOR metolachlorOR metribuzinOR MevinphosOR milbemycinOR molinateOR MonocrotophosOR monomethylarsonic acidOR N, N- diethylphenylacetamide OR N-(3, 5dichlorophenyl) succinimideOR N-bromoacetamideOR n- hexanalOR Naled OR neem oilOR neosaxitoxinOR Niclosamide OR nitrofenOR nonachlor OR norbormideOR norflurazoneOR nornicotine OR octamethyl pyrophosphoramideOR oryzalinOR ParaoxonOR ParaquatOR ParathionOR pendimethalin OR pentachlorobenzeneOR PentachlorophenolOR PermethrinOR phenothrinOR phenthoateOR phentin acetate OR Phenylmercuric Acetate OR phenylmercuric nitrate, basicOR Phenylmercury CompoundsOR Phenylphosphonothioic Acid 2-Ethyl 2-(4-Nitrophenyl) EsterOR Phorate OR phosaloneOR PhosmetOR PhosphamidonOR phosphineOR phosphinothricinOR phoxim OR Picloram OR Piperonyl ButoxideOR pirimicarbOR pirimiphos methylOR precocene IIOR prochlorazOR procymidoneOR profenofosOR PrometryneOR propachlorOR PropanilOR PropoxurOR PyrethrinsOR pyriminil OR quinalphos OR quintozene OR RotenoneOR S, S'-(2-(dimethylamino)-1, 3-propanediyl)thiosulfuric acid ester OR SimazineOR sodium chlorateOR spinosadOR sulfamic acidOR sulfometuron methyl OR tebufenozideOR TemefosOR terbutryneOR terbutylazineOR terthienyl OR tetrachloroisophthalonitrileOR TetrachlorvinphosOR tetramethrinOR thallium sulfate OR ThiophanateOR ThiramOR ToxapheneOR triadimefon OR Triallate OR TrichlorfonOR triclopyrOR triflumuron OR Trifluralin OR vinclozolin OR Warfarin OR zinc phosphide OR Zineb OR Ziram)

(LIMITS: HUMAN, 1/1/2006 - 1/10/2012)

# 付録 II. データ抽出データベースの説明

Study ID	これは、研究の主要な影響ごとに順次与えられる研究の固有 ID で ある。
PUBMED _ID	これがその研究の PUBMED ID である(EMBASE の ID が提供されてい ない場合に PUBMED ID が提供され、PUBMED ID が提供されていない 場合は研究のタイトルが提供された)。
筆頭著者	筆頭著者の姓
ジャーナル	研究が掲載された雑誌
年	出版年
围	研究実施国
場所(大陸)	研究実施大陸
募集期間	研究参加者を募集した期間
ばく露期間(妊娠前、乳児期、小児期、成	農薬ばく露が発生した発育期(妊娠前、妊娠期、乳児期、小児
人期、妊娠期)	期、思春期、成人期)
追跡期間	前向き/後ろ向き研究の追跡予定期間
追跡期間(最大)	前向き/後ろ向き研究の最大追跡期間は年単位である。
追跡期間(年)(中央値/平均値)	前向き/後ろ向き研究の平均または中央値の追跡期間(年)。
研究の種類(コホート、コホート内症例対	疫学研究のデザイン:コホート、コホート内症例対照、症例対
照、症例対照、横断的)	照、横断的
コホート名	疫学研究の名称
年齢(年)(範囲/平均/中央値)	調査対象となった集団の年齢(平均年齢または中央値年齢を提示 することを好む。データは、別段の記載がない限り、年単位で表 示される。
性別(男性の割合)	調査対象集団における男性の割合
評価された有効成分	試験で定義・命名された試験で評価された農薬
有効成分のカテゴリー	農薬が分類されている化学的または機能的な農薬の分類
認可状況	EU 域内で認可された農薬有効成分(2013/09/06) Yes/No/NA (NA=該 当なし)
バイオマーカー名	農薬ばく露のバイオマーカー名(測定した場合)
対照定義	症例対照研究における対照群の定義
農薬の共ばく露(測定値)	その研究では、他の共ばく露農薬に関する情報を提供したか?(は い、いいえ)
集団の特徴	調査した集団の説明(性別、場所、病状)
ばく露の種類(職業的、環境的、両方)	農薬へのばく露源は何か:職業的(ばく露が特定の職業活動に関 連している場合);環境的(ばく露がいかなる職業活動にも関連し ていない場合(例えば、住居内での農薬使用、ガーデニングでの 農薬使用、ガーデニングに関連したばく露など);両方(職業ばく 露と環境ばく露の両方が存在する場合)。
ばく露評価の種類(直接ばく露についての 質問紙/バイオマーカー/居住履歴/職歴 /JEM/専門家評価/環境 odeling 誤植と 思われる	農薬ばく露の測定方法:直接ばく露についての質問紙(面接また は自己記入);体液中のバイオマーカーの測定;居住歴;職業歴; 職業ばく露マトリックス (JEM)。

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I Pesticide epidemiology

ばく露の定義	研究に記載されているばく露の定義
質問紙の種類	質問紙の種類(面接または自己管理)(質問紙を用いてばく露を評
	価した研究の場合、そうでない場合は該当なし)
バイオマーカーの測定(全血、血漿、尿、	バイオマーカーが測定された体液または組織(全血、血漿、尿、
母乳、胎盤、爪、毛髪、唾液、脂肪組織)	母乳、胎盤、爪、毛髪、唾液、脂肪組織など)
アッセイタイプ	バイオマーカー測定に使用した生化学的アッセイの種類
ばく露期間	年単位での農薬ばく露期間 (入手可能な場合)
小児ばく露タイプ(母、父、小児、組合わ	小児の影響に関する研究については、自己ばく露または親のばく
せ)	露(母親、父親、小児、組合わせ)を通じたばく露手段を記述
小児ばく露時期(妊娠前、妊娠期、両方)	小児の影響に関する研究では、親のばく露は妊娠前、妊娠期、ま
	たは両方の期間に行われたか?
健康影響	研究に記載されている通りの健康影響
影響の定義	研究で使用された健康影響の定義
疾患カテゴリ	疾患カテゴリ
効果推定タイプ(RR、OR、HR、 $\beta$ 、MD、	農薬と健康影響の関係を評価するための効果推定値の種類 (RR,
SMD)	OR, HR, $\beta$ , MD, SMD)
効果(2変数、連続変数)	2変数法または連続変数法で推定された効果(2変数、連続変数)
比較単位(はい/いいえ、単位増加、	効果量を算出するための比較の定義(はい/いいえ、単位増加な
	ど)
効果推定	効果推定値
SE/SD 効果推定	効果推定値の標準誤差・標準偏差
95% CI の下限	効果推定値の 95%信頼区間の下限
95% CI の上限	効果推定値の 95%信頼区間の上限
調整	効果推定値の交絡因子/変数の調整
マッチした対照	対照の変数を症例にマッチさせた (症例対照研究のみ)
サンプルサイズ	参加者総数
N 症例	症例数
N 対照	対照数
統計的手法	効果量の計算に用いられる統計的手法
試験デザイン(前向き、後ろ向き、混合、	研究デザインの前向き型または後ろ向き型(前向き、後ろ向き、
横断)	混合、横断)
包含/除外の基準の明記	研究参加者(母集団)の説明は詳細に行われていましたか?(はい
(はい、一部、いいえ)	/一部/いいえ)
著者による検出力の言及	著者らは、統計分析の前または後に論文で検出力について言及し
(はい、いいえ)	ているか?(はい/いいえ)
ばく露レベル(高、中、低)の詳細記述	農薬ばく露の定義でばく露レベル(高、中、低)の詳細記述
ばく露量の妥当な測定 :	ばく露量の測定は妥当であったか:バイオマーカー(はい);小規
(バイオマーカー(有);小規模区域の生態	模区域の生態学的測定、職種、質問紙(一部);大規模区域の生態
学的測定、職種、質問紙(一部);大規模区	学的測定に基づいた(いいえ)
域の生態学的測定に基づいた(無))	
ばく露量の測定は特定のものであったか?	ばく露量の測定は特定のものであったか?(はい);より広範で化
はい;より広範で化学的に関連したグルー プに其べく (一部)・タばな化学的及び書	学的に関連したグループに基づく(一部);多様な化学的及び毒性
プに基づく(一部);多様な化学的及び毒	学的特性の広範なグループ化に基づく(いいえ)

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性学的特性の広範なグループに基づく(い いえ)	
グループ間の配分のバランスを図る(層別 化、マッチングなど)	症例対照研究では、層別化またはマッチングによってグループ間 の配分のバランスをとる試みが行われたか? (はい/いいえ)
潜在的な交絡因子の調整を行った(はい、 いくつか、いいえ)	潜在的な交絡因子について効果量を調整したか? (はい、いくつ か、いいえ)
ばく露状況を盲検化された評価者(コホー ト研究の場合)	コホート研究では、評価者はばく露状況を盲検化されていたか? (はい/いいえ/;n/a:コホート研究でない場合は、成果なし、また は該当なし)
影響は、すべての研究参加者に一貫して実 施された有効かつ信頼性のある測定を用い て評価されたか?	影響は、すべての研究参加者に一貫して実施された有効で信頼性 のある測定を用いて評価されたか? (はい/いいえ)
サンプルサイズ(四分位数の上部[991]、 中間部、下部[104])	サンプルの大きさ
資金源の承認	著者は、資金調達の可能性があることを認めているか(はい/いい え)
大まかな品質評価	データ抽出フォームの品質評価の他のすべての列のデータを考慮 した大まかな品質評価
コメント	抽出されたデータの解釈に役立つ研究に関するコメント

# 付録 III. データ抽出データベースの参考文献

- A.S Al-Sarar, Y. Abo Bakr, G.S Al-Erimah, H.I Hussein, A.E Bayoumi. Hematological and biochemical alterations in occupationally pesticide-exposed workers of Riyadh municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology 3 (4) : 179-185, 2009 ISSN 1819-3420
- Abadi-Korek I, Stark B, Zaizov R, Shaham J. Parental occupational exposure and the risk of acute lymphoblastic leukemia in offspring in israel. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:165-174
- Abdel Rasoul GM, Abou Salem ME, Mechael AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. Neurotoxicology. 2008;29:833-838
- Abdelouahab N, Ainmelk Y, Takser L. Polybrominated diphenyl ethers and sperm quality. Reprod Toxicol. 2011;31:546-550
- Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, Spear PA, Chan HM. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of quebec (canada). Environmental research. 2008;107:380-392
- Abu Sham'a F, Skogstad M, Nijem K, Bjertness E, Kristensen P. Lung function and respiratory symptoms in male palestinian farmers. Arch Environ Occup Health. 2010;65:191-200
- Ahamed M, Anand M, Kumar A, Siddiqui MK. Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. Clinical biochemistry. 2006;39:762-766
- Ahrens W, Mambetova C, Bourdon-Raverdy N, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Zambon P. Occupational exposure to endocrine-disrupting compounds and biliary tract cancer among men. Scand J Work Environ Health. 2007;33:387-396
- Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. Diabetes care. 2011;34:1972-1979
- Al-Saleh I, Al-Doush I, Alsabbaheen A, Mohamed Gel D, Rabbah A. Levels of ddt and its metabolites in placenta, maternal and cord blood and their potential influence on neonatal anthropometric measures. The Science of the total environment. 2012;416:62-74
- Alavanja MCR, Sandler DP, Hoppin JA, Schroeder P, Lynch CF, Blair A, Mahajan R. Fonofos exposure and cancer incidence in the agricultural health study. Environmental Health Perspectives. 2006
- Albers JW, Garabrant DH, Mattsson JL, Burns CJ, Cohen SS, Sima C, Garrison RP, Richardson RJ, Berent S. Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. Toxicological sciences: an official journal of the Society of Toxicology. 2007;97:196-204

- Alderton LE, Spector LG, Blair CK, Roesler M, Olshan AF, Robison LL, Ross JA. Child and maternal household chemical exposure and the risk of acute leukemia in children with Down"s syndrome: A report from the children's oncology group. American journal of epidemiology. 2006;164:212-221
- Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009;43:7909-7915
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, Sunyer J. Thyroid disruption at birth due to prenatal exposure to betahexachlorocyclohexane. Environment international. 2008;34:737-740
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Grimalt JO, Sunyer J. Effects of pcbs, p,p'-ddt, p,p'-dde, hcb and beta-hch on thyroid function in preschool children. Occupational and environmental medicine. 2008;65:452-457
- Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jorgensen E, Kjaerstad MB, Baelum J, Nielsen JB, Skakkebaek NE, Main KM. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 2008;116:566-572
- Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, Silverman DT, Alavanja MC. Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort. International journal of cancer. Journal international du cancer. 2009;124:2495-2500
- Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental ddt exposure in young men living in a malaria area in the limpopo province, south africa. Journal of andrology. 2007;28:423-434
- Araoud M, Neffeti F, Douki W, Najjar MF, Kenani A. Paraoxonase 1 correlates with butyrylcholinesterase and gamma glutamyl transferase in workers chronically exposed to pesticides. Journal of occupational health. 2010;52:383-388
- Arcury TA, Feldman SR, Schulz MR, Vallejos Q, Verma A, Fleischer AB, Jr., Rapp SR, Davis SF, Preisser JS, Quandt SA. Diagnosed skin diseases among migrant farmworkers in North Carolina: Prevalence and risk factors. Journal of agricultural safety and health. 2007;13:407-418
- Arguelles LM, Liu X, Venners SA, Ronnenberg AG, Li Z, Yang F, Yang J, Xu X, Wang X. Serum folate and DDT isomers and metabolites are inversely associated in Chinese women: A cross-sectional analysis. Journal of the American College of Nutrition. 2009;28:380-387
- Aronson KJ, Wilson JW, Hamel M, Diarsvitri W, Fan W, Woolcott C, Heaton JP, Nickel JC, Macneily A, Morales A. Plasma organochlorine levels and prostate cancer risk. Journal of exposure science & environmental epidemiology. 2010;20:434-445
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of ddt and their association with reproductive hormones in adult men from northern thailand. The Science of the total environment. 2006;355:98-105
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand. Environment international. 2006;32:554-559

- Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, Schwarzschild MA, Thun MJ. Pesticide exposure and risk for Parkinson's disease. Annals of neurology. 2006;60:197-203
- Avivar Oyonarte C. , Duran Salas I. , Molina Arrebola M.A. , Castilla Alcala J.A. , Olea Serrano N. , Fernandez Cabrera M. Pesticide exposure and decreased sperm count. Revista del Laboratorio Clinico (2010) 3:1 (4-11).
- Axmon A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Zvyezday V, Ludwicki JK, Jonsson BA, Toft G, Bonde JP, Hagmar L. Time to pregnancy as a function of male and female serum concentrations of 2, 2'4, 4'5, 5'-hexachlorobiphenyl (cb-153) and 1, 1-dichloro-2, 2-bis (p-chlorophenyl)-ethylene (p, p'-dde). Hum Reprod. 2006;21:657-665
- Azmi MA, Naqvi SN, Akhtar K, Moinuddin, Parveen S, Parveen R, Aslam M. Effect of pesticide residues on health and blood parameters of farm workers from rural gadap, karachi, pakistan. Journal of environmental biology / Academy of Environmental Biology, India. 2009;30:747-756
- Baharuddin MR, Sahid IB, Noor MA, Sulaiman N, Othman F. Pesticide risk assessment: A study on inhalation and dermal exposure to 2,4-d and paraquat among malaysian paddy farmers. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:600-607
- Bahena-Medina LA, Torres-Sanchez L, Schnaas L, Cebrian ME, Chavez CH, Osorio-Valencia E, Hernandez RM, Lopez-Carrillo L. Neonatal neurodevelopment and prenatal exposure to dichlorodiphenyldichloroethylene (dde): A cohort study in Mexico. Journal of exposure science & environmental epidemiology. 2011;21:609-614
- Bailey HD, Armstrong BK, de Klerk NH, Fritschi L, Attia J, Scott RJ, Smibert E, Milne E. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. International journal of cancer. Journal international du cancer. 2011;129:1678-1688
- Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, Le ND. Prostate cancer risk and exposure to pesticides in British Columbia farmers. The Prostate. 2011;71:168-183
- Baranska M, Van Amelsvoort L, Birindelli S, Fustinoni S, Corsini E, Liesivuori J, Van Loveren H. Association of pesticide exposure, vaccination response, and interleukin-1 gene polymorphisms. Human & experimental toxicology. 2008;27:709-713
- Barczyk A, Sozanska E, Pierzchala W. [the influence of occupational exposure to pesticides on the frequency of chronic obstructive pulmonary diseases]. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2006;59:596-600
- Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in new jersey. The Science of the total environment. 2010;408:790-795
- Barry KH, Koutros S, Berndt SI, Andreotti G, Hoppin JA, Sandler DP, Burdette LA, Yeager M, Freeman LE, Lubin JH, Ma X, Zheng T, Alavanja MC. Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk. Environ Health Perspect. 2011;119:1726-1732
- Barry KH, Koutros S, Lubin JH, Coble JB, Barone-Adesi F, Beane Freeman LE, Sandler DP, Hoppin JA, Ma X, Zheng T, Alavanja MC. Methyl bromide exposure and cancer risk in the agricultural health study. Cancer causes & control: CCC. 2012;23:807-818

- Bayrami M, Hashemi T, Malekirad AA, Ashayeri H, Faraji F, Abdollahi M. Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. Toxicology and industrial health. 2012;28:90-96
- Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, Sandler DP, Kamel F. Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. Environ Health Perspect. 2011;119:1610-1615
- Behrens T, Lynge E, Cree I, Lutz JM, Eriksson M, Guenel P, Merletti F, Morales-Suarez-Varela M, Afonso N, Stengrevics A, Fevotte J, Sabroe S, Llopis-Gonzalez A, Gorini G, Hardell L, Stang A, Ahrens W. Pesticide exposure in farming and forestry and the risk of uveal melanoma. Cancer causes & control: CCC. 2012;23:141-151
- Beltrame D, Lo Cascio N, Miotto D, Mapp CE, De Rosa E, Boschetto P. [occupational exposure and chronic heart failure severity]. Giornale italiano di medicina del lavoro ed ergonomia. 2007;29:438-439
- Bergonzi R, De Palma G, Specchia C, Dinolfo M, Tomasi C, Frusca T, Apostoli P. Persistent organochlorine compounds in fetal and maternal tissues: Evaluation of their potential influence on several indicators of fetal growth and health. The Science of the total environment. 2011;409:2888-2893
- Bertrand KA, Spiegelman D, Aster JC, Altshul LM, Korrick SA, Rodig SJ, Zhang SM, Kurth T, Laden F. Plasma organochlorine levels and risk of non-hodgkin lymphoma in a cohort of men. Epidemiology. 2010;21:172-180
- Beseler C, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:1005-1013
- Beseler CL, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures among private pesticide applicators enrolled in the agricultural health study. Environ Health Perspect. 2008;116:1713-1719
- Bhalli JA, Khan QM, Haq MA, Khalid AM, Nasim A. Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis. 2006;21:143-148
- Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, Doody DR, Fish S, Needham LL, Chen C, Schwartz SM. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: A population-based case-control study. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:2012-2018
- Blanco-Munoz J, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Bassol S, Cebrian ME, Lopez-Flores I, Ruiz-Perez I. Effect of exposure to p,p'-dde on male hormone profile in mexican flower growers. Occupational and environmental medicine. 2012;69:5-11
- Blanco-Munoz J, Morales MM, Lacasana M, Aguilar-Garduno C, Bassol S, Cebrian ME. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of morelos, Mexico. Hum Reprod. 2010;25:1787-1795
- Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Cause-specific mortality of dutch chlorophenoxy herbicide manufacturing workers. Occupational and environmental medicine. 2010;67:24-31

- Boers D, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Campo L, Bosetti C, La Vecchia C, Vergieva T, Tarkowski M, Liesivuori J, Steerenberg P, van Loveren H. Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the europit field studies. Human & experimental toxicology. 2008;27:721-727
- Bonde JP, Toft G, Rylander L, Rignell-Hydbom A, Giwercman A, Spano M, Manicardi GC, Bizzaro D, Ludwicki JK, Zvyezday V, Bonefeld-Jorgensen EC, Pedersen HS, Jonsson BA, Thulstrup AM. Fertility and markers of male reproductive function in Inuit and European populations spanning large contrasts in blood levels of persistent organochlorines. Environ Health Perspect. 2008;116:269-277
- Bonner MR, Coble J, Blair A, Beane Freeman LE, Hoppin JA, Sandler DP, Alavanja MC. Malathion exposure and the incidence of cancer in the agricultural health study. American journal of epidemiology. 2007;166:1023-1034
- Bonner MR, Williams BA, Rusiecki JA, Blair A, Beane Freeman LE, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC. Occupational exposure to terbufos and the incidence of cancer in the agricultural health study. Cancer causes & control: CCC. 2010;21:871-877
- Bonvicini F, Marcello N, Mandrioli J, Pietrini V, Vinceti M. Exposure to pesticides and risk of amyotrophic lateral sclerosis: A population-based case-control study. Annali dell'Istituto superiore di sanita. 2010;46:284-287
- Borkowski WJ, Riederer A, Prapamontol T. Neurological evaluation of newborn infants of mothers working in citrus groves in northern Thailand. International journal of occupational and environmental health. 2011;17:135-143
- Bornman R, de Jager C, Worku Z, Farias P, Reif S. Ddt and urogenital malformations in newborn boys in a malarial area. BJU international. 2010;106:405-411
- Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics. 2010;125:e1270-1277
- Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr DB, Eskenazi B. Prenatal exposure to organophosphate pesticides and iq in 7-year-old children. Environ Health Perspect. 2011;119:1189-1195
- Bräuner EV, Sørensen M, Gaudreau E, LeBlanc A, Eriksen KT, Tjønneland A, Overvad K, Raaschou-Nielsen O. A prospective study of organochlorines in adipose tissue and risk of non-hodgkin lymphoma. Environmental Health Perspectives. 2011;120:105-111
- Brender JD, Felkner M, Suarez L, Canfield MA, Henry JP. Maternal pesticide exposure and neural tube defects in Mexican Americans. Annals of epidemiology. 2010;20:16-22
- Bretveld R, Zielhuis GA, Roeleveld N. Time to pregnancy among female greenhouse workers. Scandinavian Journal of Work, Environment & Health. 2006;32:359-367
- Bretveld RW, Hooiveld M, Zielhuis GA, Pellegrino A, van Rooij IA, Roeleveld N. Reproductive disorders among male and female greenhouse workers. Reprod Toxicol. 2008;25:107-114
- Brighina L, Frigerio R, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Farrer MJ, Lincoln SJ, Checkoway H, Rocca WA, Maraganore DM. Alpha-synuclein, pesticides, and Parkinson disease: A case-control study. Neurology. 2008;70:1461-1469

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Brooks K, Hasan H, Samineni S, Gangur V, Karmaus W. Placental p,p'dichlorodiphenyldichloroethylene and cord blood immune markers. Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2007;18:621-624
- Brouwers MM, Feitz WF, Roelofs LA, Kiemeney LA, de Gier RP, Roeleveld N. Risk factors for hypospadias. European journal of pediatrics. 2007;166:671-678
- Browne RO, Moyal-Segal LB, Zumsteg D, David Y, Kofman O, Berger A, Soreq H, Friedman A. Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2006;20:1733-1735
- Brucker-Davis F, Ducot B, Wagner-Mahler K, Tommasi C, Ferrari P, Pacini P, Boda-Buccino M, Bongain A, Azuar P, Fenichel P. [environmental pollutants in maternal milk and cryptorchidism]. Gynecologie, obstetrique & fertilite. 2008;36:840-847
- Brucker-Davis F, Ferrari P, Boda-Buccino M, Wagner-Mahler K, Pacini P, Gal J, Azuar P, Fenichel P. Cord blood thyroid tests in boys born with and without cryptorchidism: Correlations with birth parameters and in utero xenobiotics exposure. Thyroid: official journal of the American Thyroid Association. 2011;21:1133-1141
- Brucker-Davis F, Wagner-Mahler K, Bornebusch L, Delattre I, Ferrari P, Gal J, Boda-Buccino M, Pacini P, Tommasi C, Azuar P, Bongain A, Fenichel P. Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from nice area (france). Chemosphere. 2010;81:169-176
- Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A, Kurzenne JY, Mas JC, Fenichel P. Cryptorchidism at birth in nice area (France) is associated with higher prenatal exposure to pcbs and dde, as assessed by colostrum concentrations. Hum Reprod. 2008;23:1708-1718
- Brulls C., Niggemann H., Weissbach W., Dott W., Fischer M., Merk H.F., Blomeke B., Isselstein J., Ilgner, Westhofen M., Wiesmuller G.A.. Pilot study on living conditions and living factors investigated in patients suffering from self-reported multiple chemical sensitivity, fragrance allergies or polyposis nasi. Atemwegs- und Lungenkrankheiten (2008) 34:5 (187-198)
- Buck Louis GM, Rios LI, McLain A, Cooney MA, Kostyniak PJ, Sundaram R. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere. 2011;85:1742-1748
- Burdorf A, Brand T, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA. The effects of workrelated maternal risk factors on time to pregnancy, preterm birth and birth weight: The generation r study. Occupational and environmental medicine. 2011;68:197-204
- Burns JS, Williams PL, Sergeyev O, Korrick SA, Lee MM, Revich B, Altshul L, Del Prato JT, Humblet O, Patterson DG, Turner WE, Starovoytov M, Hauser R. Serum concentrations of organochlorine pesticides and growth among russian boys. Environ Health Perspect. 2012;120:303-308
- Bustamante Montes LP, Waliszewski S, Hernandez-Valero M, Sanin-Aguirre L, Infanzon-Ruiz RM, Janas AG. [prenatal exposure to organochlorine pesticides and cryptorchidism]. Ciencia & saude coletiva. 2010;15 Suppl 1:1169-1174

- Carbone P, Giordano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamanca I. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural Sicily. International journal of andrology. 2007;30:3-13
- Carmichael SL, Herring AH, Sjodin A, Jones R, Needham L, Ma C, Ding K, Shaw GM. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80:641-646
- Carozza SE, Li B, Wang Q, Horel S, Cooper S. Agricultural pesticides and risk of childhood cancers. International journal of hygiene and environmental health. 2009;212:186-195
- Cha ES, Lee YK, Moon EK, Kim YB, Lee YJ, Jeong WC, Cho EY, Lee IJ, Hur J, Ha M, Lee WJ. Paraquat application and respiratory health effects among South Korean farmers. Occupational and environmental medicine. 2012;69:398-403
- Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in india. Journal of occupational health. 2009;51:488-497
- Chang CK, Astrakianakis G, Thomas DB, Seixas NS, Ray RM, Gao DL, Wernli KJ, Fitzgibbons ED, Vaughan TL, Checkoway H. Occupational exposures and risks of liver cancer among shanghai female textile workers--a case-cohort study. International journal of epidemiology. 2006;35:361-369
- Chang YL, Li J, Yao SQ, Hu WN, Jiang SF, Guo Z, Yang L, Li DD, Li YM, Liu Y. [a casecontrol study on serum organochlorines residues, genetic polymorphisms of glutathione s- transferase t1 and the risks of breast cancer]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2008;29:763-766
- Charles LE, Burchfiel CM, Fekedulegn D, Gu JK, Petrovitch H, Sanderson WT, Masaki K, Rodriguez BL, Andrew ME, Ross GW. Occupational exposure to pesticides, metals, and solvents: The impact on mortality rates in the Honolulu heart program. Work. 2010;37:205-215
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational exposures and movement abnormalities among japanese-american men: The honolulu-asia aging study. Neuroepidemiology. 2006;26:130-139
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Sanderson WT, Petrovitch H. Occupational and other risk factors for hand-grip strength: The honolulu-asia aging study. Occupational and environmental medicine. 2006;63:820-827
- Chatzi L, Alegakis A, Kruger-Krasagakis S, Lionis C. Skin symptoms and work-related skin symptoms among grape farmers in crete, greece. American journal of industrial medicine. 2006;49:77-84
- Chatzi L, Alegakis A, Tzanakis N, Siafakas N, Kogevinas M, Lionis C. Association of allergic rhinitis with pesticide use among grape farmers in crete, greece. Occupational and environmental medicine. 2007;64:417-421
- Chen SC, Wong RH, Shiu LJ, Chiou MC, Lee H. Exposure to mosquito coil smoke may be a risk factor for lung cancer in Taiwan. Journal of epidemiology / Japan Epidemiological Association. 2008;18:19-25

- Chen Z, Robison L, Giller R, Krailo M, Davis M, Davies S, Shu XO. Environmental exposure to residential pesticides, chemicals, dusts, fumes, and metals, and risk of childhood germ cell tumors. International journal of hygiene and environmental health. 2006;209:31-40
- Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, Durand G, Cordier S. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the pelagie birth cohort. Environ Health Perspect. 2011;119:1034-1041
- Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008;168:298-310
- Chitra GA, Muraleedharan VR, Swaminathan T, Veeraraghavan D. Use of pesticides and its impact on health of farmers in south india. International journal of occupational and environmental health. 2006;12:228-233
- Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-hodgkin lymphoma. Blood. 2006;108:1363-1369
- Christensen CH, Platz EA, Andreotti G, Blair A, Hoppin JA, Koutros S, Lynch CF, Sandler DP, Alavanja MC. Coumaphos exposure and incident cancer among male participants in the agricultural health study (ahs). Environ Health Perspect. 2010;118:92-96
- Cocco P, Brennan P, Ibba A, de Sanjose Llongueras S, Maynadie M, Nieters A, Becker N, Ennas MG, Tocco MG, Boffetta P. Plasma polychlorobiphenyl and organochlorine pesticide level and risk of major lymphoma subtypes. Occupational and environmental medicine. 2008;65:132-140
- Cockburn M, Mills P, Zhang X, Zadnick J, Goldberg D, Ritz B. Prostate cancer and ambient pesticide exposure in agriculturally intensive areas in california. American journal of epidemiology. 2011;173:1280-1288
- Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environ Health Perspect. 2007;115:1442-1447
- Cohn BA, Cirillo PM, Christianson RE. Prenatal ddt exposure and testicular cancer: A nested case-control study. Arch Environ Occup Health. 2010;65:127-134
- Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. Ddt and breast cancer in young women: New data on the significance of age at exposure. Environ Health Perspect. 2007;115:1406-1414
- Cole DC, Wainman B, Sanin LH, Weber JP, Muggah H, Ibrahim S. Environmental contaminant levels and fecundability among non-smoking couples. Reprod Toxicol. 2006;22:13-19
- Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2009;51:1212-1219
- Colt JS, Davis S, Severson RK, Lynch CF, Cozen W, Camann D, Engels EA, Blair A, Hartge P. Residential insecticide use and risk of non-hodgkin's lymphoma. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:251-257

- Colt JS, Rothman N, Severson RK, Hartge P, Cerhan JR, Chatterjee N, Cozen W, Morton LM, De Roos AJ, Davis S, Chanock S, Wang SS. Organochlorine exposure, immune gene variation, and risk of non-hodgkin lymphoma. Blood. 2009;113:1899-1905
- Cooney MA, Buck Louis GM, Hediger ML, Vexler A, Kostyniak PJ. Organochlorine pesticides and endometriosis. Reprod Toxicol. 2010;30:365-369
- Cooney MA, Daniels JL, Ross JA, Breslow NE, Pollock BH, Olshan AF. Household pesticides and the risk of wilms tumor. Environmental Health Perspectives. 2006;115:134-137
- Cooper GS, Parks CG, Schur PS, Fraser PA. Occupational and environmental associations with antinuclear antibodies in a general population sample. Journal of toxicology and environmental health. Part A. 2006;69:2063-2069
- Cornelis C, Schoeters G, Kellen E, Buntinx F, Zeegers M. Development of a gis-based indicator for environmental pesticide exposure and its application to a belgian case-control study on bladder cancer. International journal of hygiene and environmental health. 2009;212:172-185
- Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of california. American journal of epidemiology. 2009;169:919-926
- Cote S, Ayotte P, Dodin S, Blanchet C, Mulvad G, Petersen HS, Gingras S, Dewailly E. Plasma organochlorine concentrations and bone ultrasound measurements: A crosssectional study in peri-and postmenopausal inuit women from greenland. Environmental health: a global access science source. 2006;5:33
- Cox S, Niskar AS, Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. Environ Health Perspect. 2007;115:1747-1752
- Crawford JM, Hoppin JA, Alavanja MC, Blair A, Sandler DP, Kamel F. Hearing loss among licensed pesticide applicators in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:817-826
- Cupul-Uicab LA, Gladen BC, Hernandez-Avila M, Weber JP, Longnecker MP. Dde, a degradation product of ddt, and duration of lactation in a highly exposed area of mexico. Environ Health Perspect. 2008;116:179-183
- Cupul-Uicab LA, Hernandez-Avila M, Terrazas-Medina EA, Pennell ML, Longnecker MP. Prenatal exposure to the major ddt metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) and growth in boys from mexico. Environmental research. 2010;110:595-603
- Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberte C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in quebec, canada. Environmental research. 2008;108:387-392
- Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in inuit adults. Environ Health Perspect. 2009;117:1380-1386

- Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect. 2009;117:1014-1020
- Dallaire R, Muckle G, Rouget F, Kadhel P, Bataille H, Guldner L, Seurin S, Chajes V, Monfort C, Boucher O, Thome JP, Jacobson SW, Multigner L, Cordier S. Cognitive, visual, and motor development of 7-month-old guadeloupean infants exposed to chlordecone. Environmental research. 2012;118:79-85
- Damgaard IN, Skakkebæk NE, Toppari J, Virtanen HE, Shen H, Schramm K-W, Petersen JH, Jensen TK, Main KM. Persistent pesticides in human breast milk and cryptorchidism. Environmental Health Perspectives. 2006;114:1133-1138
- Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. Pop levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from uppsala, sweden. Environment international. 2010;36:180-187
- Dassanayake T, Gawarammana IB, Weerasinghe V, Dissanayake PS, Pragaash S, Dawson A, Senanayake N. Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2009;120:1693-1698
- Dayton SB, Sandler DP, Blair A, Alavanja M, Beane Freeman LE, Hoppin JA. Pesticide use and myocardial infarction incidence among farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2010;52:693-697
- De Fleurian G, Perrin J, Ecochard R, Dantony E, Lanteaume A, Achard V, Grillo JM, Guichaoua MR, Botta A, Sari-Minodier I. Occupational exposures obtained by questionnaire in clinical practice and their association with semen quality. Journal of andrology. 2009;30:566-579
- de Jager C, Aneck-Hahn NH, Bornman MS, Farias P, Leter G, Eleuteri P, Rescia M, Spano M. Sperm chromatin integrity in ddt-exposed young men living in a malaria area in the limpopo province, south africa. Hum Reprod. 2009;24:2429-2438
- De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, Dombrowski C, Rousseau F, Sanchez VD, Bailey JL. Reduced seminal parameters associated with environmental ddt exposure and p,p'-dde concentrations in men in chiapas, mexico: A cross- sectional study. Journal of andrology. 2006;27:16-27
- de Souza A, Medeiros Ados R, de Souza AC, Wink M, Siqueira IR, Ferreira MB, Fernandes L, Loayza Hidalgo MP, Torres IL. [evaluation of the impact of exposure to pesticides on the health of the rural population: Vale do taquari, state of rio grande do sul (brazil)]. Ciencia & saude coletiva. 2011;16:3519-3528
- Delancey JO, Alavanja MC, Coble J, Blair A, Hoppin JA, Austin HD, Beane Freeman LE. Occupational exposure to metribuzin and the incidence of cancer in the agricultural health study. Annals of epidemiology. 2009;19:388-395
- Delport R, Bornman R, MacIntyre UE, Oosthuizen NM, Becker PJ, Aneck-Hahn NH, de Jager C. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to ddt. Environ Health Perspect. 2011;119:647-651
- Demers PA, Davies HW, Friesen MC, Hertzman C, Ostry A, Hershler R, Teschke K. Cancer and occupational exposure to pentachlorophenol and tetrachlorophenol (canada). Cancer causes & control: CCC. 2006;17:749-758

- Den Hond E, Dhooge W, Bruckers L, Schoeters G, Nelen V, van de Mieroop E, Koppen G, Bilau M, Schroijen C, Keune H, Baeyens W, van Larebeke N. Internal exposure to pollutants and sexual maturation in flemish adolescents. Journal of exposure science & environmental epidemiology. 2011;21:224-233
- Deng F, Tao FB, Liu DY, Xu YY, Hao JH, Sun Y, Su PY. Effects of growth environments and two environmental endocrine disruptors on children with idiopathic precocious puberty. European journal of endocrinology / European Federation of Endocrine Societies. 2012;166:803-809
- Dennis LK, Lowe JB, Lynch CF, Alavanja MC. Cutaneous melanoma and obesity in the agricultural health study. Annals of epidemiology. 2008;18:214-221
- Dennis LK, Lynch CF, Sandler DP, Alavanja MC. Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. Environ Health Perspect. 2010;118:812- 817
- Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, Shepherd S. Pesticide/environmental exposures and parkinson's disease in east texas. Journal of agromedicine. 2008;13:37-48
- Dhooge W, Den Hond E, Koppen G, Bruckers L, Nelen V, Van De Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, Van Larebeke N. Internal exposure to pollutants and body size in flemish adolescents and adults: Associations and dose-response relationships. Environment international. 2010;36:330-337
- Dhooge W, den Hond E, Koppen G, Bruckers L, Nelen V, van de Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, van Larebeke N. Internal exposure to pollutants and sex hormone levels in flemish male adolescents in a cross-sectional study: Associations and dose-response relationships. Journal of exposure science & environmental epidemiology. 2011;21:106-113
- Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, Semple S, Dick S, Counsell C, Mozzoni P, Haites N, Wettinger SB, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A. Environmental risk factors for Parkinson's disease and Parkinsonism: The geoparkinson study. Occupational and environmental medicine. 2007;64:666-672
- Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, Mertens I, Van Gaal L. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring). 2011;19:709-714
- Djordjevic M, Sazdanovic P, Djordjevic G, Jovanovic B. Morbidity in newborns exposed to organophosphorus pesticides. Medicinski pregled. 2010;63:414-417
- Dugas J, Nieuwenhuijsen MJ, Martinez D, Iszatt N, Nelson P, Elliott P. Use of biocides and insect repellents and risk of hypospadias. Occupational and environmental medicine. 2010;67:196-200
- Duk-Hee Lee, Michael W. Steffes, Andreas Sjodin, Richard S. Jones, Larry L. Needham, David R., Jacobs Jr. Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. PLoS ONE 2011 ; 6(1): e15977

- Eckerman DA, Gimenes LS, de Souza RC, Galvao PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in brazil. Neurotoxicology and teratology. 2007;29:164-175
- Eggesbo M, Stigum H, Longnecker MP, Polder A, Aldrin M, Basso O, Thomsen C, Skaare JU, Becher G, Magnus P. Levels of hexachlorobenzene (hcb) in breast milk in relation to birth weight in a norwegian cohort. Environmental research. 2009;109:559-566
- El-Helaly M, Abdel-Elah K, Haussein A, Shalaby H. Paternal occupational exposures and the risk of congenital malformations--a case-control study. International journal of occupational medicine and environmental health. 2011;24:218-227
- Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, Alperovitch A, Tzourio C. Professional exposure to pesticides and parkinson disease. Annals of neurology. 2009;66:494-504
- Elobeid MA, Padilla MA, Brock DW, Ruden DM, Allison DB. Endocrine disruptors and obesity: An examination of selected persistent organic pollutants in the nhanes 1999-2002 data. International journal of environmental research and public health. 2010;7:2988-3005
- Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal organophosphate metabolite and organochlorine levels and performance on the brazelton neonatal behavioral assessment scale in a multiethnic pregnancy cohort. American journal of epidemiology. 2007;165:1397-1404
- Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 2011;119:1182-1188
- English RG, Perry M, Lee MM, Hoffman E, Delport S, Dalvie MA. Farm residence and reproductive health among boys in rural South Africa. Environment international. 2012;47:73-79
- Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for nonhodgkin lymphoma including histopathological subgroup analysis. International journal of cancer. Journal international du cancer. 2008;123:1657-1663
- Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, Jewell NP. In utero exposure to dichlorodiphenyltrichloroethane (ddt) and dichlorodiphenyldichloroethylene (dde) and neurodevelopment among young mexican american children. Pediatrics. 2006;118:233-241
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate pesticide exposure and neurodevelopment in young mexican-american children. Environ Health Perspect. 2007;115:792-798
- Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Jr., Mainous AG, 3rd. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and ddt with diabetes in the 1999-2002 national health and nutrition examination survey. Environmental research. 2007;103:413-418
- Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination survey. Environment international. 2010;36:398-401

F. Giordano, V. Dell'Orco, G. Galante, F. Giannandrea,

- Fang F, Quinlan P, Ye W, Barber MK, Umbach DM, Sandler DP, Kamel F. Workplace exposures and the risk of amyotrophic lateral sclerosis. Environ Health Perspect. 2009;117:1387-1392
- Farooq U, Joshi M, Nookala V, Cheriyath P, Fischman D, Graber NJ, Stellman SD, Muscat J. Self-reported exposure to pesticides in residential settings and risk of breast cancer: A case- control study. Environmental health : a global access science source. 2010;9:30
- Farr SL, Cai J, Savitz DA, Sandler DP, Hoppin JA, Cooper GS. Pesticide exposure and timing of menopause: The agricultural health study. American journal of epidemiology. 2006;163:731-742
- Fatemeh Tohidia, Farzaneh Farrokhib, Ali Taravatic. Effects of pesticide on the thyroid hormones of pesticide sprayers living in Mazandaran. Abstracts. doi:10.1016/j.clinbiochem.2011.08.1063
- Fear NT, Hey K, Vincent T, Murphy M. Paternal occupation and neural tube defects: A casecontrol study based on the oxford record linkage study register. Paediatric and perinatal epidemiology. 2007;21:163-168
- Fear NT, Vincent TJ, King JC, MacCarthy A, Bunch KJ, Murphy MF. Wilms tumour and paternal occupation: An analysis of data from the national registry of childhood tumours. Pediatric blood & cancer. 2009;53:28-32
- Feldman AL, Johansson AL, Nise G, Gatz M, Pedersen NL, Wirdefeldt K. Occupational exposure in parkinsonian disorders: A 43-year prospective cohort study in men. Parkinsonism & related disorders. 2011;17:677-682
- Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: Results of a case- control study. Birth defects research. Part A, Clinical and molecular teratology. 2008;82:98-105
- Feng Hong qi, Yang Lin, Guo Ling, Huang Wen li, Xu Bo nan, LI Zhao Xiang, Wang Tong. A case control study on the risk factors of Yunnan endemic sudden cardiac death. Chin J Endemiol Jul 20 2005 Vol 24 No.4 ; 1000-4955 2005 04-0414-03
- Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, Hubbard A, Barr DB. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environmental Health Perspectives. 2005;114:597-602
- Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. In utero exposure to ddt and performance on the brazelton neonatal behavioral assessment scale. Neurotoxicology. 2007;28:471-477
- Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p'-dde, hcb, pcbs and reproductive hormones among men of reproductive age. Reprod Toxicol. 2012;34:429-435
- Fernandez MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez JM, Cruz M, Olea-Serrano F, Olea N. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: A nested casecontrol study. Environ Health Perspect. 2007;115 Suppl 1:8-14

- Fieten KB, Kromhout H, Heederik D, van Wendel de Joode B. Pesticide exposure and respiratory health of indigenous women in costa rica. American journal of epidemiology. 2009;169:1500-1506
- Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Jr., Checkoway H. Occupational factors and risk of Parkinson's disease: A population- based case-control study. American journal of industrial medicine. 2010;53:217-223
- Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, Cheng CW. Pesticide exposure on southwestern Taiwanese with mnsod and nqo1 polymorphisms is associated with increased risk of parkinson's disease. Clinica chimica acta; international journal of clinical chemistry. 2007;378:136-141
- Fortes C, Mastroeni S, Melchi F, Pilla MA, Alotto M, Antonelli G, Camaione D, Bolli S, Luchetti E, Pasquini P. The association between residential pesticide use and cutaneous melanoma. Eur J Cancer. 2007;43:1066-1075
- Freeman LE, Rusiecki JA, Hoppin JA, Lubin JH, Koutros S, Andreotti G, Zahm SH, Hines CJ, Coble JB, Barone-Adesi F, Sloan J, Sandler DP, Blair A, Alavanja MC. Atrazine and cancer incidence among pesticide applicators in the agricultural health study (1994-2007). Environ Health Perspect. 2011;119:1253-1259
- Freire C, Lopez-Espinosa MJ, Fernandez M, Molina-Molina JM, Prada R, Olea N. Prenatal exposure to organochlorine pesticides and tsh status in newborns from southern spain. The Science of the total environment. 2011;409:3281-3287
- Freire C, Ramos R, Amaya E, Fernandez MF, Santiago-Fernandez P, Lopez-Espinosa MJ, Arrebola JP, Olea N. Newborn tsh concentration and its association with cognitive development in healthy boys. European journal of endocrinology / European Federation of Endocrine Societies. 2010;163:901-909
- Friesen MC, Davies HW, Teschke K, Ostry AS, Hertzman C, Demers PA. Impact of the specificity of the exposure metric on exposure-response relationships. Epidemiology. 2007;18:88-94
- Frigerio R, Sanft KR, Grossardt BR, Peterson BJ, Elbaz A, Bower JH, Ahlskog JE, de Andrade M, Maraganore DM, Rocca WA. Chemical exposures and Parkinson"s disease: A population-based case-control study. Movement disorders: official journal of the Movement Disorder Society. 2006;21:1688-1692
- Fritschi L, Glass DC, Tabrizi JS, Leavy JE, Ambrosini GL. Occupational risk factors for prostate cancer and benign prostatic hyperplasia: A case-control study in Western Australia. Occupational and environmental medicine. 2007;64:60-65
- Gabel P, Jensen MS, Andersen HR, Baelum J, Thulstrup AM, Bonde JP, Toft G. The risk of cryptorchidism among sons of women working in horticulture in Denmark: A cohort study. Environmental health: a global access science source. 2011;10:100
- Gallagher RP, Macarthur AC, Lee TK, Weber JP, Leblanc A, Mark Elwood J, Borugian M, Abanto Z, Spinelli JJ. Plasma levels of polychlorinated biphenyls and risk of cutaneous malignant melanoma: A preliminary study. International journal of cancer. Journal international du cancer. 2011;128:1872-1880
- Ganesh B, Sushama S, Monika S, Suvarna P. A case-control study of risk factors for lung cancer in mumbai, India. Asian Pacific journal of cancer prevention: APJCP. 2011;12:357-362

- Garced S, Torres-Sanchez L, Cebrian ME, Claudio L, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and child growth during the first year of life. Environmental research. 2012;113:58-62
- Gascon M, Vrijheid M, Martinez D, Ballester F, Basterrechea M, Blarduni E, Esplugues A, Vizcaino E, Grimalt JO, Morales E, Sunyer J. Pre-natal exposure to dichlorodiphenyldichloroethylene and infant lower respiratory tract infections and wheeze. The European respiratory journal. 2012;39:1188-1196
- Gaspari L, Paris F, Jandel C, Kalfa N, Orsini M, Daures JP, Sultan C. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: A nested case-control study. Hum Reprod. 2011;26:3155-3162
- Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B. Well-water consumption and parkinson's disease in rural california. Environ Health Perspect. 2009;117:1912-1918
- Gatto NM, Longnecker MP, Press MF, Sullivan-Halley J, McKean-Cowdin R, Bernstein L. Serum organochlorines and breast cancer: A case-control study among african-american women. Cancer causes & control: CCC. 2007;18:29-39
- German D, Roy A, Shalat S, Buckley B, Gearing M, Levey A, Richardson J. A ddt metabolite is elevated in the serum of alzheimer's disease patients. Alzheimer's & Dementia. 2012;8:P496
- Gian S. Jhangri, Colin L. Soskolne, Giovanni Pagano, Gerardo Botte, Patrizia Di Cintio. Alcohol and tobacco variables in the assessment of internal validity in an
- Giannandrea F, Gandini L, Paoli D, Turci R, Figa-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:780-787
- Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figa-Talamanca I. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth defects research. Part A, Clinical and molecular teratology. 2010;88:241-250
- Giordano F, Dell'Orco V, Giannandrea F, Lauria L, Valente P, Figa-Talamanca I. Mortality in a cohort of pesticide applicators in an urban setting: Sixty years of followup. International journal of immunopathology and pharmacology. 2006;19:61-65
- Giwercman A, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, Lindh C, Pedersen HS, Ludwicki JK, Lesovoy V, Shvets M, Spano M, Manicardi GC, Bizzaro D, Bonefeld- Jorgensen EC, Bonde JP. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three european cohorts. Environmental Health Perspectives. 2006;114:1348-1353
- Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S. Immune cell counts and risks of respiratory infections among infants exposed preand postnatally to organochlorine compounds: A prospective study. Environmental health : a global access science source. 2008;7:62
- Gold LS, Ward MH, Dosemeci M, De Roos AJ. Systemic autoimmune disease mortality and occupational exposures. Arthritis and rheumatism. 2007;56:3189-3201

- Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the agricultural health study. American journal of epidemiology. 2010;171:455-464
- Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of pcb congeners and chlorinated pesticides. Environ Health Perspect. 2011;119:319-325
- Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, Morse G, Carpenter DO. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native american men. Environ Health Perspect. 2009;117:1454-1460
- Grandjean P, Harari R, Barr DB, Debes F. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in ecuadorian school children. Pediatrics. 2006;117:e546-556
- Greenburg DL, Rusiecki J, Koutros S, Dosemeci M, Patel R, Hines CJ, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to captan in the agricultural health study. Cancer causes & control : CCC. 2008;19:1401-1407
- Guillette EA, Conard C, Lares F, Aguilar MG, McLachlan J, Guillette LJ. Altered breast development in young girls from an agricultural environment. Environmental Health Perspectives. 2005;114:471-475
- Gunnar Toft, Allan Flyvbjerg and Jens Peter Bonde. A Global Access Science Source. Enviromental Health 2006; 5: 32 32
- Guodong D, Pei W, Ying T, Jun Z, Yu G, Xiaojin W, Rong S, Guoquan W, Xiaoming S. Organophosphate pesticide exposure and neurodevelopment in young shanghai children. Environmental science & technology. 2012;46:2911-2917
- H Yu, X Liu, K Kezios, O Kalantzi, YWang, M Petreas, J-S Park, P Cirillio, B Cohn, P Factor- Litvak. Prenatal organochlorine exposure maternal thyroid function and neuromotor development. Am J Epidemiol. 2011;173(Suppl):S1-S316
- Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the national health and nutrition examination survey, 1999-2002. Environ Health Perspect. 2007;115:1204-1209
- Ha MH, Lee DH, Son HK, Park SK, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999-2002. Journal of human hypertension. 2009;23:274-286
- Hadjigeorgiou GM, Stefanidis I, Dardiotis E, Aggellakis K, Sakkas GK, Xiromerisiou G, Konitsiotis S, Paterakis K, Poultsidi A, Tsimourtou V, Ralli S, Gourgoulianis K, Zintzaras
- E. Low rls prevalence and awareness in central greece: An epidemiological survey. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2007;14:1275-1280
- Han Y, Xia Y, Han J, Zhou J, Wang S, Zhu P, Zhao R, Jin N, Song L, Wang X. The relationship of 3-pba pyrethroids metabolite and male reproductive hormones among nonoccupational exposure males. Chemosphere. 2008;72:785-790

- Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of parkinson's disease: A family-based casecontrol study. BMC neurology. 2008;8:6
- Handal AJ, Harlow SD, Breilh J, Lozoff B. Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. Epidemiology. 2008;19:851-859
- Handal AJ, Lozoff B, Breilh J, Harlow SD. Neurobehavioral development in children with potential exposure to pesticides. Epidemiology. 2007;18:312-320
- Harari R, Julvez J, Murata K, Barr D, Bellinger DC, Debes F, Grandjean P. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect. 2010;118:890-896
- Hardell L, Andersson SO, Carlberg M, Bohr L, van Bavel B, Lindstrom G, Bjornfoth H, Ginman C. Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:700-707
- Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. International journal of andrology. 2006;29:228-234
- Hardell L, Carlberg M, Hardell K, Bjornfoth H, Wickbom G, Ionescu M, van Bavel B, Lindstrom G. Decreased survival in pancreatic cancer patients with high concentrations of organochlorines in adipose tissue. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2007;61:659-664
- Hardell. Concentrations of organohalogen compounds and titres of antibodies to epsteinbarr virus antigens and the risk for non-hodgkin lymphoma. Oncology Reports. 2009;21
- Harley KG, Marks AR, Bradman A, Barr DB, Eskenazi B. Ddt exposure, work in agriculture, and time to pregnancy among farmworkers in california. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:1335-1342
- Hashemi N, Mirsadraee M, Shakeri MT, Varasteh AR. Prevalence of work-related respiratory symptoms in iranian farmers. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2006;13:198-202
- Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, Welsh-Bohmer KA. Occupational exposure to pesticides increases the risk of incident ad: The cache county study. Neurology. 2010;74:1524-1530
- Herin F, Boutet-Robinet E, Levant A, Dulaurent S, Manika M, Galatry-Bouju F, Caron P, Soulat JM. Thyroid function tests in persons with occupational exposure to fipronil. Thyroid: official journal of the American Thyroid Association. 2011;21:701-706
- Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. Inhalation toxicology. 2008;20:839-849
- Hernandez-Morales AL, Zonana-Nacach A, Zaragoza-Sandoval VM. [associated risk factors in acute leukemia in children. A cases and controls study]. Revista medica del Instituto Mexicano del Seguro Social. 2009;47:497-503

Hodgson S, Thomas L, Fattore E, Lind PM, Alfven T, Hellstrom L, Hakansson H, Carubelli G, Fanelli R, Jarup L. Bone mineral density changes in relation to environmental pcb exposure. Environ Health Perspect. 2008;116:1162-1166

Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA, Demers PA, Blair

- A. Exposure to multiple pesticides and risk of non-hodgkin lymphoma in men from six canadian provinces. International journal of environmental research and public health. 2011;8:2320-2330
- Hoppin JA, Umbach DM, Kullman GJ, Henneberger PK, London SJ, Alavanja MC, Sandler DP. Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the agricultural health study. Occupational and environmental medicine. 2007;64:334-341
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the agricultural health study. American journal of respiratory and critical care medicine. 2008;177:11-18
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MC, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the agricultural health study. The European respiratory journal. 2009;34:1296-1303
- Hoppin JA, Umbach DM, London SJ, Lynch CF, Alavanja MC, Sandler DP. Pesticides and adult respiratory outcomes in the agricultural health study. Annals of the New York Academy of Sciences. 2006;1076:343-354
- Hoppin JA, Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP. Pesticide use and chronic bronchitis among farmers in the agricultural health study. American journal of industrial medicine. 2007;50:969-979
- Horton MK, Rundle A, Camann DE, Boyd Barr D, Rauh VA, Whyatt RM. Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. Pediatrics. 2011;127:e699-706
- Hossain F, Ali O, D'Souza UJ, Naing DK. Effects of pesticide use on semen quality among farmers in rural areas of sabah, malaysia. Journal of occupational health. 2010;52:353-360
- Hou L, Lee WJ, Rusiecki J, Hoppin JA, Blair A, Bonner MR, Lubin JH, Samanic C, Sandler DP, Dosemeci M, Alavanja MC. Pendimethalin exposure and cancer incidence among pesticide applicators. Epidemiology. 2006;17:302-307
- Huisman M.H.B., De Jong D.E., Van Doormaa P.T.C., Vermeulen R., Heederick D., Kromhout H., Schelhaas H.J., Van Der Kooi A.J., De VISSER M., Veldink J.H., Van Den Berg A.H. Exogenous risk factors in ALS: A population-based case-control study. Amyotrophic Lateral Sclerosis (2011) 12 SUPPL. 1 (23).
- Issaragrisil S, Kaufman DW, Anderson T, Chansung K, Leaverton PE, Shapiro S, Young NS. The epidemiology of aplastic anemia in thailand. Blood. 2006;107:1299-1307
- Itoh H, Iwasaki M, Hanaoka T, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S. Serum organochlorines and breast cancer risk in japanese women: A casecontrol study. Cancer causes & control : CCC. 2009;20:567-580

- Iwasaki M, Inoue M, Sasazuki S, Kurahashi N, Itoh H, Usuda M, Tsugane S. Plasma organochlorine levels and subsequent risk of breast cancer among japanese women: A nested case-control study. The Science of the total environment. 2008;402:176-183
- J R Suarez-Lopez, J H Himes, D R Jacobs, Jr., B H Alexander, D Lazovich, M Gunnar. Secondary pesticide exposure is associated with head circumference and growth in children. Am J Epidemiol. 2012;175(11 Suppl):S1-S145
- Jansson C, Plato N, Johansson AL, Nyren O, Lagergren J. Airborne occupational exposures and risk of oesophageal and cardia adenocarcinoma. Occupational and environmental medicine. 2006;63:107-112
- Jeebhay MF, Baatjies R, Chang YS, Kim YK, Kim YY, Major V, Lopata AL. Risk factors for allergy due to the two-spotted spider mite (tetranychus urticae) among table grape farm workers. International archives of allergy and immunology. 2007;144:143-149
- Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, Wang S, Wang X. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in chinese men. Reprod Toxicol. 2011;31:171-176
- Jimenez-Jimenez FJ, de Toledo-Heras M, Alonso-Navarro H, Ayuso-Peralta L, Arevalo- Serrano J, Ballesteros-Barranco A, Puertas I, Jabbour-Wadih T, Barcenilla B. Environmental risk factors for essential tremor. European neurology. 2007;58:106-113
- Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland inuit. Diabetologia. 2008;51:1416-1422
- Jurewicz J, Hanke W, Makowiec-Dabrowska T. [low risk of reproductive disorders among female greenhouse workers--safe work conditions or health selection for the light work?]. Medycyna pracy. 2008;59:123-131
- Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and schoolaged children. Epidemiology. 2012;23:689-698
- Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I. Maternal ddt exposures in relation to fetal and 5year growth. Epidemiology. 2006;17:692-700
- Kallioniemi MK, Simola AJ, Kymalainen HR, Vesala HT, Louhelainen JK. Mental symptoms among Finnish farm entrepreneurs. Annals of agricultural and environmental medicine: AAEM. 2009;16:159-168
- Kamalesh Das, Chiranjib Nag and Mrinalkanti Ghosh. Familial, Environmental, and Occupational Risk Factors in Development of Amyotrophic Lateral Sclerosis. North American Journal of Medical Sciences 2012.
- Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed pesticide applicators in the agricultural health study. Human & experimental toxicology. 2007;26:243-250
- Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported parkinson's disease in the agricultural health study. American journal of epidemiology. 2007;165:364-374

- Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, Hoppin JA, Dosemeci M, Coble J, Lubin J, Blair A, Alavanja M. Cancer incidence among pesticide applicators exposed to trifluralin in the agricultural health study. Environmental research. 2008;107:271-276
- Karami S, Boffetta P, Rothman N, Hung RJ, Stewart T, Zaridze D, Navritalova M, Mates D, Janout V, Kollarova H, Bencko V, Szeszenia-Dabrowska N, Holcatova I, Mukeria A, Gromiec J, Chanock SJ, Brennan P, Chow WH, Moore LE. Renal cell carcinoma, occupational pesticide exposure and modification by glutathione s-transferase polymorphisms. Carcinogenesis. 2008;29:1567-1571
- Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH. Hodgkin lymphoma and pesticides exposure in men: A Canadian case-control study. Journal of agromedicine. 2012;17:30-39
- Kaufman DW, Anderson TE, Issaragrisil S. Risk factors for leukemia in Thailand. Annals of hematology. 2009;88:1079-1088
- Kelada SN, Checkoway H, Kardia SL, Carlson CS, Costa-Mallen P, Eaton DL, Firestone J, Powers KM, Swanson PD, Franklin GM, Longstreth WT, Jr., Weller TS, Afsharinejad Z, Costa LG. 5' and 3' region variability in the dopamine transporter gene (slc6a3), pesticide exposure and parkinson's disease risk: A hypothesis-generating study. Human molecular genetics. 2006;15:3055-3062
- Khanjani N, Sim MR. Maternal contamination with dichlorodiphenyltrichloroethane and reproductive outcomes in an Australian population. Environmental research. 2006;101:373-379
- Khanjani N, Sim MR. Reproductive outcomes of maternal contamination with cyclodiene insecticides, hexachlorobenzene and beta-benzene hexachloride. The Science of the total environment. 2006;368:557-564
- Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M. Gst polymorphisms, interaction with smoking and pesticide use, and risk for parkinson's disease in a japanese population. Parkinsonism & related disorders. 2010;16:447-452
- Koutros S, Andreotti G, Berndt SI, Hughes Barry K, Lubin JH, Hoppin JA, Kamel F, Sandler DP, Burdette LA, Yuenger J, Yeager M, Alavanja MC, Freeman LE. Xenobioticmetabolizing gene variants, pesticide use, and the risk of prostate cancer. Pharmacogenetics and genomics. 2011;21:615-623
- Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. Heterocyclic aromatic amine pesticide use and human cancer risk: Results from the u.S. Agricultural health study. International journal of cancer. Journal international du cancer. 2009;124:1206-1212
- Koutros S, Mahajan R, Zheng T, Hoppin JA, Ma X, Lynch CF, Blair A, Alavanja MC. Dichlorvos exposure and human cancer risk: Results from the agricultural health study. Cancer causes & control: CCC. 2008;19:59-65
- Kouznetsova M, Huang X, Ma J, Lessner L, Carpenter DO. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. Environmental Health Perspectives. 2006;115:75-79

- Kumar V, Yadav CS, Singh S, Goel S, Ahmed RS, Gupta S, Grover RK, Banerjee BD. Cyp 1a1 polymorphism and organochlorine pesticides levels in the etiology of prostate cancer. Chemosphere. 2010;81:464-468
- L. Lind, D. Lee, D.R. Jacobs, S. Salihovic, B. vanBavel, P.M. Lind. Are persistent organic pollutants associated with obesity, the metabolic syndrome or both? Abstracts / Toxicology Letters 205S (2011) S60-S179
- Lacasana M, Lopez-Flores I, Rodriguez-Barranco M, Aguilar-Garduno C, Blanco-Munoz J, Perez-Mendez O, Gamboa R, Bassol S, Cebrian ME. Association between organophosphate pesticides exposure and thyroid hormones in floriculture workers. Toxicology and applied pharmacology. 2010;243:19-26
- Lacasana M, Vazquez-Grameix H, Borja-Aburto VH, Blanco-Munoz J, Romieu I, Aguilar- Garduno C, Garcia AM. Maternal and paternal occupational exposure to agricultural work and the risk of anencephaly. Occupational and environmental medicine. 2006;63:649-656
- Laden F, Bertrand KA, Altshul L, Aster JC, Korrick SA, Sagiv SK. Plasma organochlorine levels and risk of non-hodgkin lymphoma in the nurses' health study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010;19:1381-1384
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV, Alavanja MC. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the agricultural health study. Blood. 2009;113:6386-6391
- Langer P, Kocan A, Drobna B, Susienkova K, Radikova Z, Huckova M, Imrich R, Ksinantova L, Klimes I. Polychlorinated biphenyls and testosterone: Age and congener related correlation approach in heavily exposed males. Endocrine regulations. 2010;44:109-114
- Langer P, Kocan A, Tajtakova M, Koska J, Radikova Z, Ksinantova L, Imrich R, Huckova M, Drobna B, Gasperikova D, Sebokova E, Klimes I. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: Outcome of transgenerational transmission? Chemosphere. 2008;73:1145-1150
- Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Radikova Z, Koska J, Ksinantova L, Huckova M, Imrich R, Wimmerova S, Gasperikova D, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Fish from industrially polluted freshwater as the main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere. 2007;67:S379-385
- Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. Chemosphere. 2007;69:118-127
- Lauria L, Settimi L, Spinelli A, Figa-Talamanca I. Exposure to pesticides and time to pregnancy among female greenhouse workers. Reprod Toxicol. 2006;22:425-430
- Lee DH, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and gamma glutamyltransferase: Results from the national health and examination survey 1999-2002. Clinical chemistry. 2006;52:1825-1827

- Lee DH, Jacobs DR, Jr., Steffes M. Association of organochlorine pesticides with peripheral neuropathy in patients with diabetes or impaired fasting glucose. Diabetes. 2008;57:3108-3111
- Lee DH, Jacobs DR, Kocher T. Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. Environ Health Perspect. 2008;116:1558-1562
- Lee DH, Jacobs DR, Porta M. Association of serum concentrations of persistent organic pollutants with the prevalence of learning disability and attention deficit disorder. Journal of epidemiology and community health. 2007;61:591-596
- Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetes care. 2007;30:622-628
- Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetologia. 2007;50:1841-1851
- Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr. A strong doseresponse relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. Diabetes care. 2006;29:1638-1644
- Lee DH, Lee IK, Steffes M, Jacobs DR, Jr. Extended analyses of the association between serum concentrations of persistent organic pollutants and diabetes. Diabetes care. 2007;30:1596-1598
- Lee DH, Lind L, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind PM. Associations of persistent organic pollutants with abdominal obesity in the elderly: The prospective investigation of the vasculature in uppsala seniors (pivus) study. Environment international. 2012;40:170-178
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Background exposure to persistent organic pollutants predicts stroke in the elderly. Environment international. 2012;47:115-120
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: The prospective investigation of the vasculature in Uppsala seniors (pivus) study. Diabetes care. 2011;34:1778-1784
- Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect. 2007;115:883-888
- Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose of some persistent organic pollutants predicts type 2 diabetes: A nested case-control study. Environ Health Perspect. 2010;118:1235-1242
- Lee WJ, Alavanja MC, Hoppin JA, Rusiecki JA, Kamel F, Blair A, Sandler DP. Mortality among pesticide applicators exposed to chlorpyrifos in the agricultural health study. Environ Health Perspect. 2007;115:528-534

- Lee WJ, Baccarelli A, Tretiakova M, Gorbanev S, Lomtev A, Klimkina I, Tchibissov V, Averkina O, Dosemeci M. Pesticide exposure and lung cancer mortality in leningrad province in russia. Environment international. 2006;32:412-416
- Lee WJ, Purdue MP, Stewart P, Schenk M, De Roos AJ, Cerhan JR, Severson RK, Cozen W, Hartge P, Blair A. Asthma history, occupational exposure to pesticides and the risk of non-hodgkin's lymphoma. International journal of cancer. Journal international du cancer. 2006;118:3174-3176
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC. Pesticide use and colorectal cancer risk in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:339-346
- LeVan TD, Koh WP, Lee HP, Koh D, Yu MC, London SJ. Vapor, dust, and smoke exposure in relation to adult-onset asthma and chronic respiratory symptoms: The singapore chinese health study. American journal of epidemiology. 2006;163:1118-1128
- Li J, Lu Y, Shi Y, Wang T, Wang G, Luo W, Jiao W, Chen C, Yan F. Environmental pollution by persistent toxic substances and health risk in an industrial area of china. Journal of Environmental Sciences. 2011;23:1359-1367
- Li JY, Li H, Tao P, Lei FM. [serum organochlorines pesticides level of non-occupational exposure women and risk of breast cancer:A case-control study]. Wei sheng yan jiu = Journal of hygiene research. 2006;35:391-394
- Li JY, Wu DS, Yang F, Zeng HY, Lei FM, Zhou WD, Li H, Tao P. [study on serum organochlorines pesticides (ddts) level, cyp1a1 genetic polymorphism and risk of breast cancer: A case control study]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:217-222
- Lifeng T, Shoulin W, Junmin J, Xuezhao S, Yannan L, Qianli W, Longsheng C. Effects of fenvalerate exposure on semen quality among occupational workers. Contraception. 2006;73:92-96
- Lim JS, Son HK, Park SK, Jacobs DR, Jr., Lee DH. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond). 2011;35:744-747
- Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (pops) and carotid atherosclerosis in the elderly. Environ Health Perspect. 2012;120:38-43
- Liu B, Jung KH, Horton MK, Camann DE, Liu X, Reardon AM, Perzanowski MS, Zhang H, Perera FP, Whyatt RM, Miller RL. Prenatal exposure to pesticide ingredient piperonyl butoxide and childhood cough in an urban cohort. Environment international. 2012;48:156-161
- Lizardi PS, O'Rourke MK, Morris RJ. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of pediatric psychology. 2008;33:91-101
- Lo AC, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, Omar S, Hamilton SR, Greenson JK, Abbruzzese JL. Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. Pancreas. 2007;35:120-129

- Lo AC, Soliman AS, Khaled HM, Aboelyazid A, Greenson JK. Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. Diseases of the colon and rectum. 2010;53:830-837
- Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, Hernandez-Avila M. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(pchlorophenyl)ethylene (dde) in relation to anogenital distance in male newborns from chiapas, mexico. American journal of epidemiology. 2007;165:1015-1022
- Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Zock JP, Pollan M. Occupation, exposure to chemicals, sensitizing agents, and risk of multiple myeloma in Sweden. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:3123-3127
- Lopez-Espinosa MJ, Granada A, Carreno J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from southern spain and some related factors. Placenta. 2007;28:631-638
- Lopez-Espinosa MJ, Murcia M, Iniguez C, Vizcaino E, Llop S, Vioque J, Grimalt JO, Rebagliato M, Ballester F. Prenatal exposure to organochlorine compounds and birth size. Pediatrics. 2011;128:e127-134
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, Grimalt JO, Ballester F. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. Journal of exposure science & environmental epidemiology. 2010;20:579-588
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, Marco A, Rebagliato M, Grimalt JO, Ballester F. Association between thyroid hormone levels and 4,4'dde concentrations in pregnant women (valencia, spain). Environmental research. 2009;109:479-485
- Louis ED, Factor-Litvak P, Parides M, Andrews L, Santella RM, Wolff MS. Organochlorine pesticide exposure in essential tremor: A case-control study using biological and occupational exposure assessments. Neurotoxicology. 2006;27:579-586
- Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. American journal of public health. 2011;101:63-70
- Lu JL. Comparison of pesticide exposure and physical examination, neurological assessment, and laboratory findings between full-time and part-time vegetable farmers in the Philippines. Environmental health and preventive medicine. 2009;14:345-352
- Lu JL. Occupational safety of farmers in the vegetable industry. International journal of occupational safety and ergonomics: JOSE. 2011;17:445-453
- Luis D Boada, Manuel Zumbado, Luis Alberto Henríquez-Hernández, Maira Almeida- González, Eva E Álvarez-León, Lluis Serra-Majem and Octavio P Luzardo. Complex organochlorine pesticide mixtures as determinant factor for breast cancer risk: a population- based case-control study in theCanary Islands (Spain). Environmental Health 2012, 11:28

- Lv L, Lin G, Gao X, Wu C, Dai J, Yang Y, Zou H, Sun H, Gu M, Chen X, Fu H, Bao L. Casecontrol study of risk factors of myelodysplastic syndromes according to world health organization classification in a chinese population. American journal of hematology. 2011;86:163-169
- Lv L, Lin GW, Wang XQ, Bao LM, Zou HJ. [a case-control study of risk factors for myelodysplastic syndromes]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2007;25:705-709
- Lynch SM, Mahajan R, Beane Freeman LE, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to butylate in the agricultural health study (ahs). Environmental research. 2009;109:860-868
- Lynch SM, Rusiecki JA, Blair A, Dosemeci M, Lubin J, Sandler D, Hoppin JA, Lynch CF, Alavanja MCR. Cancer incidence among pesticide applicators exposed to cyanazine in the agricultural health study. Environmental Health Perspectives. 2006;114:1248-1252
- MacCarthy A, Bunch KJ, Fear NT, King JC, Vincent TJ, Murphy MF. Paternal occupation and neuroblastoma: A case-control study based on cancer registry data for Great Britain 1962-1999. British journal of cancer. 2010;102:615-619
- MacFarlane E, Simpson P, Benke G, Sim MR. Suicide in australian pesticide-exposed workers. Occup Med (Lond). 2011;61:259-264
- Mackenzie Ross SJ, Brewin CR, Curran HV, Furlong CE, Abraham-Smith KM, Harrison V. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicology and teratology. 2010;32:452-459
- Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect. 2007;115:1780-1786
- Mahajan R, Blair A, Coble J, Lynch CF, Hoppin JA, Sandler DP, Alavanja MC. Carbaryl exposure and incident cancer in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:1799-1805
- Mahajan R, Bonner MR, Hoppin JA, Alavanja MCR. Phorate exposure and incidence of cancer in the agricultural health study. Environmental Health Perspectives. 2006;114:1205-1209
- Maluf E, Hamerschlak N, Cavalcanti AB, Junior AA, Eluf-Neto J, Falcao RP, Lorand-Metze IG, Goldenberg D, Santana CL, Rodrigues Dde O, Passos LN, Rosenfeld LG, Pitta M, Loggetto S, Ribeiro AA, Velloso ED, Kondo AT, Coelho EO, Pintao MC, de Souza HM, Borbolla JR, Pasquini R. Incidence and risk factors of aplastic anemia in latin american countries: The latin case-control study. Haematologica. 2009;94:1220-1226
- Manfo FPT, Moundipa PF, Déchaud H, Tchana AlN, Nantia EA, Zabot M-T, Pugeat M. Effect of agropesticides use on male reproductive function: A study on farmers in djutitsa (cameroon). Environmental Toxicology. 2012;27:423-432
- Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and parkinson disease. Epidemiology. 2010;21:87-94

- Marina Krstevska Konstantinova. Organochlorine pesticides in Macedonain girls Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. Organophosphate pesticide exposure and attention in young mexican-american children: The chamacos study. Environ Health Perspect. 2010;118:1768-1774
- Marzouk D. A., El Gaafary M. M., El Damaty S. I., Sabbour S. M., Mecky F. A. S., Saker M., Sayed A. M., Fahim H. I., Anwar W. A., Breast cancer and hormonal intake among Egyptian females. European Journal of Oncology (2009) 14:1 (37-51).
- Matmurodov R. J., Khalimova K. M., Raimova M. M. Polymorphism of the genes GSTM1, GSTT1, and environmental factors in the development of Parkinson's disease among representatives of the Uzbek nationality. European Journal of Neurology (2011) 18 SUPPL. 2 (501).
- McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ. Environmental exposure to polychlorinated biphenyls and p,p'-dde and sperm sex-chromosome disomy. Environ Health Perspect. 2012;120:535-540
- McDuffie HH, Quail J, Ghosh S, Pahwa P. Host factors, occupation, and testicular cancer in saskatchewan, canada: 1979-2002. Journal of agricultural safety and health. 2007;13:247-258
- McElroy JA, Gangnon RE, Newcomb PA, Kanarek MS, Anderson HA, Brook JV, Trentham- Dietz A, Remington PL. Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in wisconsin. Journal of exposure science & environmental epidemiology. 2007;17:207-214
- McGlynn KA, Abnet CC, Zhang M, Sun XD, Fan JH, O'Brien TR, Wei WQ, Ortiz-Conde BA, Dawsey SM, Weber JP, Taylor PR, Katki H, Mark SD, Qiao YL. Serum concentrations of 1, 1, 1trichloro-2, 2-bis(p-chlorophenyl)ethane (ddt) and 1, 1-dichloro-2, 2-bis(pchlorophenyl)ethylene (dde) and risk of primary liver cancer. Journal of the National Cancer Institute. 2006;98:1005-1010
- McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of the National Cancer Institute. 2008;100:663-671
- McHugh MK, Kachroo S, Liu M, D'Amelio AM, Jr., Dong Q, Hong WK, Greisinger AJ, Spitz MR, Etzel CJ. Assessing environmental and occupational risk factors for lung cancer in mexican-americans. Cancer causes & control: CCC. 2010;21:2157-2164
- Meeker JD, Altshul L, Hauser R. Serum pcbs, p,p'-dde and hcb predict thyroid hormone levels in men. Environmental research. 2007;104:296-304
- Meeker JD, Barr DB, Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Hum Reprod. 2008;23:1932-1940
- Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol. 2009;27:155-160
- Meeker JD, Barr DB, Hauser R. Thyroid hormones in relation to urinary metabolites of nonpersistent insecticides in men of reproductive age. Reprod Toxicol. 2006;22:437-442
- Meeker JD, Ravi SR, Barr DB, Hauser R. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod Toxicol. 2008;25:184-191

- Meeker JD, Ryan L, Barr DB, Hauser R. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 2006;17:61-68
- Melkonian S, Argos M, Pierce BL, Chen Y, Islam T, Ahmed A, Syed EH, Parvez F, Graziano J, Rathouz PJ, Ahsan H. A prospective study of the synergistic effects of arsenic exposure and smoking, sun exposure, fertilizer use, and pesticide use on risk of premalignant skin lesions in Bangladeshi men. American journal of epidemiology. 2011;173:183-191
- Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, Fochs S, Sunyer J. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect. 2011;119:272-278
- Menegaux F, Baruchel A, Bertrand Y, Lescoeur B, Leverger G, Nelken B, Sommelet D, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood acute leukaemia. Occupational and environmental medicine. 2006;63:131-134
- Merletti F, Richiardi L, Bertoni F, Ahrens W, Buemi A, Costa-Santos C, Eriksson M, Guenel P, Kaerlev L, Jockel KH, Llopis-Gonzalez A, Merler E, Miranda A, Morales-Suarez-Varela MM, Olsson H, Fletcher T, Olsen J. Occupational factors and risk of adult bone sarcomas: A multicentric case-control study in europe. International journal of cancer. Journal international du cancer. 2006;118:721-727
- Messaros BM, Rossano MG, Liu G, Diamond MP, Friderici K, Nummy-Jernigan K, Daly D, Puscheck E, Paneth N, Wirth JJ. Negative effects of serum p,p'-dde on sperm parameters and modification by genetic polymorphisms. Environmental research. 2009;109:457-464
- Meyer A, Alexandre PC, Chrisman Jde R, Markowitz SB, Koifman RJ, Koifman S. Esophageal cancer among brazilian agricultural workers: Case-control study based on death certificates. International journal of hygiene and environmental health. 2011;214:151-155
- Meyer KJ, Reif JS, Veeramachaneni DNR, Luben TJ, Mosley BS, Nuckols JR. Agricultural pesticide use and hypospadias in eastern arkansas. Environmental Health Perspectives. 2006;114:1589-1595
- Meyer TE, Coker AL, Sanderson M, Symanski E. A case-control study of farming and prostate cancer in african-american and caucasian men. Occupational and environmental medicine. 2007;64:155-160
- Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: An overview and some results of the italian multicenter case-control study on hematolymphopoietic malignancies. Annals of the New York Academy of Sciences. 2006;1076:366-377
- Mills KT, Blair A, Freeman LE, Sandler DP, Hoppin JA. Pesticides and myocardial infarction incidence and mortality among male pesticide applicators in the agricultural health study. American journal of epidemiology. 2009;170:892-900
- Mills PK, Yang RC. Agricultural exposures and gastric cancer risk in Hispanic farm workers in california. Environmental research. 2007;104:282-289
- Min JY, Cho JS, Lee KJ, Park JB, Park SG, Kim JY, Min KB. Potential role for organochlorine pesticides in the prevalence of peripheral arterial diseases in obese persons: Results from the national health and nutrition examination survey 1999-2004. Atherosclerosis. 2011;218:200- 206
- Miyake Y, Tanaka K, Masuzaki Y, Sato N, Ikeda Y, Chisaki Y, Arakawa M. Organochlorine concentrations in breast milk and prevalence of allergic disorders in japanese women. Chemosphere. 2011;85:374-378

- Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP, Weiderpass E, Partanen T. Parental occupational exposure to pesticides and the risk of childhood leukemia in costa rica. Scandinavian Journal of Work, Environment & Health. 2007;33:293-303
- Montgomery MP, Kamel F, Saldana TM, Alavanja MC, Sandler DP. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993-2003. American journal of epidemiology. 2008;167:1235-1246
- Morahan JM, Pamphlett R. Amyotrophic lateral sclerosis and exposure to environmental toxins: An Australian case-control study. Neuroepidemiology. 2006;27:130-135
- Mozzachio AM, Rusiecki JA, Hoppin JA, Mahajan R, Patel R, Beane-Freeman L, Alavanja MC. Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study. Environmental research. 2008;108:400-403
- Mueller BA, Kuehn CM, Shapiro-Mendoza CK, Tomashek KM. Fetal deaths and proximity to hazardous waste sites in washington state. Environ Health Perspect. 2007;115:776-780
- Multigner L, Kadhel P, Pascal M, Huc-Terki F, Kercret H, Massart C, Janky E, Auger J, Jegou B. Parallel assessment of male reproductive function in workers and wild rats exposed to pesticides in banana plantations in guadeloupe. Environmental health: a global access science source. 2008;7:40
- Multigner L, Ndong JR, Giusti A, Romana M, Delacroix-Maillard H, Cordier S, Jegou B, Thome JP, Blanchet P. Chlordecone exposure and risk of prostate cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010;28:3457-3462
- N. Murgia, M. Dell"Omo, G. Muzi, G. Brugnami, M. Biancalana, Nagayama J, Kohno H, Kunisue T, Kataoka K, Shimomura H, Tanabe S, Konishi S. Concentrations of organochlorine pollutants in mothers who gave birth to neonates with congenital hypothyroidism. Chemosphere. 2007;68:972-976
- Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, Yanagawa T, Fukushige J, Watanabe T. Immunologic effects of perinatal exposure to dioxins, pcbs and organochlorine pesticides in japanese infants. Chemosphere. 2007;67:S393-398
- Naidoo S, London L, Burdorf A, Naidoo R, Kromhout H. Spontaneous miscarriages and infant deaths among female farmers in rural south africa. Scand J Work Environ Health. 2011;37:227-236
- Narendra M, Kavitha G, Helah Kiranmai A, Raghava Rao N, Varadacharyulu NC. Chronic exposure to pyrethroid-based allethrin and prallethrin mosquito repellents alters plasma biochemical profile. Chemosphere. 2008;73:360-364
- Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environmental science & technology. 2011;45:1680-1687
- Neundorfer B. . Solvents and PARKINSON'S disease. Padiatrische Praxis (2008) 72:3 (508-510).
- Nicolas Lebas, Louise Nadon, Mounia Rhazi, Hugues Richard, Marie Desy, Marie-Elise Parent. Exposure to occupational and domestic pesticides, and prostate cancer risk: preliminary findings from a case-control study in Montreal, Canada.

- Nicolle-Mir L. Exposure to pesticides and thyroid diseases. Environmement, Risques et Sante (2010) 9:5 (381-382).
- Nicolle-Mir L. Occupational factors and Parkinson's disease. Environmement, Risques et Sante (2010) 9:5 (382-383).
- Nordby KC, Irgens LM, Kristensen P. Immunological exposures in Norwegian agriculture and pre-eclampsia. Paediatric and perinatal epidemiology. 2006;20:462-470
- Norlaily H, Azidah AK, Asrenee AR, Rohayah H, Juwita S. Proportion of dementia and its associated factors among elderly patients attending outpatient clinics of universiti sains malaysia hospital. The Medical journal of Malaysia. 2009;64:140-145
- Ochoa-Acuna H, Frankenberger J, Hahn L, Carbajo C. Drinking-water herbicide exposure in indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Perspect. 2009;117:1619-1624
- Ociepa-Zawal M, Rubis B, Wawrzynczak D, Wachowiak R, Trzeciak WH. Accumulation of environmental estrogens in adipose tissue of breast cancer patients. Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering. 2010;45:305-312
- Onishchenko GG, Mamaev IA, Guseinov GK. [impact of the area burden of agrochemicals on tuberculosis morbidity and mortality]. Problemy tuberkuleza i boleznei legkikh. 2006:30-33
- Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X, Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: Results of a french casecontrol study. Occupational and environmental medicine. 2009;66:291-298
- Orsi L, Troussard X, Monnereau A, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Clavel J. Occupation and lymphoid malignancies: Results from a french case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:1339-1350
- Ostrea EM, Jr., Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012;33:669-675
- Ozen S, Darcan S, Bayindir P, Karasulu E, Simsek DG, Gurler T. Effects of pesticides used in agriculture on the development of precocious puberty. Environmental monitoring and assessment. 2012;184:4223-4232
- P. Monica Lind, Samira Salihovic, Bert van Bavel, Lars Lind. Circulating levels of pp-DDE and hypertension. Abstracts / Toxicology Letters 211S (2012) S43-S216
- Padmaja R. Jonnalagadda\*, A. Y. E. Prasad, Katla Ashok Reddy, Challa Suresh, M. Vishnu, Vardhana Rao, Goparaju Ramya and D. Raghunatha Rao. Biochemical alterations of certain health parameters in cotton growing farmers exposed to organophosphorous and pyrethroid insecticides. African Journal of Biotechnology Vol. 9(49), pp. 8369-8377, 6 December, 2010

- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McDuffie HH, McLaughlin JR. Multiple myeloma and exposure to pesticides: A Canadian case-control study. Journal of agromedicine. 2012;17:40-50
- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR. Soft-tissue sarcoma and pesticides exposure in men: Results of a Canadian case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:1279-1286
- Pahwa P, Karunanayake CP, Spinelli JJ, Dosman JA, McDuffie HH. Ethnicity and incidence of Hodgkin lymphoma in canadian population. BMC cancer. 2009;9:141
- Pahwa P, McDuffie HH, Dosman JA, McLaughlin JR, Spinelli JJ, Robson D, Fincham S. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:264-274
- Pamphlett R. Exposure to environmental toxins and the risk of sporadic motor neuron disease: An expanded Australian case-control study. European journal of neurology: the official journal of the European Federation of Neurological Societies. 2012;19:1343-1348
- Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: An analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect. 2009;117:488-494
- Pan IJ, Daniels JL, Herring AH, Rogan WJ, Siega-Riz AM, Goldman BD, Sjodin A. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: An analysis of the pregnancy, infection, and nutrition babies study. Paediatric and perinatal epidemiology. 2010;24:262- 271
- Pant N, Kumar R, Mathur N, Srivastava SP, Saxena DK, Gujrati VR. Chlorinated pesticide concentration in semen of fertile and infertile men and correlation with sperm quality. Environmental toxicology and pharmacology. 2007;23:135-139
- Park SK, Kang D, Beane-Freeman L, Blair A, Hoppin JA, Sandler DP, Lynch CF, Knott C, Gwak J, Alavanja M. Cancer incidence among paraquat exposed applicators in the agricultural health study: Prospective cohort study. International journal of occupational and environmental health. 2009;15:274-281
- Park SK, Son HK, Lee SK, Kang JH, Chang YS, Jacobs DR, Lee DH. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among nondiabetic adults. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2010;43:1-8
- Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J, Sarto G, Howard BV. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the women's health initiative observational study. Arthritis care & research. 2011;63:184-194
- Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (ewas) on type 2 diabetes mellitus. PloS one. 2010;5:e10746
- Pathak R, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Banerjee BD. Maternal and cord blood levels of organochlorine pesticides: Association with preterm labor. Clinical biochemistry. 2009;42:746-749

- Pathak R, Mustafa M, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Association between recurrent miscarriages and organochlorine pesticide levels. Clinical biochemistry. 2010;43:131-135
- Pathak R, Mustafa MD, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Intra uterine growth retardation: Association with organochlorine pesticide residue levels and oxidative stress markers. Reprod Toxicol. 2011;31:534-539
- Patil JA, Patil AJ, Sontakke AV, Govindwar SP. Occupational pesticides exposure of sprayers of grape gardens in western Maharashtra (india): Effects on liver and kidney function. Journal of basic and clinical physiology and pharmacology. 2009;20:335-355
- Pearce MS, Hammal DM, Dorak MT, McNally RJ, Parker L. Paternal occupational exposure to pesticides or herbicides as risk factors for cancer in children and young adults: A case- control study from the north of england. Arch Environ Occup Health. 2006;61:138-144
- Pekmezovic T, Suvajdzic Vukovic N, Kisic D, Grgurevic A, Bogdanovic A, Gotic M, Bakrac M, Brkic N. A case-control study of myelodysplastic syndromes in belgrade (serbia montenegro). Annals of hematology. 2006;85:514-519
- Pereira D, Garrett C. [risk factors for Parkinson disease: An epidemiologic study]. Acta medica portuguesa. 2010;23:15-24
- Perez-Herrera N, Polanco-Minaya H, Salazar-Arredondo E, Solis-Heredia MJ, Hernandez- Ochoa I, Rojas-Garcia E, Alvarado-Mejia J, Borja-Aburto VH, Quintanilla-Vega B. Pon1q192r genetic polymorphism modifies organophosphorous pesticide effects on semen quality and DNA integrity in agricultural workers from southern mexico. Toxicology and applied pharmacology. 2008;230:261-268
- Perry MJ, Ouyang F, Korrick SA, Venners SA, Chen C, Xu X, Lasley BL, Wang X. A prospective study of serum ddt and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age. American journal of epidemiology. 2006;164:1056-1064
- Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, Barr DB, Xu X. Organophosphorous pesticide exposures and sperm quality. Reprod Toxicol. 2011;31:75-79
- Persson EC, Graubard BI, Evans AA, London WT, Weber JP, LeBlanc A, Chen G, Lin W, McGlynn KA. Dichlorodiphenyltrichloroethane and risk of hepatocellular carcinoma. International journal of cancer. Journal international du cancer. 2012;131:2078-2084
- Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, Jorgensen PJ, Budtz-Jorgensen E, Grandjean P. Impact of dietary exposure to food contaminants on the risk of parkinson's disease. Neurotoxicology. 2008;29:584-590
- Petit C, Blangiardo M, Richardson S, Coquet F, Chevrier C, Cordier S. Association of environmental insecticide exposure and fetal growth with a bayesian model including multiple exposure sources: The pelagie mother-child cohort. American journal of epidemiology. 2012;175:1182-1190
- Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R, Cordier S. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort study in Brittany, France. Environmental health: a global access science source. 2010;9:71

- Philibert A, Schwartz H, Mergler D. An exploratory study of diabetes in a first nation community with respect to serum concentrations of p,p'-dde and pcbs and fish consumption. International journal of environmental research and public health. 2009;6:3179-3189
- Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environmental research. 2007;105:364-369
- Polsky JY, Aronson KJ, Heaton JP, Adams MA. Pesticides and polychlorinated biphenyls as potential risk factors for erectile dysfunction. Journal of andrology. 2007;28:28-37
- Pombo-de-Oliveira MS, Koifman S. Infant acute leukemia and maternal exposures during pregnancy. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:2336-2341
- Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, Ferro A, Ingelido AM, Maggi A, Panici PB, De Felip E. Endometriosis and organochlorinated environmental pollutants: A case-control study on italian women of reproductive age. Environ Health Perspect. 2009;117:1070-1075
- Postuma RB, Montplaisir JY, Pelletier A, Dauvilliers Y, Oertel W, Iranzo A, Ferini-Strambi L, Arnulf I, Hogl B, Manni R, Miyamoto T, Mayer G, Stiasny-Kolster K, Puligheddu M, Ju Y, Jennum P, Sonka K, Santamaria J, Fantini ML, Zucconi M, Leu-Semenescu S, Frauscher B, Terzaghi M, Miyamoto M, Unger MM, Cochen De Cock V, Wolfson C. Environmental risk factors for rem sleep behavior disorder: A multicenter casecontrol study. Neurology. 2012;79:428-434
- Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, Kaufman D. Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific journal of public health / Asia-Pacific Academic Consortium for Public Health. 2011;23:369-377
- Prochazka M, Feychting M, Ahlbom A, Edwards CG, Nise G, Plato N, Schwartzbaum JA, Forssen UM. Occupational exposures and risk of acoustic neuroma. Occupational and environmental medicine. 2010;67:766-771
- Provost D, Cantagrel A, Lebailly P, Jaffre A, Loyant V, Loiseau H, Vital A, Brochard P, Baldi I. Brain tumours and exposure to pesticides: A case-control study in southwestern France. Occupational and environmental medicine. 2007;64:509-514
- Puertas R, Lopez-Espinosa MJ, Cruz F, Ramos R, Freire C, Perez-Garcia M, Abril A, Julvez J, Salvatierra M, Campoy C, Olea N. Prenatal exposure to mirex impairs neurodevelopment at age of 4 years. Neurotoxicology. 2010;31:154-160
- Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, Barr DB, Blair A, Rothman N, McGlynn KA. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. Environ Health Perspect. 2009;117:1514-1519
- Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;120:642-649
- Qiu XQ, Zhong QA, Zeng XY, Li YH, Nie SF. [a case-control study on congenital heart diseases with methylenetetrahydrofolate reductase gene, cystathionine beta-synthase gene, and environmental factors]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:260-263

- Quaranta MG, Porpora MG, Mattioli B, Giordani L, Libri I, Ingelido AM, Cerenzia P, Di Felice A, Abballe A, De Felip E, Viora M. Impaired nk-cell-mediated cytotoxic activity and cytokine production in patients with endometriosis: A possible role for pcbs and dde. Life sciences. 2006;79:491-498
- Quiros-Alcala L, Alkon AD, Boyce WT, Lippert S, Davis NV, Bradman A, Barr DB, Eskenazi B. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. Neurotoxicology. 2011;32:646-655
- Qureshi MM, Hayden D, Urbinelli L, Ferrante K, Newhall K, Myers D, Hilgenberg S, Smart R, Brown RH, Cudkowicz ME. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (als). Amyotrophic lateral sclerosis: official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2006;7:173-182
- R. Alarcon, M. Requena, A.F. Hernández, T. Parrón. The relationship between breast cancer and pesticide exposure in regions with differing pesticide use levels. Abstracts / Toxicology Letters 205S (2011) S180-S300
- Ragab M, Elzayadi AR, Hamdy H, Badran H, Shawky S, Emara S. 692 non viral risk factors in development of hcc among egyptian chronic liver disease patients. Journal of Hepatology. 2012;56:S274
- Rastogi SK, Singh VK, Kesavachandran C, Jyoti, Siddiqui MK, Mathur N, Bharti RS. Monitoring of plasma butyrylcholinesterase activity and hematological parameters in pesticide sprayers. Indian journal of occupational and environmental medicine. 2008;12:29-32
- Rau AT, Coutinho A, Avabratha KS, Rau AR, Warrier RP. Pesticide (endosulfan) levels in the bone marrow of children with hematological malignancies. Indian pediatrics. 2012;49:113-117
- Rauch SA, Braun JM, Barr DB, Calafat AM, Khoury J, Montesano AM, Yolton K, Lanphear BP. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012;120:1055-1060
- Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, Whyatt R. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 2011;119:1196-1201
- Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006;118:e1845-1859
- Recio-Vega R, Ocampo-Gomez G, Borja-Aburto VH, Moran-Martinez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: Association between sperm parameters and urinary pesticide levels. Journal of applied toxicology: JAT. 2008;28:674-680
- Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proceedings of the National Academy of Sciences of the United States of America. 2011;108:12770-12775

- Ribas-Fito N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. Prenatal exposure to 1,1dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-dde) in relation to child growth. International journal of epidemiology. 2006;35:853-858
- Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. Environ Health Perspect. 2007;115:447-450
- Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, Sunyer J. In utero exposure to background concentrations of ddt and cognitive functioning among preschoolers. American journal of epidemiology. 2006;164:955-962
- Richardson DB, Terschuren C, Hoffmann W. Occupational risk factors for non-hodgkin's lymphoma: A population-based case-control study in northern Germany. American journal of industrial medicine. 2008;51:258-268
- Richardson JR, Shalat SL, Buckley B, Winnik B, O'Suilleabhain P, Diaz-Arrastia R, Reisch J, German DC. Elevated serum pesticide levels and risk of parkinson disease. Archives of neurology. 2009;66:870-875
- Rignell-Hydbom A, Elfving M, Ivarsson SA, Lindh C, Jonsson BA, Olofsson P, Rylander L. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants in relation to risk of type 1 diabetes. PloS one. 2010;5:e11281
- Rignell-Hydbom A, Lidfeldt J, Kiviranta H, Rantakokko P, Samsioe G, Agardh CD, Rylander L. Exposure to p,p'-dde: A risk factor for type 2 diabetes. PloS one. 2009;4:e7503
- Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. Human & experimental toxicology. 2007;26:447-452
- Rignell-Hydbom A, Skerfving S, Lundh T, Lindh CH, Elmstahl S, Bjellerup P, Junsson BA, Strumberg U, Akesson A. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. Environmental research. 2009;109:991-996
- Riu E, Monso E, Marin A, Magarolas R, Radon K, Morera J, Andreo F, Nowak D. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. American journal of rhinology. 2008;22:361-364
- Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California central valley. Environ Health Perspect. 2007;115:1482-1489
- Rocheleau CM, Romitti PA, Sanderson WT, Sun L, Lawson CC, Waters MA, Stewart PA, Olney RS, Reefhuis J. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth defects research. Part A, Clinical and molecular teratology. 2011;91:927-936
- Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral performance of adult and adolescent agricultural workers. Neurotoxicology. 2007;28:374-380
- Rojas-Garcia AE, Medina-Diaz IM, Robledo-Marenco Mde L, Barron-Vivanco BS, Giron- Perez MI, Velazquez-Fernandez JB, Gonzalez-Arias CA, Albores-Medina A, Quintanilla-Vega B, Ostrosky-Wegman P, Rojas-Garcia MC, Perez-Herrera NE, Lopez-Flores JF. Hematological, biochemical effects, and self-reported symptoms in pesticide retailers. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:517-521

- Roldan-Tapia L, Nieto-Escamez FA, del Aguila EM, Laynez F, Parron T, Sanchez-Santed F. Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicology and teratology. 2006;28:694-703
- Romero Ramos R, Romero Gutierrez G, Abortes Monroy I, Medina Sanchez HG. [risk factors associated to female infertility]. Ginecologia y obstetricia de Mexico. 2008;76:717-721
- Rosano A, Gemelli V, Giovannelli C, Paciotti G, Sabatucci A, Spagnolo A. [fertility changes in women working in greenhouses]. La Medicina del lavoro. 2009;100:448-454
- Rossman MD, Thompson B, Frederick M, Iannuzzi MC, Rybicki BA, Pander JP, Newman LS, Rose C, Magira E, Monos D. Hla and environmental interactions in sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders. 2008;25:125-132
- Rosso AL, Hovinga ME, Rorke-Adams LB, Spector LG, Bunin GR. A case-control study of childhood brain tumors and fathers' hobbies: A children's oncology group study. Cancer causes & control: CCC. 2008;19:1201-1207
- Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and nonagricultural hispanic workers. Environmental Health Perspectives. 2006;114:691-696
- Rubin CH, Lanier A, Kieszak S, Brock JW, Koller KR, Strosnider H, Needham L, Zahm S, Harpster A. Breast cancer among alaska native women potentially exposed to environmental organochlorine chemicals. International journal of circumpolar health. 2006;65:18-27
- Rudant J, Menegaux F, Leverger G, Baruchel A, Nelken B, Bertrand Y, Patte C, Pacquement H, Verite C, Robert A, Michel G, Margueritte G, Gandemer V, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood hematopoietic malignancies: The escale study (sfce). Environ Health Perspect. 2007;115:1787-1793
- Ruder AM, Carreon T, Butler MA, Calvert GM, Davis-King KE, Waters MA, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. Exposure to farm crops, livestock, and farm tasks and risk of glioma: The upper midwest health study. American journal of epidemiology. 2009;169:1479-1491
- Ruder AM, Waters MA, Carreon T, Butler MA, Davis-King KE, Calvert GM, Schulte PA, Ward EM, Connally LB, Lu J, Wall D, Zivkovich Z, Heineman EF, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of primary intracranial gliomas in farm and rural residents. Journal of agricultural safety and health. 2006;12:255-274
- Ruder AM, Yiin JH. Mortality of us pentachlorophenol production workers through 2005. Chemosphere. 2011;83:851-861
- Rugbjerg K, Harris MA, Shen H, Marion SA, Tsui JK, Teschke K. Pesticide exposure and risk of Parkinson"s disease--a population-based case-control study evaluating the potential for recall bias. Scand J Work Environ Health. 2011;37:427-436
- Rull RP, Gunier R, Von Behren J, Hertz A, Crouse V, Buffler PA, Reynolds P. Residential proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environmental research. 2009;109:891-899

- Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. American journal of epidemiology. 2006;163:743-753
- Rusiecki JA, Hou L, Lee WJ, Blair A, Dosemeci M, Lubin JH, Bonner M, Samanic C, Hoppin JA, Sandler DP, Alavanja MC. Cancer incidence among pesticide applicators exposed to metolachlor in the agricultural health study. International journal of cancer. Journal international du cancer. 2006;118:3118-3123
- Rusiecki JA, Patel R, Koutros S, Beane-Freeman L, Landgren O, Bonner MR, Coble J, Lubin J, Blair A, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to permethrin in the agricultural health study. Environ Health Perspect. 2009;117:581-586
- Rylander L, Wallin E, Jonssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between cb-153 and p,p'-dde and hormone levels in serum in middle-aged and elderly men. Chemosphere. 2006;65:375-381
- Sagiv SK, Nugent JK, Brazelton TB, Choi AL, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and measures of behavior in infancy using the neonatal behavioral assessment scale (nbas). Environ Health Perspect. 2008;116:666-673
- Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. Environ Health Perspect. 2012;120:904-909
- Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. American journal of epidemiology. 2010;171:593-601
- Sagiv SK, Tolbert PE, Altshul LM, Korrick SA. Organochlorine exposures during pregnancy and infant size at birth. Epidemiology. 2007;18:120-129
- Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: A case-control study in Lebanon. Journal of epidemiology and community health. 2006;60:256-261
- Salameh PR, Waked M, Baldi I, Brochard P, Saleh BA. Chronic bronchitis and pesticide exposure: A case-control study in Lebanon. European journal of epidemiology. 2006;21:681-688
- Saldana TM, Basso O, Baird DD, Hoppin JA, Weinberg CR, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and hypertensive disorders during pregnancy. Environ Health Perspect. 2009;117:1393-1396
- Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. Diabetes care. 2007;30:529-534
- Samanic C, Rusiecki J, Dosemeci M, Hou L, Hoppin JA, Sandler DP, Lubin J, Blair A, Alavanja MCR. Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study. Environmental Health Perspectives. 2006;114:1521-1526
- Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD. Occupational exposure to pesticides and risk of adult brain tumors. American journal of epidemiology. 2008;167:976-985

- Sanchez A.T., Olivera R.M.P., Sanchez G.M.D.O., Dorantes G.L.. Pemphigus vulgaris. Epidemiological study and analysis of possible risk factors of mortality. Dermatologia Revista Mexicana (2006) 50:2 (50-53).
- Santibanez M, Alguacil J, de la Hera MG, Navarrete-Munoz EM, Llorca J, Aragones N, Kauppinen T, Vioque J. Occupational exposures and risk of stomach cancer by histological type. Occupational and environmental medicine. 2012;69:268-275
- Santibanez M, Vioque J, Alguacil J, de la Hera MG, Moreno-Osset E, Carrato A, Porta M, Kauppinen T. Occupational exposures and risk of pancreatic cancer. European journal of epidemiology. 2010;25:721-730
- Sanyal J, Chakraborty DP, Sarkar B, Banerjee TK, Mukherjee SC, Ray BC, Rao VR. Environmental and familial risk factors of Parkinson"s disease: Case-control study. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 2010;37:637-642
- Sartor SG, Eluf-Neto J, Travier N, Wunsch Filho V, Arcuri AS, Kowalski LP, Boffetta P. [occupational risks for laryngeal cancer: A case-control study]. Cadernos de saude publica. 2007;23:1473-1481
- Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, Hoppin JA. Maternal pesticide use and birth weight in the agricultural health study. Journal of agromedicine. 2010;15:127-136
- Sawada N, Iwasaki M, Inoue M, Itoh H, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: A nested case- control study. Environ Health Perspect. 2010;118:659-665
- Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p,p'- dde, and other toxicants in akwesasne mohawk youth. Environ Health Perspect. 2008;116:806-813
- Schell LM, Gallo MV, Ravenscroft J, DeCaprio AP. Persistent organic pollutants and antithyroid peroxidase levels in akwesasne mohawk young adults. Environmental research. 2009;109:86-92
- Schmeisser N, Behrens T, Mester B, Gottlieb A, Langner I, Ahrens W. Local cluster of germ cell cancer in a cohort of male automotive workers in germany not explained by previous or concurrent activities and exposures in farming and forestry. Cancer epidemiology. 2011;35:73-77
- Schmeisser N, Kaerlev L, Bourdon-Raverdy N, Ganry O, Llopis-Gonzalez A, Guenel P, Hardell
  L, Merletti F, Zambon P, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M,
  Ahrens W. Occupational exposure to pesticides and bile tract carcinoma in men:
  Results from a european multicenter case-control study. Cancer causes & control:
  CCC. 2010;21:1493-1502
- Schreinemachers DM. Perturbation of lipids and glucose metabolism associated with previous
  2,4-d exposure: A cross-sectional study of nhanes iii data, 1988-1994. Environmental
  health: a global access science source. 2010;9:11
- Semchuk KM, Rosenberg AM, McDuffie HH, Cessna AJ, Pahwa P, Irvine DG. Antinuclear antibodies and bromoxynil exposure in a rural sample. Journal of toxicology and environmental health. Part A. 2007;70:638-657

- Settimi L, Spinelli A, Lauria L, Miceli G, Pupp N, Angotzi G, Fedi A, Donati S, Miligi L, Osborn J, Figa-Talamanca I. Spontaneous abortion and maternal work in greenhouses. American journal of industrial medicine. 2008;51:290-295
- Seyed Jalal Emam, Maryam Salehcheh, Mohammad Hossein Haghighizadeh, Seyed Mohammad Hosein Mousavi Jazayeri. Occupational exposure to pesticides among farmers. Pak J Med Sci 2012;28(1):120-123
- Sharma E, Mustafa M, Pathak R, Guleria K, Ahmed RS, Vaid NB, Banerjee BD. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. European journal of obstetrics, gynecology, and reproductive biology. 2012;161:163-169
- Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in cyplal, gstmi, gsttl genes and organochlorine pesticides in the etiology of hypospadias. Human & experimental toxicology. 2011;30:1464-1474
- Shim YK, Mlynarek SP, van Wijngaarden E. Parental exposure to pesticides and childhood brain cancer: U.S. Atlantic coast childhood brain cancer study. Environ Health Perspect. 2009;117:1002-1006
- Shirangi A, Fritschi L, Holman CD. Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. Occupational and environmental medicine. 2008;65:719-725
- Siddharth M, Datta SK, Bansal S, Mustafa M, Banerjee BD, Kalra OP, Tripathi AK. Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. Journal of biochemical and molecular toxicology. 2012;26:241-247
- Silva SR, Martins JL, Seixas S, Silva DC, Lemos SP, Lemos PV. [congenital defects and exposure to pesticides in sao francisco valley]. Revista brasileira de ginecologia e obstetrician: revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia. 2011;33:20-26
- Skeie GO, Muller B, Haugarvoll K, Larsen JP, Tysnes OB. Differential effect of environmental risk factors on postural instability gait difficulties and tremor dominant Parkinson"s disease. Movement disorders: official journal of the Movement Disorder Society. 2010;25:1847-1852
- Slager RE, Poole JA, LeVan TD, Sandler DP, Alavanja MC, Hoppin JA. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the agricultural health study. Occupational and environmental medicine. 2009;66:718-724
- Slager RE, Simpson SL, Levan TD, Poole JA, Sandler DP, Hoppin JA. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. Journal of toxicology and environmental health. Part A. 2010;73:1382-1393
- Slater ME, Linabery AM, Spector LG, Johnson KJ, Hilden JM, Heerema NA, Robison LL, Ross JA. Maternal exposure to household chemicals and risk of infant leukemia: A report from the children's oncology group. Cancer causes & control : CCC. 2011;22:1197-1204
- Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr. 2008;97:1465-1469

- Snijder CA, Roeleveld N, Te Velde E, Steegers EA, Raat H, Hofman A, Jaddoe VW, Burdorf
  A. Occupational exposure to chemicals and fetal growth: The generation r study. Hum
  Reprod. 2012;27:910-920
- Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27:1510-1517
- Soldin OP, Nsouli-Maktabi H, Genkinger JM, Loffredo CA, Ortega-Garcia JA, Colantino D, Barr DB, Luban NL, Shad AT, Nelson D. Pediatric acute lymphoblastic leukemia and exposure to pesticides. Therapeutic drug monitoring. 2009;31:495-501
- Solomon C, Poole J, Palmer KT, Peveler R, Coggon D. Neuropsychiatric symptoms in past users of sheep dip and other pesticides. Occupational and environmental medicine. 2007;64:259-266
- Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, Jacobs DR, Jr., Lee DH. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in korea. Environment international. 2010;36:410-414
- Spinelli JJ, Ng CH, Weber JP, Connors JM, Gascoyne RD, Lai AS, Brooks-Wilson AR, Le ND, Berry BR, Gallagher RP. Organochlorines and risk of non-hodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;121:2767-2775
- Spix C, Schulze-Rath R, Kaatsch P, Blettner M. Case-control study on risk factors for leukaemia and brain tumours in children under 5 years in germany. Klinische Padiatrie. 2009;221:362-368
- Stallones L. Suicide and potential occupational exposure to pesticides, colorado 1990-1999. Journal of agromedicine. 2006;11:107-112
- Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Hoppin JA. Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the agricultural health study. Neurotoxicology and teratology. 2012;34:168-176
- Starks SE, Hoppin JA, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Gerr F. Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the agricultural health study. Environ Health Perspect. 2012;120:515-520
- Steerenberg P, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Vergieva T, Zaikov C, Pennanen S, Liesivuori J, Van Loveren H. Toxicological evaluation of the immune function of pesticide workers, a european wide assessment. Human & experimental toxicology. 2008;27:701-707
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal pcb exposure and intelligence (iq) in 9-year-old children. Environ Health Perspect. 2008;116:1416-1422
- Strom SS, Yamamura Y, Flores-Sandoval FN, Pettaway CA, Lopez DS. Prostate cancer in mexican-americans: Identification of risk factors. The Prostate. 2008;68:563-570
- Strom SS, Yamamura Y, Kantarijian HM, Cortes-Franco JE. Obesity, weight gain, and risk of chronic myeloid leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18:1501-1506

- Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spano M, Toft G, Bonde JP, Jonsson BA, Rignell-Hydbom A, Rylander L, Giwercman A, Pedersen HS, Bonefeld-Jorgensen EC, Ludwicki JK, Lesovoy V, Sakkas D, Bizzaro D. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of cb-153 and p,p'- dde in european and inuit populations. Reproduction. 2006;132:949-958
- Stuetz W, McGready R, Cho T, Prapamontol T, Biesalski HK, Stepniewska K, Nosten F. Relation of ddt residues to plasma retinol, alpha-tocopherol, and beta-carotene during pregnancy and malaria infection: A case-control study in karen women in northern thailand. The Science of the total environment. 2006;363:78-86
- Su Y, Dai Y, Lin Y, Gao X, Han Y, Zhao B. Serum organochlorine pesticide residues and risk of gallstone disease: A case-control study in xiamen. Annals of epidemiology. 2012;22:592-597
- Subahir MN, Shah SA, Zainuddin ZM. Risk factors for prostate cancer in universiti kebangsaan Malaysia medical centre: A case-control study. Asian Pacific journal of cancer prevention: APJCP. 2009;10:1015-1020
- Sunyer J, Alvarez-Pedrerol M, To-Figueras J, Ribas-Fito N, Grimalt JO, Herrero C. Urinary porphyrin excretion in children is associated with exposure to organochlorine compounds. Environ Health Perspect. 2008;116:1407-1410
- Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de Cid R, Anto JM, Torrent M. Early life environment, neurodevelopment and the interrelation with atopy. Environmental research. 2010;110:733-738
- Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goni F, Basterrechea M, Vrijheid M, Guerra S, Anto JM. Dde in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology. 2010;21:729-735
- Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2006;36:1236-1241
- Sutoluk Z, Kekec Z, Daglioglu N, Hant I. Association of chronic pesticide exposure with serum cholinesterase levels and pulmonary functions. Archives of Environmental & Occupational Health. 2011;66:95-99
- Swaen G, van Amelsvoort L, Boers D, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Pennanen S, Liesivuori J, Colosio C, van Loveren H. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: Results from the europit field study. Human & experimental toxicology. 2008;27:715-720
- Swan SH. Semen quality in fertile us men in relation to geographical area and pesticide exposure. International journal of andrology. 2006;29:62-68; discussion 105-108
- Tadevosyan N.S., Tadevosyan A.E., Petrosyan M.S. Pesticides application in agricultural of Armenia and their impact on reproductive function in humans. THE NEW ARMENIAN MEDICAL JOURNALVol. 3 (2009), N 2, 41 - 48
- Tagiyeva N, Devereux G, Semple S, Sherriff A, Henderson J, Elias P, Ayres JG. Parental occupation is a risk factor for childhood wheeze and asthma. The European respiratory journal. 2010;35:987-993

- Tan X.H. Risk factors for Parkinson disease and the path analysis: One-to-one paired design. Neural Regeneration Research (2007) 2:2 (117-120)
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson"s disease. Environ Health Perspect. 2011;119:866-872
- Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW. Occupation and risk of Parkinsonism: A multicenter case-control study. Archives of neurology. 2009;66:1106-1113
- Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. Reported residential pesticide use and breast cancer risk on long island, New York. American journal of epidemiology. 2007;165:643-651
- Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. Epidemiological study of high cancer among rural agricultural community of punjab in northern india. International journal of environmental research and public health. 2008;5:399-407
- Tiido T, Rignell-Hydbom A, Jönsson BAG, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvyezday V, Spano M, Manicardi G-C, Bizzaro D, Bonefeld- Jørgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A. Impact of pcb and p, p' dde contaminants on human sperm y:X chromosome ratio: Studies in three european populations and the inuit population in greenland. Environmental Health Perspectives. 2005;114:718-724
- Toft G, Axmon A, Lindh CH, Giwercman A, Bonde JP. Menstrual cycle characteristics in european and inuit women exposed to persistent organochlorine pollutants. Hum Reprod. 2008;23:193-200
- Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, Lindh CH, Pedersen HS, Ludwicki JK, Lesovoy V, Hagmar L, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Thulstrup AM, Bonde JP. Semen quality and exposure to persistent organochlorine pollutants. Epidemiology. 2006;17:450-458
- Toft G, Thulstrup AM, Jonsson BA, Pedersen HS, Ludwicki JK, Zvezday V, Bonde JP. Fetal loss and maternal serum levels of 2, 2', 4, 4', 5, 5'-hexachlorbiphenyl (cb-153) and 1,1-dichloro- 2,2-bis(p-chlorophenyl)ethylene (p,p'-dde) exposure: A cohort study in greenland and two european populations. Environmental health: a global access science source. 2010;9:22
- Ton TG, Longstreth WT, Jr., Koepsell TD. Environmental toxins and risk of narcolepsy among people with hla dqb1\*0602. Environmental research. 2010;110:565-570
- Tondel M, Lindh J, Jonsson P, Vrethem M, Persson B. Occupational determinants of cryptogenic polyneuropathy. Neuroepidemiology. 2006;26:187-194
- Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen Hernandez M, Garcia-Hernandez RM, Del Rio-Garcia C, Wolff MS, Lopez-Carrillo L. In utero p, p'dde exposure and infant neurodevelopment: A perinatal cohort in mexico. Environ Health Perspect. 2007;115:435-439

- Torres-Sanchez L, Schnaas L, Cebrian ME, Hernandez Mdel C, Valencia EO, Garcia Hernandez RM, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and neurodevelopment: A follow-up from 12 to 30 months of age. Neurotoxicology. 2009;30:1162-1165
- Torres-Sanchez L, Zepeda M, Cebrian ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, Lopez-Carrillo L. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Annals of the New York Academy of Sciences. 2008;1140:155-162
- Trabert B, Longnecker MP, Brock JW, Klebanoff MA, McGlynn KA. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ Health Perspect. 2012;120:478-482
- Tsai J, Kaye WE, Bove FJ. Wilms' tumor and exposures to residential and occupational hazardous chemicals. International journal of hygiene and environmental health. 2006;209:57-64
- Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Van Trong P, Nhung NT. Impacts of pesticide use on semen characteristics among rice farmers in kienxuong district, thaibinh province, vietnam. The Southeast Asian journal of tropical medicine and public health. 2007;38:569-575
- Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. Environ Health Perspect. 2009;117:1076-1082
- Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and dde in adults. Environ Health Perspect. 2007;115:1197-1203
- Twum C, Wei Y. The association between urinary concentrations of dichlorophenol pesticides and obesity in children. Reviews on environmental health. 2011;26:215-219
- Ubaidullaeva KM. [the clinical and functional features of chronic obstructive lung disease in patients with organic chlorine pesticides in blood]. Problemy tuberkuleza i boleznei legkikh. 2006:21-23
- Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, Drobna B, Trnovec T, Susienkova K, Labudova V, Gasperikova D, Langer P, Klimes I. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia. 2010;53:899-906
- Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Mdr1 gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16:1172-1177
- Vajdic CM, Fritschi L, Grulich AE, Kaldor JM, Benke G, Kricker A, Hughes AM, Turner JJ, Milliken S, Goumas C, Armstrong BK. Atopy, exposure to pesticides and risk of nonhodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;120:2271-2274
- Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP, Hoppin JA. Chronic bronchitis among nonsmoking farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:574-583

- Valikhani M, Kavusi S, Chams-Davatchi C, Daneshpazhooh M, Barzegari M, Ghiasi M, Abedini R. Pemphigus and associated environmental factors: A case-control study. Clinical and experimental dermatology. 2007;32:256-260
- Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. Prenatal concentrations of polychlorinated biphenyls, dde, and ddt and overweight in children: A prospective birth cohort study. Environ Health Perspect. 2012;120:451-457
- van Amelsvoort L, Mohren D, Slangen J, Swaen G, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Liesivuori J, Tarkowski M, Colosio C, van Loveren H. Immune effects and exposure to ethylenebisdithiocarbamate pesticides in re-entry workers in the netherlands. Human & experimental toxicology. 2008;27:693-699
- van Balen E, Font R, Cavalle N, Font L, Garcia-Villanueva M, Benavente Y, Brennan P, de Sanjose S. Exposure to non-arsenic pesticides is associated with lymphoma among farmers in spain. Occupational and environmental medicine. 2006;63:663-668
- van Bemmel DM, Visvanathan K, Beane Freeman LE, Coble J, Hoppin JA, Alavanja MC. S- ethyln,n-dipropylthiocarbamate exposure and cancer incidence among male pesticide applicators in the agricultural health study: A prospective cohort. Environ Health Perspect. 2008;116:1541-1546
- Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, Schoeters G, Desager K. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 2009;117:122-126
- Vidal JS, Vidailhet M, Derkinderen P, de Gaillarbois TD, Tzourio C, Alperovitch A. Risk factors for progressive supranuclear palsy: A case-control study in France. Journal of neurology, neurosurgery, and psychiatry. 2009;80:1271-1274
- Vidal JS, Vidailhet M, Elbaz A, Derkinderen P, Tzourio C, Alperovitch A. Risk factors of multiple system atrophy: A case-control study in french patients. Movement disorders: official journal of the Movement Disorder Society. 2008;23:797-803
- Viel JF, Floret N, Deconinck E, Focant JF, De Pauw E, Cahn JY. Increased risk of nonhodgkin lymphoma and serum organochlorine concentrations among neighbors of a municipal solid waste incinerator. Environment international. 2011;37:449-453
- Villarejo D, McCurdy SA. The California agricultural workers health survey. Journal of agricultural safety and health. 2008;14:135-146
- Villeneuve S, Cyr D, Lynge E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Fevotte J, Guenel P. Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: A case-control study in europe. Occupational and environmental medicine. 2010;67:837-844
- Vlajinac HD, Sipetic SB, Maksimovic JM, Marinkovic JM, Dzoljic ED, Ratkov IS, Kostic VS. Environmental factors and Parkinson"s disease: A case-control study in belgrade, serbia. The International journal of neuroscience. 2010;120:361-367
- Waggoner JK, Kullman GJ, Henneberger PK, Umbach DM, Blair A, Alavanja MC, Kamel F, Lynch CF, Knott C, London SJ, Hines CJ, Thomas KW, Sandler DP, Lubin JH, Beane Freeman LE, Hoppin JA. Mortality in the agricultural health study, 1993-2007. American journal of epidemiology. 2011;173:71-83

- Walker KM, Carozza S, Cooper S, Elgethun K. Childhood cancer in texas counties with moderate to intense agricultural activity. Journal of agricultural safety and health. 2007;13:9-24
- Waller SA, Paul K, Peterson SE, Hitti JE. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in washington state. American journal of obstetrics and gynecology. 2010;202:241 e241-246
- Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. European journal of epidemiology. 2011;26:547-555
- Wang J, Zhu Y, Cai X, Yu J, Yang X, Cheng J. Abnormal glucose regulation in pyrethroid pesticide factory workers. Chemosphere. 2011;82:1080-1082
- Wang P, Tian Y, Wang XJ, Gao Y, Shi R, Wang GQ, Hu GH, Shen XM. Organophosphate pesticide exposure and perinatal outcomes in shanghai, china. Environment international. 2012;42:100-104
- Wanigasuriya KP, Peiris-John RJ, Wickremasinghe R. Chronic kidney disease of unknown aetiology in Sri Lanka: Is cadmium a likely cause? BMC nephrology. 2011;12:32
- Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ Health Perspect. 2009;117:1007-1013
- Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, Altshul L, Ascherio A. Persistent organochlorine pesticides in serum and risk of parkinson disease. Neurology. 2010;74:1055-1061
- Weisskopf MG, Morozova N, O'Reilly EJ, McCullough ML, Calle EE, Thun MJ, Ascherio A. Prospective study of chemical exposures and amyotrophic lateral sclerosis. Journal of neurology, neurosurgery, and psychiatry. 2009;80:558-561
- Weselak M, Arbuckle TE, Wigle DT, Krewski D. In utero pesticide exposure and childhood morbidity. Environmental research. 2007;103:79-86
- Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D. Pre- and post-conception pesticide exposure and the risk of birth defects in an ontario farm population. Reprod Toxicol. 2008;25:472-480
- Wickerham EL, Lozoff B, Shao J, Kaciroti N, Xia Y, Meeker JD. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment international. 2012;47:80-85
- Wohlfahrt-Veje C, Andersen HR, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Smaller genitals at school age in boys whose mothers were exposed to non-persistent pesticides in early pregnancy. International journal of andrology. 2012;35:265-272
- Wohlfahrt-Veje C, Andersen HR, Schmidt IM, Aksglaede L, Sorensen K, Juul A, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Early breast development in girls after prenatal exposure to non-persistent pesticides. International journal of andrology. 2012;35:273-282
- Wohlfahrt-Veje C, Main KM, Schmidt IM, Boas M, Jensen TK, Grandjean P, Skakkebaek NE, Andersen HR. Lower birth weight and increased body fat at school age in children prenatally exposed to modern pesticides: A prospective study. Environmental health : a global access science source. 2011;10:79

- Wohlfahrt-Veje C., Main K.M., Schmidt I.M., Jensen T.K., Grandjean P., Skakkebaek N.E., Andersen H.R. Effects of prenatal exposure to modern pesticides on birth weight, growth and body composition in childhood; Interactions with maternal smoking and PON1 gene- polymorphisms. Hormone Research in Paediatrics (2011) 76 SUPPL. 2 (232-233).
- Wojtyniak BJ, Rabczenko D, Jonsson BA, Zvezday V, Pedersen HS, Rylander L, Toft G, Ludwicki JK, Goralczyk K, Lesovaya A, Hagmar L, Bonde JP. Association of maternal serum concentrations of 2, 2', 4, 4'5, 5'-hexachlorobiphenyl (cb-153) and 1, 1-dichloro-2, 2-bis (p-chlorophenyl)-ethylene (p, p'-dde) levels with birth weight, gestational age and preterm births in inuit and european populations. Environmental health : a global access science source. 2010;9:56
- Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. Environmental exposures and puberty in inner-city girls. Environmental research. 2008;107:393-400
- Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, Wetmur J. Prenatal pesticide and pcb exposures and birth outcomes. Pediatric research. 2007;61:243-250
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of acute myeloid leukemia in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:112-128
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of non-hodgkin lymphoid neoplasms in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:129-146
- Wu P.-L., Dai B.-T., Yu Z.-H., Yu J., Xian Y., Su Y.-C. Dependablity investigation of the risk factors of childhood leukaemia. Chinese Journal of Evidence-Based Medicine (2010) 10:9 (1037-1040).
- Wu T, Bhanegaonkar AJ, Flowers JW. Blood concentrations of selected volatile organic compounds and neurobehavioral performance in a population-based sample. Arch Environ Occup Health. 2006;61:17-25
- Xia Y, Han Y, Wu B, Wang S, Gu A, Lu N, Bo J, Song L, Jin N, Wang X. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertility and sterility. 2008;89:1743-1750
- Xu JX, Hoshida Y, Yang WI, Inohara H, Kubo T, Kim GE, Yoon JH, Kojya S, Bandoh N, Harabuchi Y, Tsutsumi K, Koizuka I, Jia XS, Kirihata M, Tsukuma H, Aozasa K. Life-style and environmental factors in the development of nasal nk/t-cell lymphoma: A case-control study in east asia. International journal of cancer. Journal international du cancer. 2007;120:406-410
- Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR. Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in u.S. Adults. Environ Health Perspect. 2010;118:60-66
- Xu X, Nembhard WN, Kan H, Kearney G, Zhang ZJ, Talbott EO. Urinary trichlorophenol levels and increased risk of attention deficit hyperactivity disorder among us school-aged children. Occupational and environmental medicine. 2011;68:557-561

- Yang JH, Lee YM, Bae SG, Jacobs DR, Jr., Lee DH. Associations between organochlorine pesticides and vitamin d deficiency in the u.S. Population. PloS one. 2012;7:e30093
- Yang Yang, Zeng Li-Xia, Yan Hong. Analysis of risk factors of birth defects in Shaanxi Province. Journal of XI"an Jiaotong University (Medical Sciences) 2011; Vol. 32 No. 1
- Yiin JH, Ruder AM, Stewart PA, Waters MA, Carreon T, Butler MA, Calvert GM, Davis-King KE, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of pesticide applicators and risk of glioma. Environmental health: a global access science source. 2012;11:39
- Yucra S, Gasco M, Rubio J, Gonzales GF. Semen quality in peruvian pesticide applicators: Association between urinary organophosphate metabolites and semen parameters. Environmental health: a global access science source. 2008;7:59
  - Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in peruvian pesticide sprayers. International journal of occupational and environmental health. 2006;12:355-361
  - Zakerinia M, Namdari M, Amirghofran S. The relationship between exposure to pesticides and the occurrence of lymphoid neoplasm. Iranian Red Crescent medical journal. 2012;14:337-344
  - Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. Muscle invasive bladder cancer in upper egypt: The shift in risk factors and tumor characteristics. BMC cancer. 2008;8:250
  - Zhang Y, Zhu S, Gao Y, Wang XJ, Chen T, Yang Y, Wang GQ, Hu GH, Shi R, Jin P, Tian Y. [a case-control study on correlation of pesticide exposure with childhood acute leukemia]. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2011;45:41-46
  - Zhu JL, Hjollund NH, Andersen AM, Olsen J. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: A study within the danish national birth cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:347-352
  - Zota AR, Aschengrau A, Rudel RA, Brody JG. Self-reported chemicals exposure, beliefs about disease causation, and risk of breast cancer in the cape cod breast cancer and environment study: A case-control study. Environmental health: a global access science source. 2010;9:40
  - Zschiedrich K, Konig IR, Bruggemann N, Kock N, Kasten M, Leenders KL, Kostic V, Vieregge P, Ziegler A, Klein C, Lohmann K. Mdr1 variants and risk of parkinson disease. Association with pesticide exposure? Journal of neurology. 2009;256:115-120

# 用語解説と略語

AHS:農業健康調查

ベータ推定値:線形回帰の係数

バイアス:データの作成、収集、操作及び表示に採用されたプロセスの特性に起因する、または推定 手法の誤ったサンプルデザインに起因する、データの体系的な不正確さ。

バイオマーカー:体内の化学物質の存在、生物学的反応、または健康への有害影響を監視するために 使用できる、人体内の測定可能な物質または特性。ばく露のバイオマーカーは、体内に存在す

る化学物質の量を評価するために使用される。

盲検影響評価:ばく露を評価する個人は、参加者の健康状態を盲検化する。

CARDIA (カーディア): "Coronary Artery Risk Development In Young Adults (若年成人の冠状動 脈リスク発症) "研究、多施設、集団ベースの研究。

- 症例対照研究(Case-control study):観察研究の一種で、結果の異なる2つの既存のグループを特定し、何らかの因果関係に基づいて比較する研究。症例対照研究は、ばく露状況を後ろ向きに 評価する後ろ向き研究である。
- 症例報告:個々の患者の症状、徴候、診断、治療、経過観察の詳細な報告。
- 症例集積:同様の治療を受けたばく露が知られている患者を追跡したり、ばく露と影響について医療 記録を調べたりする記述研究。これらの研究には対照群がない。

センターごとの分析:複数のセンターから募集した参加者がいる研究におけるセンターごとの分析。

- CHAMACOS (チャマコス): Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)。カリフォルニア州サリナスバレーに住む妊婦とその子供の健康に及ぼす農薬やそ の他の環境要因の関連を調べることを目的とした前向き出生コホート。
- CI: 信頼区間
- コホート研究:リスク因子を分析し、調査対象疾患を発症するまで疾患を持たない人のグループを追 跡調査する縦断的/前向き研究。
- 交絡因子(Confounders):従属変数(ばく露)と独立変数(影響)の両方と相関する(正または負の)統計モデルの外部変数。

横断研究:ある特定の時点での参加者全員の観察を行い、ばく露と影響を同じ時点で測定する研究。 生態学的研究:観察の単位が集団または共同体である研究。疾病率とばく露が各集団集積で測定さ

れ、それらの関係が調査される。

効果(2変数/連続変数):影響は2変数法(二項対立、例:がん(はい/いいえ))または連続変数法 (例:収縮期血圧(120mmHg))である。

効果推定値/サイズ:関連の強さの尺度

ESCALE: 全国登録ベースの症例対照研究「Etude sur les cancers de l'enfant」試験

- ファンネルプロット:システマティックレビューやメタアナリシスにおける出版バイアスの有無を確 認するために設計されたグラフ
- 不均一性:メタアナリシスは、類似した研究のグループから複合的な効果を推定するために使用される。しかし、治療効果の個々の推定値は偶然によって変動する;ある程度の変動は予想され

る。問題は、偶然だけで予想される以上の変動があるかどうかである。このような過度の変動 が生じる場合、それは不均一性と呼ばれる。

HR: ハザード比 (Hazard Ratio)

- I<sup>2</sup>:メタアナリシスにおける試験間の一貫性の尺度で、不均一性の測定であり、0(不均一性なし) から1(極端な不均一性)までの値をとる。
- INUENDO: "INUENDO-食品中の生物持続性有機塩素化合物とヒト生殖能、イヌイットと欧州の集団にお ける妊娠までの期間と精液の質の疫学研究"、欧州委員会(the 5th Framework Programme Quality of Life and Management of Living Resources, Key Action 4 on Environment and Health (Contract no. QLK4-CT-2001-00202)(契約番号: QLK4-CT-2001-00202)

(http://www.inuendo.dk))の支援を受けた不妊に関する欧州のプロジェクト。

IRR:発生率比

IQR:四分位間範囲

JEM:職業ばく露マトリックス

MD:平均差

- メタアナリシス (Meta-analysis):様々な統計的手法を用いて研究結果を統合し、以前の別々ではあ るが関連のある研究の結果を検索、選択、結合するプロセスまたは技術。
- Multiple testing (多重検定): 先験的に定義されていない、または先験的仮説に基づいていない、 多くの仮説を検定すること。
- 誤分類:測定誤差に起因する推定値のバイアス
- 多変量モデル:1つ以上の従属変数を持つ統計モデル。これらのモデルは通常、調査対象の分析の交 絡因子の数を調整する。
- コホート内症例対照研究:コホート内症例対照研究では、定義されたコホート内で発生した疾患の症 例が同定され、各症例の疾患発生時までに疾患を発症していないコホート内の患者の中から、 指定された数のマッチした対照が選択される。

NHANES:米国国民健康栄養調查

観察研究:観察研究とは、被験者に対する治療の効果について推論を行うもので、被験者を治療群と 対照群に割り当てるのは研究者の管理外である。

OR:オッズ比

統合効果推定値:メタアナリシスの要約した効果推定値、メタアナリシスの結果

POPs: 残留性有機汚染物質

- 前向き研究 (Prospective study): ある影響に影響を及ぼす可能性のある要因に基づいて個人 (コホ ート)のグループを選択する疫学研究
- 出版バイアス(Publication bias):研究者、編集者、製薬会社が、陽性(すなわち有意な知見を示 す)の実験結果の報告を、陰性(すなわち帰無仮説を支持する)または結論の出ない結果とは 異なる扱いをする傾向から生じたバイアス。
- 回想バイアス(Recall bias):過去の出来事や経験の記憶に対する回想の正確さや完全さの違いに よるシステマティックエラー。

残存交絡:残存交絡因子は、交絡因子が分析で十分に調整されていない場合に発生する(通常、交絡

因子が知られていないため)。

後ろ向き研究:参加した個人を何らかの影響あり(症例)と影響なし(対照)に分類する疫学研究

で、影響は特定の疾患である可能性があり、その影響と関連する可能性のある特定の因子について、その人の病歴を調べる。

- 逆因果関係:逆因果関係とは、因果関係の方向性のことで、ばく露が影響につながったのか、影響が ばく露につながったのかはわからない。
- RR:相対リスク
- ナラティブ・レビュー (Narrative review):実質的な知見や、特定のトピックに対する理論的・方 法論的な貢献を含めて、現在の知識の重要な点を調査するために書かれた論文
- SD:標準偏差

SE:標準誤差

- 代替健康影響(surrogate outcome):患者がどのように感じているか、機能しているか、または生存 しているかを直接測定するものや、ばく露の影響を予測することが期待されるものである臨床 的に意味のあるエンドポイントの代わりに試験で使用される測定値または物理的徴候。
- システマティックレビュー:関連する主要研究を特定、選択、批判的に評価し、レビューに含まれる 研究からデータを抽出、分析するために、体系的かつ明示的な方法を用いて、明確に定式化さ

れた問題に関するエビデンスのレビュー。

第一種の誤り:真の帰無仮説の誤った棄却

UFW: 米国農場労働者 (United Farm Workers)

### EXTERNAL SCIENTIFIC REPORT

Literature review on epidemiological studies linking exposure to pesticides and health effects<sup>1</sup>

### Evangelia E Ntzani, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece

#### ABSTRACT

We performed a systematic and extensive literature review of epidemiological studies examining the association between pesticide exposure and any health outcome published after 2006. We searched 43,259 citations and identified 603 published articles examining a very wide variety of outcomes and presenting over 6.000 analyses between pesticide exposure and health outcomes. We divided the different outcomes into 23 major disease categories. The largest proportion of studies pertains to cancer outcomes (N=164) and outcomes related to child health (N=84). The majority of studies were case-control studies and cross-sectional studies (N=222) and examined occupational exposure to pesticides (N=329). A wide and diverse range of pesticides was studied with studies using various definitions of pesticides; it is very hard to harmonise between studies this information. Despite the large volume of available data and the large number (>6,000) of analyses available, firm conclusions cannot be made for the majority of the outcomes studied. This observation is disappointing especially when one accounts for the large volume of research in the area. However, this observation is in line with previous studies on environmental epidemiology and in particular on pesticides which all acknowledge that such epidemiological studies suffer from many limitations and that the heterogeneity of data is such that does not allow firm conclusions to de made. We also performed updated metaanalysis for major outcomes and for those where a relevant meta-analysis published after 2006 was identified. This has only been possible for childhood leukaemia and for Parkinson's disease. For both these outcomes we found significant associations between pesticide exposure and disease in line with previous evidence.

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### KEY WORDS

Pesticides; epidemiological studies; pesticide exposure; health outcomes; mortality; case control studies; cohort studies

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Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

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# 外部科学報告書

# 農薬ばく露と健康影響に関連する疫学研究に関する文献レビュー1

**Evangelia E Ntzani, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I** Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece

### 概要

我々は、2006年以降に発表された農薬ばく露と健康影響との関連を調査した疫学研究の系統的かつ 広範な文献レビューを行った。43,259件の引用文献を検索し、非常に多様な影響を調査した603件の 論文を同定し、6,000件以上の農薬ばく露と健康影響との間の分析結果を提示した。さまざまな影響を 23の主要な疾患カテゴリーに分類した。研究の中で最も多いのは、発がん影響(N=164)と子どもの健 康に関連した影響(N=84)であった。研究の大部分は症例対照研究と横断研究(N=222)で、農薬への 職業ばく露(N=329)を調査したものであった。さまざまな農薬の定義を用いて研究が行われ、広範囲 かつ多様な農薬が研究されていたため、これらの情報を研究間で調和させることは非常に難しい。利 用可能なデータの量が多く、分析の数も多い(6,000以上)にもかかわらず、研究された結果の大部分 について確固たる結論を出すことはできなかった。これは、この分野の研究量の多さを考慮すると、特 に残念な結果である。しかし、この結果は環境疫学、特に農薬に関するこれまでの研究と一致してお り、疫学研究には多くの限界があり、データの不均一性があるために確固たる結論を出すことができ ないことを認めている。我々はまた、主要な影響及び2006年以降に発表された関連するメタアナリシ スが確認されたものについて、更新されたメタアナリシスを実施した。これは小児白血病とパーキン ソン病についてのみ可能である。これらの影響については、以前のエビデンスに沿って、農薬ばく露と 疾患との間に有意な関連があることが分かった。

• 欧州食品安全機関 (European Food Safety Authority)、2013 年

キーワード 農薬;疫学研究;農薬ばく露;健康影響;死亡率;症例対照研究;コホート研究

### 免責事項

本文書は、上記の著者として特定された機関によって作成され、採用されたものである。この作業は、欧州食品安全機関と著者 との間の契約に基づき、入札手続きを経て落札された著者のみが行ったものである。本文書は、欧州食品安全機関が従う情報公 開の原則に従って公表されている。当局が採用した成果物とはみなされない。欧州食品安全機関は、著者の権利を損なうことな く、本文書で取り上げられた問題及び結論に関して、その権利、見解及び立場を留保する。

<sup>&</sup>lt;sup>1</sup> Question Number EFSA-Q-2012-00372

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#### BACKGROUND AND TERMS OF REFERENCE

Over the last years an abundance of epidemiological studies investigating possible associations of pesticide exposure with adverse health effects on humans have become available. In these studies exposure to pesticides e.g. via inhalation, ingestion, dermal contact or across the placenta has been established as being, or suggested to be, causative for instance for cancer in various organs and tissues, disturbed neurodevelopment of children, allergies, decreased fertility (male and female), birth defects and Parkinson's disease.

However, for many adverse health effects that are attributed to pesticide exposure contradictive or ambiguous studies also exist. Studies vary generally greatly in design (e.g. case control versus cohort studies), sample size and in many cases exposures are rather estimated or assumed than actually determined.

A comprehensive up-to-date literature collection and review covering relevant publications from 1<sup>st</sup> January 2006 to 31<sup>st</sup> March 2012 should be carried out in which also the quality of these studies is evaluated.

The objectives of the contract resulting from the present procurement procedure are as follows:

Objective 1: To collect and compile scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated.

Objective 2: To review and evaluate each collected study in regard to its qualitative aspects (e.g. the corner points of the investigations).

Objective 3: Provision of a database and a report of epidemiological studies.

This contract was awarded by EFSA to: The Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Grecce.

Contractor: The Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Grecce.

Contract title: Literature review on epidemiological studies linking exposure to pesticides and health effects.

Contract number: CFT/EFSA/PRAS/2012/04 - CT 01.

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#### 背景と参考文献

ここ数年の間に、農薬ばく露とヒトへの有害健康影響との関連を調査する疫学研究が豊富に行われ るようになった。これらの研究では、例えば、吸入、経口、経皮及び経胎盤による農薬ばく露が、様々 な臓器や組織のがん、小児の神経発達障害、アレルギー、生殖能力の低下(男性と女性)、先天異常、 パーキンソン病などの原因となる、あるいはその可能性が示唆されていることが立証されている。

しかし、農薬ばく露に起因する多くの有害健康影響については、矛盾した、あるいは曖昧な研究も存 在する。研究は一般的にデザイン(例:症例対照研究とコホート研究)、サンプルサイズ、そして多く の場合、ばく露量は実際の測定値ではなく、むしろ推定値または想定値である。

2006年1月1日から2012年3月31日までの関連出版物を網羅した包括的な最新の文献収集とレビュー を実施し、これらの研究の質も評価すべきである。

今回の調達手続きによる契約の目的は以下の通りである。

目的1:農薬ばく露とヒトへの有害健康影響との関連が調査された科学的出版物を収集・集積する。

目的2:収集された各研究の質的側面(調査のコーナーポイントなど)をレビューし、評価する。

目的3:疫学研究のデータベースと報告書の提供。

本契約は、EFSAが次の者に授与した。イオアニナ大学医学部衛生・疫学部 (Ioannina, Grecce) 契約者: イオアニナ医科大学衛生・疫学部、イオアニナ大学、(Ioannina, Grecce)。 契約タイトル: 農薬へのばく露と健康影響を関連付ける疫学研究の文献調査 契約番号: CFT/EFSA/PRAS/2012/04 - CT 01

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### INTRODUCTION AND OBJECTIVES

This project aims to systematically collect, review and appraise epidemiological studies carried out to investigate possible links of pesticide exposure to health-related outcomes in order to improve understanding of already established or suggested associations with adverse effects in humans. The review focuses on all exposure types either through occupation or in general population with a particular focus on investigating sources of heterogeneity. In particular, we have collected scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated. The available evidence is under review and evaluation with regard to its qualitative aspects. Finally, a database of studies, which examine adverse health effect of pesticides, was compiled.

The final report is structured around health outcome categories and is linked to a *data extraction database*. In the methods we provide a detailed documentation of the search criteria and search strategy used for the literature review and the study selection process. This section also describes the analytical framework with the detailed documentation on the selected exposure and indicators of exposure and the surrogate and clinical outcomes examined. We present the results of the literature search with the full list of eligible studies and the contents of the *data extraction database*. We also present the results of the outcomes and pesticides examined and conclusions based on the literature review findings.

#### BACKGROUND AND AIMS

Pesticides have been widely used against pests that can damage crops such as insects, fungi, rodents, noxious, weeds, in order to prevent or reduce losses and improve product quality, for many years. Their use is very popular; in 2006 and 2007, the world used approximately 5.2 billion pounds of pesticides. However, despite their extensive use, and the associated benefits from pesticide use, there have been concerns on adverse effects in human health as these chemicals are designed to have adverse biological effects on target organisms. Indeed, there is evidence between pesticide use and adverse health outcomes such as cancers, neurodegenerative disease and birth defects; however, results so far have been inconsistent and firm conclusions cannot be drawn for several pesticides.

The aim of this review is to systematically collect, review and appraise epidemiological studies carried out to investigate possible links of pesticide exposure to health-related outcomes. This review includes all exposure types either through occupation or in the general population with a particular focus on investigating sources of heterogeneity. In particular, we have collected and compiled scientific publications in which possible links between pesticide exposure and adverse human health effects have been investigated. The available evidence has been reviewed and evaluated with regard to its qualitative aspects and data from each eligible study has been extracted. Finally, a database of studies, which examine adverse health effects of pesticides, has been compiled with the aim to facilitate the continuous update of results.

The aforementioned aims constitute a stimulating task due to the methodological challenges of environmental epidemiology and pesticide exposure in particular and the vast volume of the peerreviewed literature. Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

### 序文と目的

本プロジェクトは、すでに確立されている、あるいは示唆されているヒトにおける有害影響との関 連についての理解を深めるために、農薬ばく露と健康関連影響との関連を調査するために実施された 疫学研究を体系的に収集し、レビューし、評価することを目的としている。このレビューでは、特に食 い違いの原因を調査することに焦点を当て、職業を通じた、あるいは一般集団におけるすべてのばく 露タイプに焦点を当てている。特に、農薬ばく露とヒトへの有害健康影響との関連が調査された科学 的出版物を収集した。利用可能なエビデンスは、その質的側面に関してレビューと評価が行われてい る。最後に、農薬の有害健康影響を調査した研究のデータベースを作成した。

最終報告書は、健康影響カテゴリーを中心に構成され、データ抽出データベースとリンクしている。 方法のセクションでは、文献レビューと研究の選択プロセスで使用した検索基準と検索戦略の詳細な 文書を提供している。このセクションでは、選択されたばく露とばく露指標及び検討された代用及び 臨床影響に関する詳細な文書を用いた分析的枠組みについても記述している。対象となる研究の完全 なリストとデータ抽出データベースの内容を含めた文献検索の結果を提示する。また、検討した影響 と農薬の結果及び文献レビューの結果に基づく結論を提示する。

### 背景と目的

農薬は、昆虫、真菌、野鼠、不快生物、雑草などの作物にダメージを与えることができる有害生物に 対して広く使用されており、長年にわたり、損失を防止または削減し、作物の品質を向上させるために 使用されている。2006年と2007年には、世界で約52億ポンドの農薬が使用された。しかし、農薬は広範 囲に使用されており、それに関連して農薬の使用による利益があるにもかかわらず、これらの化学物 質は対象生物に有害影響を及ぼすように設計されているため、ヒトへの有害健康影響が懸念されてい る。実際、農薬の使用とがん、神経変性疾患、先天異常などの健康有害影響との間にはエビデンスがあ るが、これまでのところ結果には一貫性がなく、いくつかの農薬について確固たる結論を出すことは できていない。

本レビューの目的は、農薬ばく露と健康関連影響との関連を調査するために実施された疫学研究を 体系的に収集し、レビューし、評価することである。このレビューでは、特に食い違いの原因を調査す ることに重点を置いて、職業を通じた、または一般集団におけるすべてのばく露タイプを対象として いる。特に、農薬ばく露とヒトへの有害健康影響との関連が調査された科学的出版物を収集し、まとめ た。利用可能なエビデンスは質的側面に関してレビューされ評価され、妥当な研究からデータが抽出 されている。最後に、結果の継続的な更新を容易にする目的で、農薬の有害健康影響を調査した研究の データベースを作成した。

前述の目的は、特に環境疫学と農薬ばく露の方法論的課題と、膨大な量の査読付き文献のため、刺激 的な課題を構成している。

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### MATERIALS AND METHODS

### 1. Search strategy

A comprehensive literature search was conducted of peer-reviewed original research pertaining to pesticide exposure and any health outcome. The search strategy was designed so as to identify observational epidemiologic studies published between 1<sup>st</sup> of January 2006 to 30<sup>th</sup> of September 2012 and examining the relationship between pesticide exposures during critical exposure time windows (preconception, pregnancy, childhood, adulthood) and any health-related outcome as discussed previously. The search strategy was developed to search primarily the MEDLINE (1950–to date), and EMBASE (Excerpta Medica Database; 1980 to-date) databases as well as TOXNET (Toxicology Data Network; U.S. National Library of Medicine 2012), OpenSigle (2012), and ProQuest Digital Dissertations and Theses (2012) as supplemental searches.

# 2. Search algorithm for original studies in MEDLINE and EMBASE

This systematic review aimed to identify studies examining any clinical outcome or valid biomarker acting as surrogate for a clinical outcome that has been associated with exposure to pesticides. In order to achieve maximum sensitivity, we did not include any outcome-related search terms in the search algorithm that we developed. For the formation of the search algorithm, we concentrated on pesticides related terms, identified through the MEDLINEMESH terms and EMBASE classification trees on pesticides. In MEDLINE, the MESH terms of pesticides and pesticides (pharmacological action) were examined. Similarly, we examined the pesticide term in the EMBASE Emtree index. We have looked for pesticide categories (i.e. insecticides, herbicides, fungicide etc.) and for specific pesticide names as described in the literature or as pharmacological terms (e.g. DDT or Dichlorodiphenyltrichloroethane) in order to be comprehensive. We have also examined the search terms used in published systematic reviews on pesticide exposure during the past 10 years and looked for any additional terms.

Our first constructed algorithm was long including all aforementioned terms. We piloted different searches and shortened the search to improve the sensitivity of the algorithm with modest impact on the precision. All searches were limited to Humans and to publication date after 1<sup>st</sup> of January 2006.

The long list of pesticide names provided from the MESH database for pesticides pharmacological names only provided 2,270 citations on top of the pesticides related words search (pesticid\* OR pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields] OR "pesticides"[Pharmacological Action]) in MEDLINE. Examination of 200 from those 2,270 citations showed that these did not include epidemiological studies and referred to chemical studies on the substances and chemical formation of pesticides. We therefore adopted the search algorithm including the generic terms. The algorithm was constructed in EMBASE as the database provides a function to study MEDLINE and EMBASE simultaneously (see textbox below). The following algorithm was developed:

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### 材料と方法

### 1. 検索戦略

包括的な文献検索を実施し、農薬ばく露と健康影響に関する査読付きの原著研究を対象とした。検 索戦略は、2006年1月1日から2012年9月30日までに発表された観察疫学研究で、重要なばく露期間(妊 娠前、妊娠期、小児期、成人期)における農薬ばく露と健康関連影響との関係を調査したものを特定す るように設計された。検索戦略は、主にMEDLINE (1950年から現在まで)、EMBASE (Excerpta Medica Database、1980年から現在まで)データベースを使用し、TOXNET (Toxicology Data Network、U.S. National Library of Medicine 2012)、OpenSigle (2012)及びProQuest Digital Dissertations and Theseses (2012)を補足検索として使用した。

### 2. MEDLINE 及び EMBASE における原著論文の検索アルゴリズム

本システマティックレビューは、農薬へのばく露と関連した臨床影響または臨床影響の代用となる 有効なバイオマーカーを調査した研究を特定することを目的とした。最大限の感度を得るために、我々 が開発した検索アルゴリズムには影響に関連する検索用語は含まれていない。検索アルゴリズムの構 築にあたっては、MEDLINEMESHの用語とEMBASEの農薬分類ツリーを用いて同定された農薬関連の用語に 集中した。MEDLINEでは、農薬と農薬(薬理作用)のMESH用語を調べた。同様に、EMBASE Entreeインデ ックスの農薬用語を調べた。農薬のカテゴリー(例えば、殺虫剤、除草剤、殺菌剤など)と、文献に記 載されている特定の農薬名、または薬理学的用語(例えば、DDTまたはジクロロジフェニルトリクロロ エタン)を網羅的に調べた。また、過去10年間の農薬はく露に関する出版されたシステマティックレビ ューで使用された検索用語を調べ、追加の用語を調べた。

最初に構築したアルゴリズムは、前述のすべての用語を含む長いものであった。異なる検索を試験 的に行い、精度への影響を最小限に抑えながらアルゴリズムの感度を向上させるために検索を短縮し た。すべての検索はヒトに限定し、2006年1月1日以降の出版日に限定した。

農薬理学的名称のMESHデータベースから提供された農薬名の長いリストは、MEDLINEの農薬関連 語検索 (pesticid\* OR pesticides"[MeSH Terms] OR "pesticides"[All Fields] OR "pesticide"[All Fields] OR "pesticides"[Pharmacological Action])の上位2,270件の引用のみを提供していた。そ のうち2,270件の引用のうち200件を調査したところ、疫学研究は含まれておらず、農薬の物性や化学 構造に関する化学的研究を参照していることが判明した。そこで、一般用語を含む検索アルゴリズム を採用した。アルゴリズムは、データベースがMEDLINEとEMBASEを同時に検索する機能を提供している ため、EMBASEで構築した(下記のテキストボックスを参照)。以下のようなアルゴリズムを構築した。

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 Table 1:
 Search algorithm for EMBASE and MEDLINE

pesticid\* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid\* OR 'fungicide'/exp OR herbicid\* OR 'herbicide'/exp OR insecticid\* OR 'insecticide'/exp OR molluscacid\* OR 'molluscacide'/exp OR molluscacid\* OR 'molluscacide'/exp OR rodenticid\* OR 'rodenticide'/exp OR carbamate' OR 'carbamate'/exp OR pyrethroid\* OR 'pyrethroid'/exp OR 'agricultural chemical'/exp AND [humans]/lim AND [2006-2013]/py

The algorithm resulted in 43,259 citations in EMBASE and MEDLINE combined. Of those, 14,539 were unique to EMBASE. The algorithm includes all pesticides related terms and subcategories used either as emtree entries with the explode option and also as text words. The explode option ensures that when a term has any more specific, or narrower, index terms within the Emtree thesaurus, they are also automatically retrieved as part of the search. Terms such as organochlorine, glyphosate, paraquat and maneb were excluded as they are part of the pesticide tree of the explode option and are searched. Inclusion of these terms would lead to the same set of results. Figure 1 below shows examples of the indexing trees in EMBASE for some of our search terms.

Lines	Celline
a generate being	- a pharmitalit and thogs
a showing during	
a particula C (20.07 Paulos)	- environmental shamical
a aliminant	applicational chemical C ID and Terring
<ul> <li>A statistical drawdraw</li> </ul>	- centerial
+ attracts and	
a antonio totalia	-a catboot
a lower	· · · · · · · · · · · · · · · · · · ·
+ + interimination	- ettadent anlah
+ 4 United and 10 million	the second second second second second second
+ futured	+ statematic charles
+ + bratim	
+ Seland	···· + Intilizer
÷ + 162608	- Ident
+ + metale	
a leccolet april	- e famosett
+ minute	- CADAD
+ + REALINGTING ANTIONY	+ Contrativity of the
all a specialization political	
+ annual model	e Guittszane
A A DEPOLATION	

Figure 1: Examples of Emtree classification trees

#### 3. Supplemental searches

The database of TOXNET, which lists databases on toxicology, hazardous chemicals, environmental health, and toxic releases, was also searched to identify any information missed from previous search in MEDLINE and EMBASE. We used only the Databases, which look for references in the biomedical literature (i.e. the Toxicology Literature Online (TOXLINE) and the Developmental Toxicology Literature (DART)). The remaining TOXNET databases provided summaries of Chemical, Toxicological, and Environmental Data per chemical substance and were not relevant to this search. For TOXLINE and DART, we used the generic terms "Pesticide OR Pesticides" as longer search algorithms with the inclusion of pesticides subcategories had only minor impact on the number of references identified. The searches were limited to publication dates after 2006, excluding references identified through MEDLINE. The function to identify chemical synonyms to the search term was enabled. Overall, 893 references were retreived from TOXLINE and 34 from DART.

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# 表1: EMBASE 及び MEDLINE の検索アルゴリズム

pesticid\* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid\* OR 'fungicide'/exp OR herbicid\* OR 'herbicide'/exp OR insecticid\* OR 'insecticide'/exp OR molluscacid\* OR 'molluscacide'/exp OR molluscicide'/exp OR molluscicide'/exp OR rodenticid\* OR 'rodenticide'/exp OR carbamat\* OR 'carbamate'/exp OR pyrethroid\* OR 'pyrethroid/exp OR 'chlorinated hydrocarbon//exp OR 'agricultural chemical//exp AND [humans]/lim AND [2006-2013]/py

このアルゴリズムにより、EMBASE と MEDLINE を合わせて 43,259 件の引用が行われた。そのうち 14,539 件は EMBASE に固有のものであった。このアルゴリズムには、分割オプションを使用して Emtree エン トリとして、またテキストワードとして使用された農薬関連の用語とサブカテゴリがすべて含まれて いる。分割オプションを使用すると、用語が Emtree のシソーラス内でより具体的な、またはより狭い インデックス用語を持つ場合、それらも検索の一部として自動的に検索される。有機塩素、グリホサー ト、パラコート、マネブなどの用語は、分割オプションの農薬ツリーの一部であり、検索されるため、 除外された。これらの用語を含めると、同じ結果が得られる。以下の図1は、EMBASE で検索された用 語のインデックスツリーの例を示している。

Real of the second s	
<ul> <li>o chemicale and druge</li> </ul>	Emtree
<ul> <li>o environmental, industrial and domestic chemicals</li> </ul>	<ul> <li>chemicals and drugs</li> </ul>
environmental chemical perficide TI 220-02 finantia	<ul> <li>environmental, industrial and domestic chemicals</li> <li>o environmental chemical</li> </ul>
<ul> <li>analeximusin</li> <li>a aluminam atomatiste</li> </ul>	agricultural chemical C 22,403 Records
<ul> <li>estituitsuspert</li> <li>estesisuttoide</li> </ul>	-o <u>captafol</u>
	-o carboxin
H -+ Extlemeta.zealcide	-o dinitro ortho cresol
# + strenutellast     # fumicant	-o ethylene.oxide
e - e targoide	<ul> <li>ethylmercuric_chloride</li> </ul>
+ futfutil	-o fenamiphos
iii -= tetticide iii -= transticide	• o fertilizer
- a laccode agent	-o folget
at -a mathematik	-o mancozeb
<ul> <li>a praecositiorine pesticide</li> </ul>	e maneb
<ul> <li>a precipiositate precipios</li> <li>a precipiositate</li> </ul>	-o paclobutrazol
H a potenticide	o guintazene

図1:Emtree の分類ツリーの例

# 3. 補足検索

また、毒性学、有害化学物質、環境衛生、有害物質排出に関するデータベースを掲載しているTOXNET のデータベースを検索し、MEDLINEやEMBASEでの前回の検索で見逃した情報を特定した。生物医学文献 (Toxicology Literature Online (TOXLINE)及びDevelopmental Toxicology Literature (DART))の 文献を検索するデータベースのみを使用した。残りのTOXNETデータベースは、化学物質ごとの化学的 データ、毒物学的データ、環境データのサマリーを提供しており、今回の検索には関係なかった。 TOXLINEとDARTについては、「Pesticide OR Pesticides」という一般的な用語を使用したが、農薬のサ ブカテゴリを含む検索アルゴリズムが長くなっても、同定された参考文献の数にわずかな影響しかな かったためである。検索は2006年以降の出版日に限定し、MEDLINEで同定された文献は除外した。検索 語の化学物質の同義語を特定する機能を有効にした。全体では、TOXLINEから893件、DARTから34件の文 献が検索された。

また、ヨーロッパで出された灰色文献[非商業出版物](論文)の書誌的参考文献700,000件を収録したSystem for Information on Grey Literature in Europe (OpenSigle)も調べた。2006年以降に出

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We also looked into the System for Information on Grey Literature in Europe (OpenSigle), which includes 700.000 bibliographical references of grey literature (paper) produced in Europe. There were no bibliographical references on pesticides (search term pesticid\*) published after 2006.

We have also constructed a search algorithm to search the ProQuest Digital Dissertations and Theses database. We excluded from our search articles published in scholarly journals as those will have been identified through MEDLINE and EMBASE. We used the search term "pesticide\* AND health" and limited our search to specific subjects (environmental science OR public health OR environmental health OR epidemiology OR pesticides OR nutrition OR occupational health) and to publication dates between 2006 and 2012. This search strategy resulted in 1,713 results. Results were numerous when no subject limits were used (12,135) or when the term "health" was excluded from the initial algorithm (18,195).

Finally, the reference lists of all identified eligible studies and systematic reviews are scanned during data extraction for additional references.

#### 4. Search for literature systematic reviews and meta-analysis

We also performed targeted searches for systematic reviews and meta-analysis in relation to specific outcomes. We restricted the search for reviews on those outcomes where more than 4 studies had been identified and we performed targeted searches in MEDLINE using the name of the outcome along with the keywords "systematic review OR meta-analysis" limited to the title or the abstract of the paper.

### 5. Structure of this report

This report is structured around health outcome categories and provides the results for each outcome group separately. A section on general conclusions is presented at the end. At the end of each section on outcomes and tables and figures are presented to allow ease of reading. Also, the ID numbers of each eligible article are referenced throughout the text. These correspond to the ID for each health outcome group in the *data extraction database* which has been provided as a separate file to this report. The ID is defined with an abbreviation for the specific health outcome and a study number.

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版された農薬(検索語pesticid\*)に関する書誌的文献はなかった。

また、ProQuest Digital Dissertations and Thesesデータベースを検索するための検索アルゴリズ ムを構築した。MEDLINEやEMBASEで検索した結果、学術雑誌に掲載された論文は検索対象から除外した。 検索語を"pesticide\* AND health "とし、特定の主題(環境科学 OR 公衆衛生 OR 環境保健 OR 疫学 OR 農薬 OR 栄養 OR 労働衛生)と、2006年から2012年までの出版日に限定して検索を行った。この検 索戦略の結果、1,713件の検索結果が得られた。主題制限が行われていない場合(12,135)、または「健 康」という用語が最初のアルゴリズムから除外されている場合(18,195)には、多くの検索結果が得ら れた。

最後に、特定されたすべての妥当な研究とシステマティックレビューの参考文献リストをデータ抽 出の際にスキャンして追加の参考文献を探した。

# 4. 文献システマティックレビューとメタアナリシスの検索

また、特定の健康影響に関連したシステマティックレビューやメタアナリシスを対象とした検索を 行った。MEDLINEでは、4件以上の研究が確認された健康影響を対象としたレビューに限定し、健康影響 名と「システマティックレビュー OR メタアナリシス」というキーワードを用いて、論文のタイトル または要約に限定して検索を行った。

### 5. 本報告書の構成

本報告書は、健康影響のカテゴリーを中心に構成されており、各健康影響のグループの結果を個別 に提供している。最後に一般的な結論のセクションが提示されている。また、各健康影響のセクション の最後には、読みやすいように表と図を掲載している。また、本文中では各対象論文の ID 番号を参 照している。これらは、本報告書に別ファイルとして提供されているデータ抽出データベースの各健 康影響グループの ID に対応している。IDは、特定の健康影響の略語と研究番号で定義されている。

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### Table 2: Summary of recourses searched, search terms and references identified

			N referen
Database		Limits	ces
MEDLINE	pesticid* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'insecticide'/exp OR insecticid* OR 'insecticide'/exp OR molluscacid* OR 'molluscacide'/exp OR molluscacid* OR 'molluscicide'/exp OR rodenticid* OR 'rodenticide'/exp OR carbamat* OR 'carbamate'/exp OR pyrethroid* OR 'pyrethroid'/exp OR or 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp pesticid* OR 'pesticide'/exp OR 'chemical pest control'/exp OR fungicid* OR 'fungicide'/exp OR herbicid* OR 'herbicide'/exp OR molluscacid* OR 'molluscacide'/exp OR molluscacid* OR 'molluscacide'/exp OR carbamat* OR 'molluscacide'/exp OR carbamat* OR 'molluscacide'/exp OR carbamat* OR 'rodenticide'/exp OR carbamat* OR	Humans, Publication date: 2006-2012	28,729
EMBASE	'pyrethroid'/exp OR 'chlorinated hydrocarbon'/exp OR 'agricultural chemical'/exp	Humans, Publication date: 2006- 2012, no references identified through MEDLINE Publication date: 2006-2012, no references identified through	14,530
TOXLINE	Pesticide OR Pesticides	references identified through MEDLINE Publication date: 2006-2012, no references identified through	893
DART	Pesticide OR Pesticides	MEDLINE	34
OpenSigle	Pesticide*	Publication date: 2006-2012 Publication date: 2006-2012, Subjects (environmental science, public health, environmental health, epidemiology, pesticides, nutrition, occupational health), no articles published in scholarly	0
ProQuest	Pesticide* AND health	journals	1,713 Total: 45,899

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# 表2:検索されたリソース、検索語及び特定された参照先の概要

データ ベース	检索条件	Limits	文献数
	pesticid* OR 'pesticide'/exp OR 'chemical pest		
	control'/exp OR fungicid* OR 'fungicide'/exp		
	OR herbicid* OR 'herbicide'/exp OR insecticid*		
	OR 'insecticide'/exp OR molluscacid* OR'		
	molluscacide'/exp OR molluscicid* OR		
	'molluscicide'/exp OR rodenticid* OR		
	'rodenticide'/exp OR carbamat* OR		
	'carbamate'/exp OR pyrethroid* OR		
	'pyrethroid'/exp OR 'chlorinated		
	hydrocarbon'/exp OR 'agricultural		
MEDLINE	chemical'/exp	ヒト, 公開日:2006~2012	28,72
	pesticid* OR 'pesticide'/exp OR 'chemical pest		
	control'/exp OR fungicid* OR 'fungicide'/exp		
	OR herbicid* OR 'herbicide'/exp OR insecticid*		
	OR 'insecticide'/exp OR molluscacid* OR'		
	molluscacide'/exp OR molluscicid* OR		
	'molluscicide'/exp OR rodenticid* OR		
	'rodenticide'/exp OR carbamat* OR		
	'carbamate'/exp OR pyrethroid* OR		
	'pyrethroid'/exp OR 'chlorinated	ヒト, 公開日:2006~2012,	
	hydrocarbon'/exp OR 'agricultural	MEDLINE で確認された文献は無	
EMBASE	chemical'/exp	ℓ <sup>™</sup> ₀	14, 53
		公開目:2006~2012年、MEDLINE	
TOXLINE	Pesticide OR Pesticides	で確認された文献は無い。	89
		公開日:2006~2012年、MEDLINE	
DART	Pesticide OR Pesticides	で確認された文献は無い。	3-
OpenSigle	Pesticide*	掲載時期:2006年~2012年	
		掲載時期:2006年~2012年、	
		主題(環境科学、公衆衛生、環	
		境保健、疫学、農薬、栄養、労	
		働衛生)、専門的学術誌に掲載	
ProQuest	Pesticide* AND health	された論文は無い。	1,71
			合計
			45, 89

### 6. Selection of studies

All titles identified through the literature search of various databases were screened to identify studies, which evaluated the association between pesticides and health outcomes including any surrogate outcome. All abstracts of the selected titles are then screened in duplicate to identify epidemiological studies linking pesticide exposure to any health outcome including surrogate outcome. Both primary studies and systematic reviews or meta-analyses are selected. Articles that potentially meet eligibility criteria at the abstract screening stage have been retrieved and the full text articles have been reviewed in duplicate for eligibility. The reason for rejection of all full text articles has been recorded.

#### 6.1. Eligibility criteria for full text articles

We included observational studies assessing the association between pesticide exposure and healthrelated outcomes. We included cohort, cross-sectional and case- control studies. We included studies performed in humans published from 1<sup>st</sup> of January 2006 to 30<sup>th</sup> of September 2012. Animal studies and studies performed in human cells have been excluded. We had no language, population or geographical restrictions. To enhance totality of the evidence, all types of pesticides have been considered. Exposure to pesticides was defined as reported use of pesticides by the study participant or by government registry data (self administrated questionnaires, interviewer administrated questionnaires, job exposure matrix (JEM)), by residential status (proximity to pesticide exposure), by detecting biomarkers associated with pesticide exposure or by any other means as defined by each study. Eligible health-related outcomes such as neurocognitive scales, or laboratory surrogate outcomes with an established association with clinical outcomes, such as liver enzymes.

Narrative reviews, case-series and case-reports (studies without control populations) are excluded. We also excluded studies assessing the health-related effect of pesticide poisoning or accidental high-dose pesticide exposure. We have excluded studies with no availability of sufficient quantitative information reported in the article (e.g. effect estimates) so that effect sizes or measures of associations can be calculated. Whenever reports pertained to the same study at different follow-up periods and examining the same outcome, we retained the one with the longer follow-up to avoid data duplication. We also excluded studies that referred to fertilizers (exploded from the algorithm term "agricultural chemical") as well as studies referred to the adverse effects of substances used as therapy for various medical conditions such as warfarin for anticoagulation or agents used in the treatment of scabies. Solvents and other non-active ingredients in pesticides/herbicides were not considered eligible. We excluded studies that investigate the various effects of Agent Orange on chemical warfare veterans as they represent cases of very high dose exposures. Finally, studies which examined the association between exposure and biomarkers of exposure were also not considered eligible as they do not examine health outcomes. Finally, following consultation with EFSA, we excluded studies/analyses investigating exposure to pesticides: arsenic,  $\alpha_i$ ,  $\beta_i$ , hexachlorocyclohexane (HCH), lead, dioxins and dioxin-like compounds including polychlorinated biphenyls (PCBs) as these chemicals were not considered relevant for the current project.

Regarding systematic reviews and meta-analyses, we considered all systematic reviews and metaanalyses that systematically assessed the effect of pesticide exposure to health-related outcomes, regardless of the pesticide, exposure window and outcome assessed. We included all publications where a systematic approach was endorsed (systematic literature search, assessment of methodological characteristics of the included studies and, if a meta-analysis was performed, the use of standard analytical tools including the use of a weighted summary estimate and a formal appraisal of heterogeneity). Narrative reviews are excluded.

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#### 研究の選択

様々なデータベースの文献検索で特定されたすべてのタイトルをスクリーニングし、農薬と代替健 康影響を含む健康影響との関連を評価した研究を特定した。次に、選択したタイトルのすべての要約 を重複してスクリーニングし、農薬ばく露と代替健康影響を含むあらゆる健康影響との関連を評価す る疫学研究を特定した。主要研究とシステマティックレビューまたはメタアナリシスの両方を選択す る。要約審査の段階で妥当性基準を満たす可能性のある論文を検索し、全文論文を重複して審査して 妥当性を確認した。すべての全文論文の却下理由は記録されている。

### 6.1. 全文論文の妥当性基準

農薬ばく露と健康関連影響との関連を評価する観察研究を対象とした。コホート研究、横断研究、症 例対照研究を対象とした。2006 年 1 月 1 日から 2012 年 9 月 30 日までに発表されたヒトを対象 とした研究を対象とした。動物を対象とした研究及びヒトの細胞を対象とした研究は除外した。言語、 集団、地理的制限はなかった。エビデンスの全体性を高めるために、すべての種類の農薬を考慮した。 農薬へのばく露は、研究参加者による農薬の使用報告、または政府登録データ(自己管理質問紙、面接 官管理質問紙、職業ばく露マトリックス(JEM))、居住状況(農薬ばく露への近接性)、農薬ばく露 に関連するバイオマーカーの検出、または各研究で定義されたその他の手段によって定義されたもの とした。妥当な健康関連影響は、腫瘍形成やパーキンソン病などの「主要な」臨床影響、神経認知スケ ールなどの臨床代替健康影響、肝酵素などの臨床影響との関連が確立されている代用実験的影響とし た。

ナラティブレビュー、症例集積、症例報告(対照集団のない研究)は除外した。また、農薬中毒また は不慮の高用量農薬ばく露による健康関連の影響を評価する研究も除外した。論文で報告された十分 な量的情報(効果推定値など)が入手できない研究は除外し、効果量や関連の尺度を計算できるように した。異なる追跡期間で同じ研究に関連する報告があり、同じ結果を調査している場合は、データの重 複を避けるために、追跡期間が長い方の研究を保持した。また、肥料に言及した研究(アルゴリズム用 語「農薬」から分解)や、抗凝固剤のワルファリンや疥癬の治療に用いられる薬剤など、様々な病状の 治療に用いられる物質の副作用に言及した研究も除外した。農薬・除草剤に含まれる溶剤などの非有 効成分は対象外とした。化学戦経験者に対するオレンジ剤の様々な影響を調査した研究は、極高用量 ばく露の事例であるため除外した。最後に、ばく露とばく露のバイオマーカーとの関連を調査した研 究も、健康への影響を調査していないため、対象外とした。最後に、EFSAとの協議により、ヒ素、アル ファ及びベータへキサクロロシクロへキサン(HCH)、鉛、ポリ塩化ビフェニル(PCB)を含むダイオキ シン類などの農薬へのばく露を調査した研究/分析は、本プロジェクトには関係がないと考えられる ため除外した。

システマティックレビュー及びメタアナリシスについては、評価された農薬、ばく露城、影響にかか わらず、健康関連影響に対する農薬ばく露の影響をシステマティックに評価したすべてのシステマテ ィックレビュー及びメタアナリシスを調査した。系統的アプローチ(系統的文献検索、対象とした研究 の方法論的特徴の評価、メタアナリシスが実施された場合には重み付け要約推定値の使用や不均一性 の形式的評価を含む標準的な分析ツールの使用)が承認されているすべての出版物を対象とした。ナ ラティブレビューは除外する。

#### 6.2. Quality control measures

The pilot literature searches have all been performed in duplicate. In addition, the first 500 results of all title searches were performed in duplicate and results were compared between investigators, which displayed high levels of agreement. The kappa statistic for agreement was 0.78. Two independent research group members performed in duplicate the abstract screening, the full text screening and the data extraction. All discrepancies are resolved by consensus or by a third arbitrator.

#### 6.3. Data extraction database

We have constructed and tested the *data extraction database* with data extraction items and quality assessment items that were implemented through the whole process. The data extraction database has been structured in 7 domains: Reference, Time period, Study characteristics, Exposure assessment, Outcomes, Statistical analysis and Quality assessment (separate database file). The first 6 domains pertain to information directly extracted from the full-texts of eligible studies and would be primarily used to select studies for quantitative synthesis and aid quantitative synthesis. Studies contribute one row in the database for each outcome examined and for each exposure examined. When studies present various definitions of exposure we select for data extraction the most comprehensive definition of exposure and subsequently the one with the largest sample size. However when studies include any type of quantitative information for different biomarkers used for the identification of the same chemical substance e.g. p,p'-DDT and p,p'-DDE for dichlorodiphenyltrichloroethane (DDT), they are all reported in separate rows. When studies present data in subgroups (e.g. males and females) we extract only their main analysis (whole group) unless the data is only presented in subgroups in which case multiple rows are presented. Analyses regarding different pesticide classes and different health outcomes are extracted individually. Appendix II explains all the items used in the data extraction database.

The data extraction form was validated through a robust and systematic procedure. Specifically, various versions of the form were validated after blinded loops of extracting information for studies randomly selected from the database. We opted for the maximum agreement while preserving the comprehensiveness of the database. Two investigators extract each item independently and discrepancies are resolved with discussion.

#### 6.4. Quality appraisal

The last part of the *data extraction database*, concerns the methodological appraisal of each eligible paper. The areas that we have focused are the study design, the study population, the level of details in exposure definition and the methods of exposure measurement and the specificity of the measurement. These are crucial questions to be asked in exposure assessment epidemiology. We have also focused on the efforts undertaken to account for confounders through matching or multivariable models, blinded exposure assessment and well-defined and valid outcome assessment. We have also looked at whether the source of funding was acknowledged. The elements of the methodological appraisal were considered from the RTI item bank. The RTI item bank is a practical and validated item bank for evaluating the risk of bias and precision of observational studies of interventions or exposure included in systematic evidence reviews. The questions were adapted to reflect exposure assessment in green, orange and red with green representing low risk of bias and red high risk of bias. Table 3

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### 6.2. 品質管理対策

パイロット文献検索はすべて重複して実施した。さらに、すべてのタイトル検索の最初の500件の結 果は重複して行い、研究者間で結果を比較し、高いレベルの一致を示した。同意度のカッパ統計量は 0.78であった。2人の独立した研究グループのメンバーが、要約スクリーニング、全文スクリーニング、 データ抽出を重複して行った。すべての不一致は、合意または第三者の判定によって解決した。

### 6.3. データ抽出データベース

全工程を通じて実行されたデータ抽出項目と品質評価項目を用いて、データ抽出データベースの構 築及び確認を行った。データ抽出データベースは7つのドメイン:参考文献、期間、試験特性、ばく露 評価、影響、統計分析、品質評価(別々のファイル)で構成されている。最初の6つの領域は、対象 となる研究の全文から直接抽出された情報に関連しており、主に定量的統合のための研究を選択し、 定量的統合を支援するために使用される。研究は、調査された各影響と調査された各ばく露について、 データベースの一行を提供している。研究が様々なばく露の定義を提示している場合には、最も包括 的なばく露の定義をデータ抽出のために選択し、それに続いてサンプルサイズが最も大きいものを選 択する。しかし、同じ化学物質の同定に使用される異なるバイオマーカーの定量的情報を含む研究、例 えばジクロロジフェニルトリクロロエタン(DDT)の p,p'-DDT と p,p'-DDE のように、それらはすべ て別々の行で報告されている。研究がサブグループでデータを提示している場合(例:男性と女性) は、データがサブグループでのみ提示されている場合は複合論争が提示されている場合を除き、主分 析(グループ全体)のみを抽出している。異なる農薬クラスと異なる健康影響に関する分析は、個別に 抽出されている。付録IIでは、データ抽出データベースで使用されたすべての項目を説明している。

データ抽出フォームは、妥当で体系的な手順を経て検証された。具体的には、データベースから無作 為に選択された研究の情報を抽出するプラインドループの後、様々なバージョンのフォームを検証し た。データベースの網羅性を維持しつつ、最大の一致度が得られるものを選択した。各項目について は、2名の研究者がそれぞれ独立して抽出し、不一致は議論によって解決している。

### 6.4. 品質評価

データ抽出データベースの最後の部分は、対象となる各論文の方法論的評価に関するものである。 ここでは、研究デザイン、研究集団、ばく露の定義の詳細度、ばく露測定の方法、測定の特異性などに 焦点を当てている。これらはばく露評価の疫学において問われる重要な問題である。我々はまた、マッ チングモデルや多変量モデル、盲検化されたばく露評価、十分に定義された有効な影響評価によって 交絡因子を考慮するための努力にも焦点を当ててきた。また、資金源が認められているかどうかにも 注目した。方法論的評価の要素は RTI 項目バンクから調査した。RTI 項目バンクは、システマティッ ク・エビデンスレビューに含まれる介入またはばく露の観察研究のバイアスのリスクと精度を評価す るための実用的で妥当性のある項目バンクである。質問はばく露評価を反映するように適応されてい る。質的評価の質問では、質的評価への回答を一貫して緑、オレンジ、赤で色分けし、緑はバイアスの リスクが低く、赤はバイアスのリスクが高いことを表している。以下の表 3 は、それぞれどの回答が 低リスク、高リスクと考えられたかを説明したものである。しかし、品質評価の質問は、各研究に関連

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below explains which answers were considered low and high risk respectively. However, the quality appraisal questions should be interpreted with caution, as they are only suggestive of the risk of bias associated with each study. There may be studies which score high in this quality assessment and still have a high risk of bias and vice versa. A final column was constructed to grade the overall quality of the studies in low, intermediate and high. This classification was based on the answers to the methodological assessment questions as explained in Table 3.

#### Table 3: Methodological assessment of eligible studies

Question	High risk.	Low risk
Study design (prospective, retrospective, mixed, cross-sectional)	Retrospective, mixed, NA	Prospective
Inclusion/exclusion criteria clearly stated (yes, partially, no)	No	Yes
Authors mention power calculations (yes, no)		Yes
Level of detail in describing exposure (high, medium, low)	Low	High
Robust measurement of exposure. (biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	No	Yes
Were measures of exposure specific? yes; based on broader, chemically- related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	No	Yes
Attempt to balance the allocation between the groups (e.g., through stratification, matching)	No	Yes
Adjustment performed for potential confounders (yes, some, no)	No	Yes
Assessors blinded to exposure status (for cohort studies)	No	Yes
Outcomes assessed using valid and reliable measures, implemented		
consistently across all study participants?	No	Yes
Sample size	Low	Тор
Rough quality assessment	>6 answers high risk.	>6 asnwers low risk

#### 6.5. Quantitative synthesis

Quantitative synthesis of the results was only attempted when there were more than 4 studies per examined outcome and when there was no substantial heterogeneity among the published evidence. The presence and extent of heterogeneity was assessed by the I<sup>4</sup> (ranging from 0% to 100%) (Ioannidis 2007). We have summarized the RR/OR estimates using fixed and random-effects models (Lau 1997). Fixed-effects models assume that there is a common underlying effect and the variability observed is attributed to chance alone; random effects models acknowledge that true between-study heterogeneity exists and take into account the presence of heterogeneity into their calculations. In the absence of heterogeneity, fixed-and random-effects models yield the same results. Publication bias was assessed using funnel plots and visual inspection of the results.

For each outcome with more than 5 eligible studies quantitative synthesis was attempted. We did not include data from the same cohort study; either presented in the same or in different publications in the same meta-analysis when the groups compared were not mutually exclusive. For each outcome with more than 4 studies, we also looked for previously published meta-analyses to compare results and to interpret our findings in the context of previous studies. Meta-analyses were found through a) systematic reviews and meta-analyses identified through our literature review and b) targeted searches in PUBMED to identify published meta-analyses for each outcome of interest. We attempted to update any previously published meta-analyses with our results when the meta-analysis a) included studies published by 2006 and b) when outcome and exposure definitions were comparable with the definitions used in this report. Finally, we also plotted funnel plots to visually inspect asymmetry when more than 10 studies were include in the meta-analysis.

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するバイアスのリスクを示唆しているに過ぎないので、注意して解釈する必要がある。この品質評価 のスコアが高くても、バイアスのリスクが高い研究もあれば、その逆もあるかもしれない。最後の列 は、研究の全体的な質を低、中、高で評価するために作成された。この分類は、表3で説明した方法論 的評価の質問に対する回答に基づいている。

### 表3:対象研究の方法論的評価

営費	高リスク	低リスク
研究デザイン(有望、回顧約、混合、新進的)	回顧的、混合、該 当なし	有望
触外基準が明確に記載されている (はい、部分的に、いいえ)	1112	1261
署者は電力計算について言及しています(はい、いいえ)		TELS
暴霧の記述の詳細レベル(高、中、係)	任	商
暴霧のロバストな測定(パイオマーカー(有):小面積生態学的尺度、 職種、アンケート(部分的):大面積生態学的尺度(無)に基づいてい る。)	いいえ	(zL)
曝霧の尺度は特定のものだったか?はい:より広範な化学的に関連したグループに基づいて(部分的)。多様な化学的および毒性学的特性の 広範なグループに基づいて(いいえ)。	いいえ	(zt)
グループ間の配分のパランスを図る (層別化、マッチングなど)。	いいえ	1261
層在的な交綿因子の顕整を行った(はい、いくつか、いいえ)。	いいえ	IZLN
被ばく状態に盲袂化された評価者(コホート研究の場合)	いいえ	1211
有効かつ信頼性の高い尺度を用いて評価された結果は、すべての研究 参加者に一貫して実施されているか?	11112	1211
サンプルサイズ	绝	最大
ラフな品質評価	6 以上の回答で高り スク	6 以上の回答で低リス ク

### 6.5. 定量的な統合

結果の定量的な統合は、調査された影響ごとに4件以上の研究があり、発表されたエビデンス間に実 質的な不均一性がない場合にのみ試みられた。不均一性の存在と程度は、I2(0%から100%の範囲)で 評価した(Ioannidis 2007)。固定効果モデルとランダム効果モデルを用いて、RR/ORの推定値をまと めた(Lau 1997)。固定効果モデルでは、共通の基礎となる効果が存在し、観察された変動は偶然のみ に起因すると想定している。不均一でない場合、固定効果モデルとランダム効果モデルでは同じ結果 が得られる。出版バイアスは、ファンネルプロットと結果の視覚的精査を用いて評価した。

5件以上の妥当研究がある各影響について、定量的な統合を試みた。比較するグループが相互に排他 的でない場合には、同じメタアナリシスにおいて、同じコホート研究からのデータはなかった。4件以 上の研究がある各影響については、結果を比較し、以前の研究の背景の中で我々の知見を解釈するた めに、以前に発表されたメタアナリシスも探した。メタアナリシスは、a)文献レビューで同定されたシ ステマティックレビュー及びメタアナリシス及びb)調査対象の各影響について公表されているメタア ナリシスを同定するために、PUBMDBDでの標的検索によって発見された。メタアナリシスのa) 2006年ま でに発表された研究が含まれており、b)影響及びばく露の定義が本報告書で使用されている定義と同 等である場合には、以前に発表されたメタアナリシスを我々の結果で更新するよう試みた。最後に、10 件以上の研究がメタアナリシスに含まれている場合に非対称性を視覚的精査するためにファンネルプ ロットをプロットした。

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### RESULTS

### 7. Overall results

This section focuses on the literature review results including flowcharts with number of studies screened and deemed eligible as well as number of excluded studies and corresponding reasons. It also provides an overview of the studies identified and their main characteristics.

#### 7.1. Selection process for individual studies

Of the 43,259 retrieved citations, 40,477 were excluded at the title screening level. Of the 2,782 remaining titles, a further 1,654 were excluded after the abstract screening. We thus deemed eligible, 1,128 citations to be scrutinized at the full-text level of which 1,101 were original research articles and 27 were systematic reviews or meta-analyses. Of the 1,101 original articles, 184 were excluded (Figure 2). For few (101) publications were the full text (or abstract for conference presentations) has not been found online, we sought the full text through letters to authors and investigations from our library. This has not been possible for 58 studies for which we extracted information from the abstract only.

Main reasons for exclusion at the full-text level pertained to: no quantitative information/ data (these were mainly abstract presentations or comments/ editorials which did not present any quantitative information for the association between pesticides and health outcomes, n=108); duplicate records (n=28), no implied use of pesticides (n=18), studies on poisoning or accidental very high doses (n=11), reviews with no primary data (n=11), no data on health outcomes (n=8). Supplemental searches did not succeed to provide additional references as they resulted in a large number of policy documents, grant applications documents and studies already retrieved. Supplemental searches through reference lists of identified studies and especially the reference lists of identified systematic reviews will continue during data extraction and any new identified studies will be added to the current list of eligible studies. During full text screening and data abstraction a further 301 studies were excluded. The main reason for exclusion was no eligible pesticide, such as Polychlorinated Biphenyls (PCBs) (Figure 2). Overall, 602 individual publications were eligible for inclusion in the present review. These 602 publications correspond to 6,479 different analyses, which are also present in the *data extraction database*.

# 結果

### 7. 全体の結果

このセクションでは、スクリーニングされ、妥当と判断された研究の数、除外された研究の数とそれ に対応する理由を示すフローチャートを含む文献レビューの結果に焦点を当てている。また、同定さ れた研究とその主な特徴の概要についても説明する。

### 7.1. 個々の研究の選択プロセス

検索された43,259件の引用のうち、40,477件がタイトルのスクリーニングレベルで除外された。残 りの2,782タイトルのうち、さらに1,654タイトルが要約スクリーニングで除外された。その結果、1,128 件の引用文献のうち、1,101件が原著論文であり、27件がシステマティックレビューまたはメタアナリ シスであることが判明した。1,101 本の原著論文のうち 184 本が除外された(図 2)。また、全文(ま たは学会発表のための要約)がオンラインで見つからない出版物(101 件)については、著者への手紙 や蔵書からの調査により全文を検索したが、要約のみから情報を抽出した58研究については、これが 不可能であった。

全文レベルで除外した主な理由は次の通りである:定量的な情報/データがない(これらは主に、農 薬と健康影響との関連に関する定量的な情報を提示していない要約発表またはコメント/論説であっ た、n=108);記録の重複(n=28)、農薬の使用が示唆されていない(n=18)、中毒または不慮の極高 用量ばく露に関する研究(n=11)、主要データのないレビュー(n=11)、健康影響に関するデータがな い(n=8)。補足検索では、大量の政策文書、助成金申請書、研究が既に検索されたため、追加の参考 文献を提供することはできなかった。識別された研究の参照リスト、特に識別されたシステマティッ クレビューの参照リストからの補足検索は、データ抽出の間も継続され、新たに識別された研究は、現 在の妥当な研究リストに追加される。全文スクリーニングとデータ抽出の際に、さらに301研究が除外 された。除外の主な理由は、ポリ塩化ビフェニル(PCB)などの妥当な農薬がなかったことであった(図 2)。全体として、602の個別の出版物が本レビューに含めることができた。これらの602の出版物は、 6,479の異なる分析に対応しており、データ抽出データベースにも存在する。

Any enquiries related to this output should be addressed to pesticides.ppr@efsa.europa.eu

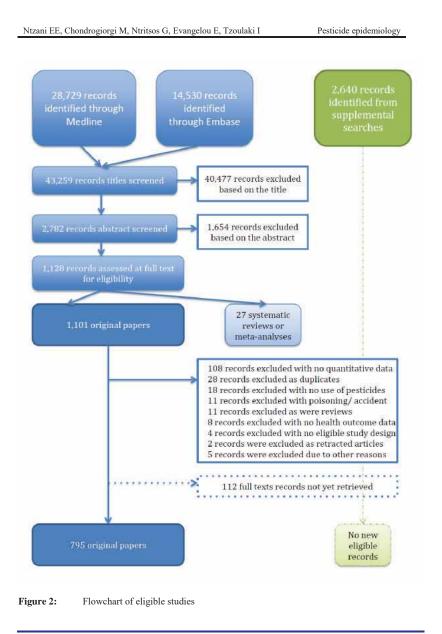
Suggested citation: Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I, 2013. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA supporting publication 2013:EN-497, 159 pp.

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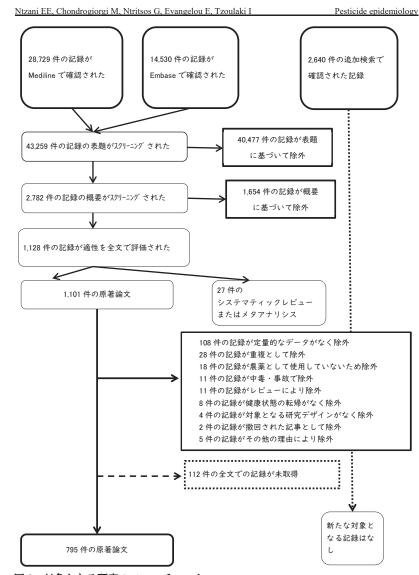


図2:対象となる研究のフローチャート

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#### 7.2. Evidence map tables and outcomes examined

We observed a great variety of assessed outcomes covering a wide range of pathophysiologies. "Hard" clinical outcomes as well as many surrogate outcomes are present in the database reflecting the different methodologies endorsed to approach the assessed clinical research questions. We divided the different outcomes into 23 major disease categories (Table 4 and Figure 3). The largest proportion of studies pertains to cancer outcomes (N=164) and outcomes related to child health (N=84). Table 4 corresponds to the Evidence map Table and shows the outcome mapping of the project describing all outcomes that have been associated with pesticide exposure between 2006 and 2012 and their frequency.

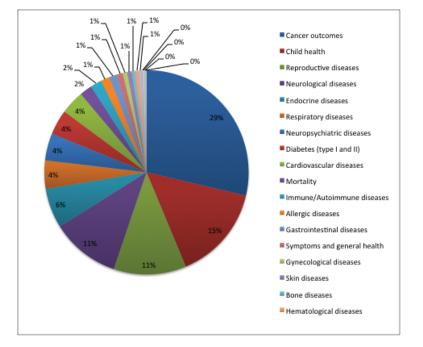


Figure 3: Major outcome categories and corresponding percentage of studies examining those outcomes among the eligible publications

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# 7.2. エビデンスマップ表と調査した影響

我々は、幅広い病態生理をカバーする多種多様な評価された影響を調査した。このデータベースに は、評価された臨床研究上の質問にアプローチするために承認された様々な方法論を反映して、「ハー ド」な臨床影響と多くの代替健康影響が存在している。我々は、異なる影響を23の主要な疾患カテゴリ ーに分類した(表4及び図3)。研究の中で最も多いのは、発がん(N=164)と小児の健康に関連する影 響(N=84)である。表4はエビデンスマップ表に対応し、2006 年から 2012 年の間に農薬ばく露に関 連したすべての影響とその頻度を記述したプロジェクトの影響マッピングを示したものである。

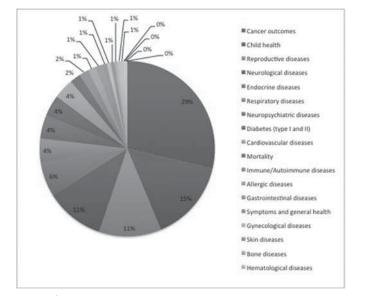


図3:対象となる論文のうち、主要な影響カテゴリーとその影響を調査した研究の 割合

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 Table 4:
 Evidence map table including all major outcome categories examined by eligible studies.

Major outcome	N studies
Cancer outcomes	164
Child health	84
Reproductive diseases	64
Neurological diseases	61
Endocrine diseases	35
Mental and psychomotor development	32
Respiratory diseases	25
Neuropsychiatric diseases	15
Diabetes (type I and II)	22
Cardiovascular diseases	31
Hematological diseases	15
Mortality	11
Immune/Autoimmune diseases	10
Allergic diseases	8
Gastrointestinal diseases	7
Symptoms and general health	5
Gynecological diseases	4
Skin diseases	4
Bone diseases	3
Kidney diseases	3
Benign tumors	1
Dental diseases	1
Men health	1
Metabolic diseases	1

#### 7.3. Characteristics of eligible studies

The eligible studies were published from 2006 to 2012. The observed distribution of the publication year of the eligible studies indicates an approximately equal distribution of studies throughout the past 5 years (Figure 4). Of note, we expected a considerable presence of the results of the various reports of the Agricultural Health Study (AHS), the largest to-date observational study performed in the field. Indeed, the AHS publications (n=42) represent a recognizable proportion of the included studies (7%). Another 22 studies come form the cross-sectional National Health and Nutrition Examination Survey (NHANES) cohorts.

The majority of studies were case-control studies (N=222) and cross-sectional studies (Figure 5) and examined occupational exposure to pesticides (N=329). Almost half of the studies (N=285) were based in America (Figure 6). The most frequent method of pesticide assessment was measurement of biomarker or use of self reported questionnaire (Figure 7). Approximately half (N=261) studies were classified as 'high' in the methodological assessment.

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## 表4:対象となる研究で調査されたすべての主要な影響カテゴリーを含むエビデン スマップ表

主な影響	研究数
発がん	164
小児の健康	84
生殖器疾患	64
神経疾患	61
内分泌疾患	35
精神・精神運動発達	32
呼吸器系疾患	25
精神神経疾患	15
糖尿病 (I型・II型)	22
循環器疾患	31
血液疾患	15
死亡率	11
免疫・自己免疫疾患	10
アレルギー性疾患	8
消化器疾患	7
症状と全身の健康	5
婦人科系の疾患	4
皮膚疾患	4
骨の疾患	3
腎臓の疾患	3
良性腫瘍	1
歯科疾患	1
男性の健康	1
代謝性疾患	1

### 7.3. 対象となる研究の特徴

対象となる研究は 2006 年から 2012 年までに発表されたものである。対象研究の発表年の分布を 見ると、過去 5 年間でほぼ均等に分布していることがわかる(図 4)。特筆すべきは、現場で実施さ れた今日までの観察研究の中で最大のものである Agricultural Health Study (AHS)の様々な報告書 の結果がかなりの割合で存在することである。実際、AHSの出版物 (n=42) は、含まれている研究の7% を占めている。その他の22件の研究は、国民健康・栄養調査 (NHANES) コホートの横断研究である。

研究の大部分は症例対照研究(N=222)と横断研究(図5)であり、農薬への職業ばく露(N=329)を 調査していた。研究のほぼ半数(N=285)はアメリカを拠点とした研究であった(図6)。農薬の評価方 法は、バイオマーカーの測定、または自記式質問紙の使用が最も多かった(図7)。約半数(N=261)の 研究が方法論的評価で「高」に分類された。

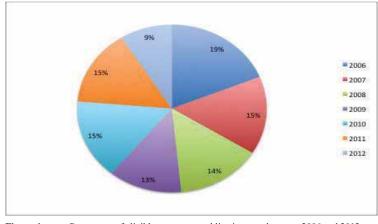
広範囲で多様な農薬が研究されており、様々な農薬の定義を用いた研究が行われている。また、公表 されている文献のかなりの割合が、欧州連合(EU)や先進国のほとんどで使用が承認されていない農薬 に焦点を当てていることも予想される。このような研究は、農薬の長期残留の根拠や、開発途上国での 継続的な農薬使用の根拠に関わるものであることを認識している。例えば、DDTとその代謝物のみに焦 点を当てた研究は、対象となる研究のほぼ10%を占めている。

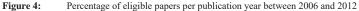
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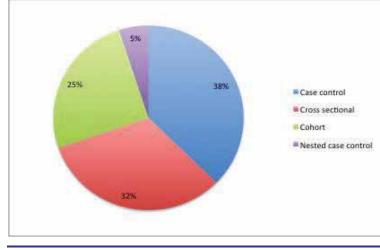
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A wide and diverse range of pesticides was studied with studies using various definitions of pesticides; it is very hard to harmonise between studies this information. We also anticipated a considerable proportion of the published literature to be focusing on pesticides no longer approved for use in the European Union and in most of the developed countries. We acknowledge that this research lies on the rational of pesticide long-term residuals, as well as of the continuing use of these pesticides in developing countries. For example, studies focusing solely on DDT and its metabolites constitute almost 10% of the eligible studies.



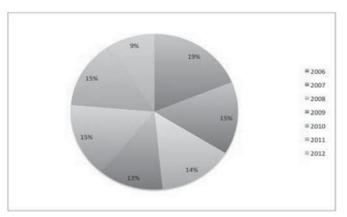




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### 図4:2006年から2012年までの出版年度ごとの対象論文の割合

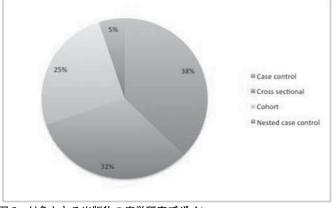


図5:対象となる出版物の疫学研究デザイン

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### Figure 5: Epidemiological study designs of eligible publications

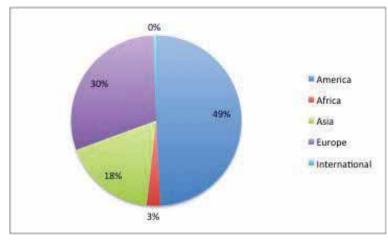


Figure 6: Location (continent) where eligible epidemiological studies were conducted

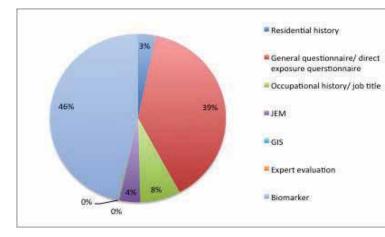


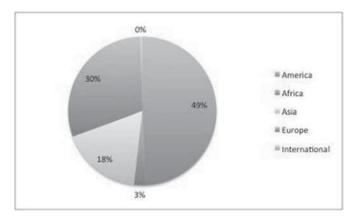
Figure 7: Method of exposure assessment in eligible epidemiological studies



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# 図6:対象となる疫学研究が実施された地域(大陸)の状況

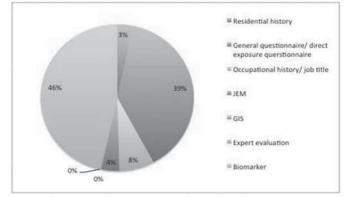


図7:対象となる疫学研究におけるばく露評価の方法

7.4. Systematic literature review of systematic reviews and meta-analysis

Throughout our search strategy we also identified systematic reviews and meta-analyses. Overall, 21 different eligible reviews were identified published after 2006. The outcomes examined are shown in Table 5 below. Most reviews examined cancer related outcomes and some claimed positive associations between pesticides and examined outcome. The reviews are discussed in relevant outcome categories along with the individual studies.

 Table 5:
 List of systematic reviews and meta-analyses identified in the literature review

			Author Journal Dublication
Outcome	N studies	Authors claim association	Author, Journal, Publication year
Outcome	N studies	Autions cidini association	Sutedja NA et al, 2009
			Kamel F et al. 2012
Amyotrophic lateral sclerosis	3	No	Malek et al, 2012
Cancers	11	NO	
Breast cancer	1	No	Khaniani Natal 2007
Di east cancer	Ι	NU	Khanjani N et al, 2007 Infante-Rivard C et al, 2007
Childhood cancer	2	Yes	
Childhood cancer	2	res	Vinson F et al, 2011
			Wingle DT et al, 2009
			Turner et al, 2010
			Van Maele-Fabry G et al,
			2010
			Van Maele-Fabry G et al,
			2011
			Bailey HD et al, 2011
Childhood Leukaemia	6	Yes	Turner MC et al, 2011
Multiple cancers	1	Yes	Cooper et al, 2008
Prostate cancer	1	Yes	Budnik LT et al, 2012
Multiple health outcomes	1	Yes	Koureas M et al, 2012
			Ismail AA et al, 2012
Neurobehavioral	2	No	Li AA et al, 2012
			Van der Mark M et al, 2012
			Van Maele Fabry G et al,
Parkinson disease	2	Yes	2012
Reproductive	1	No	Shirangi A, 2011
			Snijder CA et al, 2012
Time to pregnancy	1	Yes	

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7.4. システマティックレビューとメタアナリシスのシステマティック文献レビュー 検索戦略を通じて、システマティックレビューとメタアナリシスも同定した。全体として、2006年以 降に出版された21の異なる妥当なレビューが同定された。調査した影響を以下の表5に示す。ほとんど のレビューではがんに関連した影響を調査しており、いくつかのレビューでは農薬と調査した影響と の間に明確な関連があると主張していた。これらのレビューは、個々の研究とともに、関連する影響カ テゴリーで議論されている。

# 表5:文献レビューで確認されたシステマティックレビューとメタアナリシスのリ スト

影響		研究数	著者が主張する関連	著者・雑誌・出版年
筋萎縮性側索硬化症		3	No	Sutedja NA et al, 2009 Kamel F et
				al, 2012
				Malek et al, 2012
がん		11		
	乳がん	1	No	Khanjani N et al, 2007
/]-	児がん	2	Yes	Infante-Rivard C et al, 2007
				Vinson F et al, 2011
小児	白血病	6	Yes	Wingle DT et al, 2009 Turner et al,
				2010
				Van Maele-Fabry G et al, 2010
				Van Maele-Fabry G et al, 2011
				Bailey HD et al, 2011 Turner MC et
				al, 2011
多発	性がん	1	Yes	Cooper et al, 2008
前立	腺がん	1	Yes	Budnik LT et al, 2012
複合的健康影響		1	Yes	Koureas M et al, 2012
神経行動学		2	No	Ismail AA et al, 2012
				Li AA et al, 2012
パーキンソン病		2	Yes	Van der Mark M et al, 2012 Van Maele
				Fabry G et al, 2012
生殖性		1	No	Shirangi A, 2011
妊娠までの期間		1	Yes	Snijder CA et al, 2012

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### 8. Cancer Outcomes

Overall, 164 publications examined the effect of pesticide exposure on cancer outcomes, contributing more than 2000 separate analyses. As seen with other outcomes, the diversity of exposure definition is remarkable and poses special challenges to data synthesis. Only 36 out of the 164 were prospective cohort studies and other 13 were nested case-controls; the overwhelming majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. Also, out of the 49 prospective analyses, 30 (61%) were from the same prospective associations comes from a single population. The sample size of the analyses was often small; it ranged between 24 and 82,596 participants (median 301). In addition, 33 studies had information on biomarkers of exposure and only 7 assessed occupational exposures through job exposure matrix (JEM). Common limitations in studies included small sample sizes, self-reported exposure, potential for high false positive rates due to multiple testing (studies test multiple hypothesis without adjusting for multiple testing and therefore results are likely to be false positives), and retrospective design. A wide variety or pesticides were assessed, with many studies examining organochlorine insecticides.

The different cancer categories examined are presented in Table 6 along with the number of studies contributing to each outcome category and a recommendation for quantitative synthesis. Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for some cancer subgroups.

#### 8.1. Hematological neoplasms

#### 8.1.1. Leukemias

Overall, 26 studies (and 2 abstracts) examined associations between pesticide exposure and various forms of leukaemia. Fourteen out of these 26 studies were reports from the AHS with some overlapping results and examination of different pesticide groups. Only 2 studies, both on DDE (ID CAN 063, ID CAN 064) examined residential exposure and all the remaining studies examined occupation exposure to pesticides. Twelve out of 99 different analyses were statistically significant with effect sizes across all studies ranging between 6.1 and 0.2. Statistically significant results come from 7 different studies; with the exception of the AHS all were of modest to low quality. Table 7 shows summarised results across studies that reported information on the same pesticide class. The vast majority of results are non-significant and of small effect sizes. Figure 8 shows random effect meta-analyses keeping analyses with largest sample size form each study. The meta-analysis resulted in a non-significant pooled effect (OR 1.26, 95% CI 0.93, 1.71) and had modest heterogeneity. Previous meta-analyses on occupational exposure to pesticides and leukaemia were published in 2008 and 2007 (Merhi 2007, Van Maele-Fabry 2008). The overall summary effect estimates from previous meta-analyses suggested that there is a significantly positive, albeit weak, association between occupational exposure to pesticides and all hematopoietic cancers. But both reports acknowledged a wide range of limitations including the lack of sufficient data about exposure information and other risk factors for hematopoietic cancer and unclear definition of exposure and of leukemia type.

#### 8. 発がん

全体では164の出版物が農薬ばく露の発がんへの影響を調査し、2,000以上の個別の分析に貢献した。 他の影響に見られるように、ばく露の定義の多様性は驚くべきものであり、データ統合に特別な問題 をもたらしている。164件のうち36件のみが前向きコホート研究であり、他の13件はコホート内症例対 照研究であった。エビデンスの圧倒的多数は後ろ向き症例対照分析から来ており、ばく露測定におい てリコールバイアスがかかりやすい。また、49の前向き分析のうち、30(61%)は同じ前向き研究であ るAgricultural Health Study (AHS)からのものであり、この前向きコホートを超えたエビデンスは 限られている。これは重要な観察であり、前向きな関連のエビデンスの60%は単一の集団から得られ ているという事実を強調している。分析のサンプルサイズはしばしば小さく、参加者数は24~82,596 人(中央値301人)であった。さらに、33の研究ではばく露のバイオマーカーに関する情報が得られて おり、職業ばく露マトリックス(JEM)を用いて職業ばく露を評価したのは7件のみであった。研究に共 通する制限事項としては、サンプルサイズが小さいこと、ばく露が自己申告であること、多重検定によ る高い偽陽性率の可能性(多重検定を調整せずに複数の仮説を検定しているため、結果が偽陽性にな る可能性がある)及び後ろ向きなデザインなどが挙げられる。多くの研究で有機塩素系殺虫剤を調査 しており、多種多様な農薬が評価された。

調査されたさまざまながんのカテゴリーを、各影響カテゴリーに寄与した研究の数及び定量的統合 の推奨事項とともに表6に示す。データの不均一性と同定された研究数が少ないため、データの統計的 統合(メタアナリシス)は一部のがんサブグループについてのみ実施された。

### 8.1. 造血器新生物

### 8.1.1. 白血病

全体では、26件の研究(及び2件の要約)が農薬ばく露と様々な形態の白血病との関連を調査した。 これら26件の研究のうち14件はAHSからの報告であり、結果が重複していたり、異なる農薬群の調査が 行われていたりした。DDEに関する2件の研究(ID CAN\_063、ID CAN\_064)のみが住居ばく露を調査して おり、残りの研究はすべて農薬への職業ばく露を調査したものである。99の異なる分析のうち12の研 究は、6.1と0.2の間にあるすべての研究の効果量で統計的に有意であった。統計的に有意な結果が得 られたのは7つの研究であり、AHSを除くすべての研究の品質は中等度から低度であった。表7 は、同 じ農薬クラスに関する情報を報告した研究の結果をまとめたものである。結果の大部分は、有意では なく、効果量が小さいものであった。図8は、ランダム効果メタアナリシスを示しており、各研究で最 大のサンプルサイズで分析を行っている。このメタアナリシスでは、有意ではない統合効果(OR 1.26、 95% CI 0.93、1.71)が得られ、中等度の不均一性を有していた。農薬への職業ばく露と自血病に関す る以前のメタアナリシスからの全体的な要約効果推定値は、農薬への職業ばく露とすべての造血器がん との間には、弱いながらも有意に明確な関連があることを示唆していた。しかし、両報告とも、ばく露 情報や造血器腫瘍の他のリスク因子に関する十分なデータが不足していること、ばく露の定義や白血 病型の定義が不明確であることなど、幅広い限界があることを認めている。

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#### 8.1.2. Hodgkin lymphoma

Seven studies examined the associations between pesticide exposure and Hodgkin lymphoma. All studies assessed exposure through questionnaires, one studies had large sample size and all studies were retrospective. A wide range of pesticides classes was examined which did not allow any meaningful synthesis of the results. Twelve out of 75 separate analyses were statistically significant with effect sizes ranging from 8.4 to 0.4 across all analyses. We attempted random effects meta-analysis keeping only the Agricultural Health Study (AHS) analysis with the largest sample size. The result was not statistically significant and had high heterogeneity which can be attributed to the range of different pesticide classes examined by each study (Figure 9).

#### 8.1.3. Other lymphomas

A very wide variety of definitions of lymphomas other than Hodgkin lymphoma were used in 44 studies of which 21 were reports from the Agricultural Health Study (AHS) and 2 from the BC (British Columbia) sawmill workers cohort study. Studies examined broad definitions of lymphomas and lymphoproliferative syndromes (ID CAN\_047, ID CAN\_049, ID CAN\_074) and other examined more specific definitions of follicular lymphoma, diffuse large cell lymphoma and peripheral T-cell lymphoma. Twenty-one studies provided effect sizes between pesticide exposure and broad definitions of Non-Hodgkin lymphomas. Five of those studies were prospective (ID CAN\_063, ID CAN\_064, ID CAN\_067, ID CAN\_064, ID CAN\_067, ID CAN\_064, ID CAN\_067, ID CAN\_064, ID CAN\_065, ID CAN\_064, ID CAN\_052). However, the later analyses were all on organochlorine pesticides with only few significant results (6 analyses among a total of 35 analyses) without any firm evidence for associations. In the AHS, large and significant effect size was observed between butylate use and Non-Hodgkin lymphomas (RR 2.94, 95% 1.49–5.96, p=0.002; high vs. no exposure). However, again the AHS in the same publication has examined ten different outcomes and results need adjustment for multiple testing.

#### 8.1.4. Multiple myeloma

Also, 11 studies examined associations between pesticides and multiple myeloma, myelodysplastic syndromes and monoclonal gammopathy of undetermined significance. These studies were generally heterogeneous and no quantitative synthesis was suggested. Overall, some analyses were statistically significant, but those were mainly from the French case control study (ID CAN\_049) which presented overall 147 separate analyses and results are prone to bias. The AHS also reported significant associations between permethrin, dieldrin, Carbon-tetrachloride/carbon disulfide mix and Chlorthalonil but again these were amongst 52 other analyses and regulire cautious interpretation. One study, reported very high significant effect size of 7.3 for myelodysplastic syndrome (ID CAN\_070) but the quality of the study was poor and adjustment of covariates very limited and results were not replicated by other studies on the same phenotype.

#### 8.2. Prostate cancer

Overall, 39 studies (in 260 analyses) examined the effects of pesticide exposure on prostate cancer. One study was a conference abstract which provided little data on methodology to allow meaningful appraisal of its results (ID CAN\_107). Also, 25 of those 39 studies were studies from the AHS population with some overlapping results. For example, two studies (ID CAN\_022, ID CAN\_106) examined interactions between pesticide exposure and genetic variants in relation to prostate cancer. These AHS studies presented the same main effects for pesticide exposure; effects were largely null and, if anything, significant inverse effects were found e.g. for carbaryl, chlordane, metalachlor and

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#### 8.1.2. ホジキンリンパ腫

7件の研究が農薬ばく露とホジキンリンパ腫との関連を調査した。すべての研究は質問紙を用いてば く露を評価しており、1件の研究はサンプル数が多く、すべての研究は後ろ向きであった。広範囲の農 薬クラスが調査されたが、結果の意味のある統合はできなかった。75の個別分析のうち12の分析が統 計的に有意であり、すべての分析において効果量は8.4から0.4の範囲であった。我々は、最大のサンプ ルサイズを持つAgricultural Health Study (AHS)分析のみを残して、ランダム効果メタアナリシス を試みた。その結果は統計的に有意ではなく、各研究で調査された農薬クラスが異なることに起因す る可能性がある高い不均一性を有していた(図9)。

### 8.1.3. その他のリンパ腫

ホジキンリンパ腫以外のリンパ腫については、非常に多様な定義が44件の研究で使用されており、 そのうち21件は農業健康調査(Agricultural Health Study: AHS)からの報告で、2件はBC(プリティ ッシュコロンビア州)製材所の労働者コホート研究からの報告であった。研究ではリンパ腫及びリン パ増殖性症候群(ID CAN\_047、ID CAN\_049、ID CAN\_074)の広範な定義が調査され、他の研究では濾胞 性リンパ腫、びまん性大細胞リンパ腫及び末梢性T細胞リンパ腫のより具体的な定義が調査された。21 件の研究では、農薬ばく露と非ホジキンリンパ腫の幅広い定義との間の効果量が報告された。これら の研究のうち5件は前向き(ID CAN\_063、ID CAN\_064、ID CAN\_067、ID CAN\_118、ID CAN\_121)であり、 7件はばく露のバイオマーカーとの関連を調査した(ID CAN\_056、ID CAN\_057、ID CAN\_064、ID CAN\_065、 ID CAN\_067、ID CAN\_060、ID CAN\_052)。しかし、それ以降の分析はすべて有機塩素系農薬に関するも のであり、関連を示す確固たるエビデンスがないまま、有意な結果が得られたのはわずかであった(全 35 回の分析のうち 6 回の分析)。AHSでは、プチル酸塩の使用と非ホジキンリンパ腫との間に大きな 有意な効果量が観察された(RR 2.94、95%1.49-5.96、p=0.002;高ばく露と非ばく露との間に有意な 効果量が観察された)。しかし、同じ出版物のAHSは10種類の異なる影響を調査しており、結果は複数 の試験のための調整が必要であることを繰り返している。

### 8.1.4. 多発性骨髄腫

また、11件の研究では、農薬と多発性骨髄腫、骨髄異形成症候群及び意義不明の単クローン性免疫グ ロブリン血症との関連が調査された。これらの研究は全般的に異質であり、定量的な統合は示唆され なかった。全体的に、いくつかの分析は統計的に有意であったが、それらは主にフランスの症例対照研 究(ID CAN\_049)からのものであり、147の個別の分析が行われており、結果にバイアスがかかりやす い。AHSはまた、ペルメトリン、ディルドリン、四塩化炭素/二硫化炭素の混合物、クロルタロニルとの 間の有意な関連を報告しているが、これらは他の52分析の中に含まれており、慎重な解釈が必要であ る。ある研究では、骨髄異形成症候群(ID CAN\_070)に対して7.3という非常に高い有意な効果量が報 告されているが、研究の質が悪く、共変量の調整が非常に限られており、同じ表現型の他の研究で結果 が再現されていなかった。

### 8.2. 前立腺がん

全体では、39件の研究(260件の分析)で農薬ばく露が前立腺がんに及ぼす影響が調査された。その うちの1件は学会発表の要約であり、その結果を評価するための方法論に関するデータがほとんど提供 されていなかった(ID CAN\_107)。また、これら39件の研究のうち25件はAHS集団を対象とした研究で あり、いくつかの結果が重複していた。例えば、2つの研究(ID CAN\_022、ID CAN\_106)では、農薬ば く露と前立腺がんとの関連における遺伝子変異との相互作用が調査されていた。これらのAHS研究では、 農薬ばく露の主な効果は同じであったが、効果はほとんど無効であり、もしあるとすれば、カルバリ ル、クロルデン、メタクロルなどでは有意な逆効果が認められた。残りのAHS研究では、特定の農薬間

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others. The remaining AHS studies, examined associations between specific pesticides, again showing no statistically significant associations between any of the examined pesticides and prostate cancer with the exception of a weak significant effect between butylate exposure and prostate cancer. The remaining evidence stems from, rather small and of modest quality, retrospective studies. Most studies (ID CAN 103, ID CAN 101, ID CAN 100, ID CAN 094, ID CAN 143, ID CAN 142) examined the effects of organochlorines with largely small and non-significant results. Two studies (IDs CAN 099, ID CAN 095) showed high significant increased risk associated with pesticide exposure and prostate cancer but both studies were of low quality, had very broad definitions of exposure and results need cautious interpretation and do not match with those reported from well conducted large prospective studies (e.g. AHS). Notably, one population-based case-control study (ID CAN 104) in a highly exposure area found strong association of ambient exposure to methyl bromide with prostate cancer risk, but the study did not observe evidence for exposure-response. In summary, most evidence for prostate cancer risk in relation to pesticide exposure concerns the effect of organoclorines with studies showing weak non-significant effects. A meta-analysis (Maele-Fabry 2003) on occupational exposure to pesticides and prostate cancer was also identified published. The pooled effect estimate, based on 22 epidemiological studies, was 1.13 (95% CI 1.04 to 1.22) with substantial heterogeneity across studies. In addition, the studies reviewed contained insufficient qualitative and quantitative information on exposure in order to distinguish the influence of pesticides from other occupational, environmental, and lifestyle factors (Maele-Fabry 2003). Overall, there is no evidence supporting an association between pesticide exposure and prostate cancer.

#### 8.3. Lung cancer

Thirty studies contributing 45 analyses examined associations between pesticide exposure and lung cancer; previously published meta-analysis was not identified. Again, 23 out of 30 published studies and 30 of the 45 analyses were analyses of the AHS. Amongst the 50 different analyses of the AHS, only one statistically significant result was observed. Three studies examined broad pesticides definition as their exposure (ID CAN\_080, ID CAN\_082, ID CAN\_083), one studied mosquito coil burns (ID CAN\_081), while the remaining studies examined a range of different pesticides with an emphasis on organochlorine insecticides. The diversity of pesticide categories and the repeated use of the same cohort population (AHS) in more than half of the studies does not allow for quantitative synthesis. Notably, the association between mosquito coil burn and lung cancer was statistically significant with large effect size (3.78 (1.55, 6.90); yes vs. no use) but the study is relative small, retrospective with limited examination of confounders and of overall modest quality. Two case-control studies (ID CAN\_082, ID CAN\_082) reported over a two-fold increased risk of lung cancer for occupational exposure to pesticides but individual pesticides were not examined. Another case-control study (ID CAN\_080) failed to replicate these observations between pesticide exposure and lung cancer mortality. Overall, the evidence on pesticide exposure and lung cancer is limited and inconclusive.

#### 8.4. Childhood cancer

#### 8.4.1. Childhood hematological neoplasms

Overall, 17 studies (and one abstract) which examined childhood hematological neoplasms in relation to pesticide exposure were identified. All 17 studies examined childhood leukemia and 4 of them also included other hematological neoplasms.

Previous meta-analysis on childhood leukemia concentrated on studies which assessed residential exposure to pesticides only. All studies that were included in the meta-analyses and were published after 2006 have been identified by our search which confirms that we identified all available evidence.

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の関連を調査したが、いずれの農薬と前立腺がんとの間にも統計的に有意な関連は認められなかった が、ブチル酸塩へのばく露と前立腺がんとの間には弱い有意な影響が認められた。残りのエビデンス は、小規模で質の低い後ろ向き研究から得られたものである。ほとんどの研究(ID CAN\_103、ID CAN\_101、 ID CAN\_100、ID CAN\_094、ID CAN\_143、ID CAN\_142)では、有機塩素の影響を調査したが、ほとんどが 小規模で有意ではない結果であった。2件の研究(ID CAN\_099、ID CAN\_095)では、農薬ばく露と前立 腺がんに関連した高く有意に増加したリスクが示されたが、いずれの研究も質が低く、ばく露の定義 が非常に広く、結果は慎重な解釈が必要であり、適切に実施された大規模な前向き研究(AHSなど)で 報告されたものとは一致しない。特筆すべきは、ばく露量の多い地域で行われた1件の集団ベースの症 例対照研究(ID CAN\_104)では、臭化メチルへのばく露と前立腺がんリスクとの間に強い関連が認めら れたが、この研究ではばく露反応関係を示すエビデンスは観察されなかった。まとめると、農薬ばく露 に関連した前立腺がんリスクに関するほとんどのエビデンスは有機塩素の影響に関係しており、研究 では有意ではない弱い影響が示されている。農薬への職業ばく露と前立腺がんに関するメタアナリシ ス (Maele-Fabry 2003) も発表されている。22の疫学研究に基づく統合効果推定値は1.13 (95%CI 1.04 ~1.22) であり、研究間でかなりの不均一性があった。さらに、レビューされた研究には、農薬の影響 を他の職業的、環境的、生活習慣的要因と区別するためのばく露に関する質的及び量的情報が不十分 であった(Maele-Fabry 2003)。全体として、農薬ばく露と前立腺がんとの関連を支持するエビデンス はない。

#### 8.3. 肺がん

30件の研究が45の分析を行っており、農薬ばく露と肺がんとの関連を調べているが、これまでに発 表されたメタアナリシスは確認されなかった。ここでも、公表された30件の研究のうち23件、45件の分 析のうち30件がAHSの分析であった。AHSの50種類の分析のうち、統計的に有意な結果が観察されたの は1件のみであった。3つの研究では、ばく露としての農薬の定義が広く(ID CAN\_080、ID CAN\_082、ID CAN\_083)、1つの研究では蚊取り線香の火傷を調査し(ID CAN\_081)、残りの研究では有機塩素系殺虫 剤に重点を置いて様々な農薬を調査していた。殺虫剤のカテゴリーが多様であること、半数以上の研 究で同じコホート集団(AHS)を繰り返し使用していることから、定量的な統合はできなかった。特筆 すべきは、蚊取り線香の火傷と肺がんとの関連は、大きな効果量(3.78(1.55、6.90);使用あり vs 使用なし)で統計的に有意であったが、この研究は相対的に小規模であり、交絡因子の調査が限られた 後ろ向きであり、全体的に質は中等度であった。2件の症例対照研究(ID CAN\_082, ID CAN\_082)では、 農薬への職業ばく露による肺がんリスクの2倍以上の増加が報告されているが、個々の農薬については 調査されていない。別の症例対照研究(ID CAN\_080)では、農薬ばく露と肺がん死亡率との間のこれら の観察を再現することはできなかった。全体として、農薬ばく露と肺がんに関するエビデンスは限ら れており、結論は出ていない。

### 8.4. 小児がん

### 8.4.1. 小児の造血器新生物

全体として、農薬ばく露に関連して小児の造血器新生物を調査した17件の研究(及び1件の要約)が 同定された。17件の研究はすべて小児白血病を対象としており、そのうち4件には他の造血器新生物も 含まれていた。

小児白血病に関するこれまでのメタアナリシスは、農薬への住居内ばく露のみを対象とした研究に 集中していた。メタアナリシスに含まれ、2006年以降に発表されたすべての研究が我々の検索で同定 され、利用可能なすべてのエビデンスが同定されたことを確認した。特定された研究は、国の登録ベー スの症例対照研究ESCALE (Etude sur les cancers de l'enfant) (ID CAN\_073) とNorthern Region Young Persons' Malignant Disease Registry (ID CAN\_120) の2つの研究を除いて、一般的に小規模な

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Identified studies were generally small with the exception of two studies on national registries, the Northern Region Young Persons' Malignant Disease Registry (ID CAN 120) and the national registry-based case-control study ESCALE (Etude sur les cancers de l'enfant) (ID CAN 073). Results from these studies should be cautiously interpreted, despite their large sample size, due to the large number of hypothesis examined (high false positive rate); each study reported 42 and 64 separate analysis respectively. All were case control studies and vast majority examined residential exposures with few studies on occupation exposure identified. Although most studies assessed use of, or exposure to, pesticides or pesticide subgroups (insecticides, herbicides, fungicides), some studies also attempted to collect information on specific pesticides (ID CAN 031, ID CAN 032) and one study (ID CAN 032) assessed biomarker levels. There were few data regarding frequency or duration of pesticide use, with most studies reporting only "ever/never" use of/exposure to the pesticide of interest. Although confounding is difficult to assess because there are few established risk factors for childhood hematological neoplasms, most studies examined or adjusted for at least a range of sociodemographic and maternal characteristics. Almost all studies assessed pesticide exposure separately for preconception, pregnancy, and childhood time windows. One study of very low quality and incomplete statistical analyses results examined all exposure time windows and other 2 (ID CAN 073, ID CAN 044) examined preconception and pregnancy jointly.

Three studies were excluded from further quantitative analyses: study ID CAN\_040 was excluded due to lack of CIs; study ID CAN\_030 due to duplicate data from Northern California Childhood Leukemia Study (duplicate with ID CAN\_031), and study ID CAN\_037 due to a unique study population (Down syndrome cases only). We divided the quantitative synthesis of results by the time period (window of exposure).

#### 8.4.1.1. Exposure during pregnancy

Seven studies had information for pesticide exposures during pregnancy. Eleven out of 86 analyses were statistically significant corresponding to 5 studies which all examined acute leukaemia as outcome of interest. Largest effect estimates were reported from the national registry-based casecontrol study ESCALE (Etude sur les cancers de l'enfant). Insecticide use during pregnancy was significantly associated with childhood acute leukemia (OR = 2.1; 95% CI, 1.7–2.5) and paternal household use of pesticides was also related to acute leukemia (OR = 1.5; 95% CI, 1.2–1.8) in this study. We performed a series of quantitative synthesis of results. We first selected analyses with the largest sample size within each published report and synthesized results (Figure 10). This analysis was associated with large heterogeneity ( $I^{2}>80\%$ ) as each study had different exposure assessment (type of pesticide and parental route of exposure) and variability in outcome assessment. The remaining metaanalysis in Figure 10 show synthesis or results based on pesticide class examined in an effort to harmonize results with the previously published meta-analysis (Turner 2010) on 'Residential Pesticides and Childhood Leukemia'. We performed quantitative synthesis of all studies on insecticides and pesticides identified in this systematic review and subsequently we updated the previously published meta-analysis keeping only studies assessing residential exposure. Overall, the results show modest heterogeneity across studies, which can be attributed to variability in pesticide exposure definition, outcome definition, definition of exposure time windows etc. However, the metaanalysis show a consistent increased risk of childhood leukemia associated with exposure to unspecified pesticides and insecticide (Summary OR=1.69; 96% CI=1.35, 2.11). Our updated metaanalysis resulted in more conservative results compared to the meta-analysis published in 2010 but still supported an association between exposure to pesticides during pregnancy and childhood leukaemia. Still the evidence merits careful interpretation as there were concerns around publication bias in the original meta-analysis, the studies are typically small and the exposure is measured through non-validated self-reported questionnaires that are prone to misclassification. Funnel plot shows relative symmetry around studies of small size. Further evidence from large studies, using valid

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ものであった。これらの研究の結果は、サンブルサイズが大きいにもかかわらず、調査された仮説の数 が多い(偽陽性率が高い)ため、慎重に解釈されるべきである。各研究はそれぞれ42件、64件の個別の 分析結果を報告している。すべての研究は症例対照研究であり、大多数は住居内ばく露を調査してお り、職業ばく露に関する研究はほとんど見出されなかった。ほとんどの研究では、農薬または農薬のサ ブグループ(殺虫剤、除草剤、殺菌剤)の使用またはばく露を評価していたが、一部の研究では特定の 農薬に関する情報の収集を試みており(ID CAN\_031、ID CAN\_032)、1つの研究(ID CAN\_032)ではバ イオマーカーレベルを評価していた。農薬の使用頻度や使用期間に関するデータはほとんどなく、ほ とんどの研究では、対象となる農薬の「今までに使用したことがあるかないか」」「使用したことがある かないか」のみを報告している。小児の造血器新生物のリスク因子がほとんど確立されていないため、 交絡因子の評価は困難であるが、ほとんどの研究では、少なくとも様々な社会人口統計学的及び母性 の特性を調査または調整している。ほとんどすべての研究では、農薬はく露を妊娠前、妊娠期、小児期 に分けて評価している。非常に質が低く、統計分析の結果が不完全であった1件の研究では、すべての ばく露期間を調査し、他の2件(ID CAN\_073, ID CAN\_044)では、妊娠前と妊娠期を合わせて調査して いる。

研究ID CAN\_040はCIが不足していたため除外され、研究ID CAN\_030はNorthern California Childhood Leukemia Studyのデータが重複していたため(ID CAN\_031と重複)、研究ID CAN\_037は研 究集団が特殊であったため(ダウン症患者のみ)、3つの研究が定量分析から除外された。結果の定量 的統合を期間(ばく露の時間域)ごとに分けた。

### 8.4.1.1 妊娠期のばく露

7件の研究では妊娠期の農薬ばく露に関する情報が得られた。86件中11件の分析で統計的に有意な結 果が得られたのは5件であり、すべての研究で急性白血病を対象とした結果であった。最大の効果推定 値は、国の登録ベースの症例対照研究ESCALE (Etude sur les cancers de l'enfant) から報告されて いる。本研究では、妊娠期の殺虫剤使用は小児急性白血病と有意に関連し(OR = 2.1:95%CI, 1.7-2.5)、父方の住居での殺虫剤使用も急性白血病と関連し(OR = 1.5;95%CI, 1.2-1.8)、また、父方 の住居での殺虫剤使用も急性白血病と関連した(OR = 1.5;95%CI, 1.2-1.8)。結果の一連の定量的 統合を行った。まず、各発表報告書の中で最大のサンプルサイズを持つ分析を選択し、結果を統合した (図 10)。この分析は、各研究でばく露評価(農薬の種類と親のばく露経路)が異なり、影響評価に ばらつきがあったため、大きな不均一性(I2>80%)と関連していた。図10の残りのメタアナリシスは、 「住居用農薬と小児白血病」に関する以前に発表されたメタアナリシス(Turner 2010)と結果を調和 させるために、調査した農薬のクラスに基づいた統合または結果を示している。我々は、この系統的レ ビューで確認された殺虫剤及び農薬に関するすべての研究を定量的に統合し、その後、住居ばく露を 評価した研究のみを残して、以前に発表されたメタアナリシスを更新した。全体的に、結果は研究間で 中等度の不均一性を示しており、これは農薬ばく露の定義、影響の定義、ばく露時間域の定義などにば らつきがあることに起因していると考えられる。しかし、メタアナリシスでは、特定されていない農薬 及び殺虫剤へのばく露に関連した小児白血病のリスクの一貫した増加を示した(要約0R=1.69;96% CI=1.35,2.11)。我々の更新されたメタアナリシスでは、2010年に発表されたメタアナリシスと比較 して、より保守的な結果となったが、妊娠期の農薬へのばく露と小児白血病との関連は依然として支 持されている。しかし、元のメタアナリシスでは出版バイアスの懸念があったこと、研究の規模が一般 的に小さいこと、ばく露は誤分類されやすい検証されていない自記式質問紙で測定されていることな どから、エビデンスは慎重に解釈する必要がある。ファンネルプロットは、小規模な研究を中心とした 相対的な対称性を示している。住居用殺虫剤への出生前ばく露を減らすことに公衆衛生上のメリット があるかどうかを確認するためには、過去のばく露の有効なバイオマーカーを用いた大規模研究から のさらなるエビデンスが必要である。

biomarkers of past exposure are needed to confirm whether there is public health merit in reducing prenatal exposure to residential pesticides.

#### 8.4.1.2. Preconception

Four studies examined preconception as the time window of exposure (ID CAN\_032, ID CAN\_043, ID CAN\_073, ID CAN\_120) but none reported statistically significant results.

#### 8.4.1.3. Childhood

Seven studies with information on exposure during childhood were identified (ID CAN\_031, ID CAN\_032, ID CAN\_035, ID CAN\_036, ID CAN\_041, ID CAN\_043, ID CAN\_133). One study examined Endosulfan, which is no longer in use; the study was of very low quality and was not considered further. Meta-analysis of these studies is shown in Figure 14 below. Two analyses are presented A) one on identified studies from 2006 onwards based on the analysis of the largest sample size in each report (any pesticide) and B) an update on the 2010 meta-analysis on pesticide exposure during childhood and childhood leukemia. The meta-analysis on any pesticides had modest heterogeneity whereas the updated meta-analysis, which was restricted to residential exposure and insecticides/ unspecified pesticides only, displayed no heterogeneity in its results. The results of the updated meta-analysis are more conservative than the original meta-analysis but still very close to the pooled estimates reported in 2010 (Figure 14). Funnel plots indicated considerable symmetry around results. Overall, there is some evidence for association between childhood exposure to pesticide and childhood leukemia but this is weaker than exposure during pregnancy and requires more evidence from well-conducted large birth cohorts to draw firm conclusions.

#### 8.4.2. Lymphomas

Evidence beyond leukaemia for childhood hematological neoplasms comes only from 3 studies, which reported many analyses (IDs CAN\_073, ID CAN\_120, ID CAN\_133) among which analyses for Non-Hodgkin and Hodgkin lymphomas. All analyses were not statistically significant and had weak effect estimates.

#### 8.4.3. Other childhood cancers

Seven studies on other childhood cancers were identified. Four studies examined brain cancer (ID CAN\_006, ID CAN\_011, ID CAN\_089, ID CAN\_133), one childhood germ cell tumor (ID CAN\_114) and two examined a range of childhood cancers (ID CAN\_120, ID CAN\_133). Significant associations were only observed for brain cancers but again these pertain to only a small subset of many analyses and cannot be informative at this stage.

#### 8.5. Colorectal cancer

Overall, 26 identified studies examined associations between pesticide exposure and colorectal cancer in 207 analyses. Separate analyses for colon cancer and rectum cancer were available in 24 and 11 studies respectively. A very large body of evidence comes from the AHS study, which examined all these 3 outcomes for associations with 194 out of the 207 identified analyses on colorectal cancer examining 50 different pesticides with no adjustments for multiple testing. Out of these 194 analyses, only 7 were statistically significantly positively associated with the outcome (Carbaryl, Aldicarb,

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## 8.4.1.2. 妊娠前

4つの研究では、ばく露の時期として妊娠前を調査したが(ID CAN\_032、ID CAN\_043、ID CAN\_073、ID CAN\_120)、いずれも統計的に有意な結果の報告ではなかった。

### 8.4.1.3. 小児期

小児期のばく露に関する情報を有する7件の研究が確認された(ID CAN\_031、ID CAN\_032、ID CAN\_035、 ID CAN\_036、ID CAN\_041、ID CAN\_043、ID CAN\_133)。1件の研究では、現在使用されていないエンド スルファンを調査したが、その研究は非常に質が低く、これ以上の調査は行われなかった。これらの研 究のメタアナリシスを以下の図14に示す。2つの分析が提示されており、A) 各報告書の最大サンプルサ イズの分析に基づいて2006年以降に同定された研究(任意の農薬)についての分析と、B) 小児期及び 小児白血病における農薬ばく露に関する2010年のメタアナリシスについての更新である。あらゆる農 薬に関するメタアナリシスでは中等度の不均一性があったのに対し、更新されたメタアナリシスでは、 住居ばく露と殺虫剤/特定されていない農薬のみに限定されており、結果に不均一性は見られなかった。 更新されたメタアナリシスの結果は、元のメタアナリシスよりも保守的であるが、それでも2010年に 報告された統合推定値に非常に近いものであった(図14)。ファンネルプロットでは、結果にかなりの 対称性があることが示されている。全体的には、小児期の農薬ばく露と小児白血病との間には関連を 示すいくつかのエビデンスがあるが、これは妊娠期のばく露よりも弱く、確固とした結論を出すため には、適切に実施された大規模な出生コホートからのより多くのエビデンスが必要である。

#### 8.4.2. リンパ腫

白血病以外の小児の造血器新生物に関するエビデンスは3件の研究から得られているのみであり、その中には非ホジキンリンパ腫とホジキンリンパ腫に関する分析を含む多くの分析が報告されている (ID: CAN\_073、ID: CAN\_120、ID: CAN\_133)。すべての分析は統計的に有意ではなく、効果推定値も 弱いものであった。

### 8.4.3. その他の小児がん

その他の小児がんに関する研究が7件同定された。4件の研究では脳腫瘍(ID CAN\_006、ID CAN\_011、 ID CAN\_089、ID CAN\_133)、1件の小児生殖細胞腫瘍(ID CAN\_114)、2件の研究では様々な小児がん (ID CAN\_120、ID CAN\_133)が調査された。有意な関連が観察されたのは脳腫瘍のみであったが、これ らは多くの分析のごく一部のサブセットに関連しており、現段階では情報を得ることはできない。

### 8.5. 大腸がん

全体では、26件の研究で農薬ばく露と大腸がんとの関連性が 207 件の分析で調査された。大腸がん と直腸がんについては、それぞれ24件と11件の研究で別々の分析が行われた。非常に多くのエビデン スがAHS研究から得られている。この研究では、50種類の農薬を用いた大腸がんに関する207の分析の うち194の分析で、これら 3 つの影響発現事象すべてとの関連性が調査されており、多重試験の調整 は行われていない。これら194の分析のうち、影響発現事象と統計的に有意に関連したのは7つの分析 のみであった(カルバリル、アルジカルブ、トキサフェン、ペンディメタリン、ジプロピルチオカルバ ミン酸 S-エチル (EPTC)、イマゼタピル、フォノフォス)が、偽陽性確率が高いため、解釈には注意 が必要である。27の研究が発表されているにもかかわらず、全体的には7つの異なる集団からのエビデ ンスしか得られていない。この事実は、大腸がんに関連してこれらの研究のそれぞれで分析された異 なる農薬の範囲とともに、結果の意味のある定量的な統合を可能にするものではない。表8は、複数の 論文における1つのコホートからの重複データの公表の程度を示しており、結果の一貫性が良好である

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Toxaphene, Pendimethalin, S-Ethyl dipropylthiocarbamate (EPTC), imazethapyr and Fonofos) but need to be interpreted with caution due to high false positive probability. Despite the fact that 27 published studies were identified, overall the evidence comes from only 7 different populations. This fact along with the range of different pesticides analysed by each of these studies in relation to colon cancer does not allow meaningful quantitative synthesis of the results. Table 8 shows the extent of publication of duplicate data from one cohort in multiple papers and shows good consistency of results. Previous meta-analyses on colorectal cancer and pesticide exposure have not been identified. Overall, the evidence for pesticides and colorectal cancer is very limited and current state of the literature does not support associations between pesticides and colorectal cancer.

### 8.6. Skin cancer

Seventeen studies examined associations between melanoma and pesticide exposure. The majority of studies assessed organochlorine pesticides. Again, 14 out of 17 studies on melanoma were results from the AHS examining in each paper different pesticides categories and different definitions of exposure with some supplication of results present. Of the 26 different analyses of the AHS, 8 were statistically significant and all stemming from the same publication (ID CAN\_085) on dose response relationships for 50 agricultural pesticides with cutaneous melanoma. The study reported significant associations between cutaneous melanoma and maneb/mancozeb ( $\geq$  63 exposure days: OR = 2.4; 95% CI, 1.2–4.9; trend *p* = 0.006), parathion ( $\geq$  56 exposure days: OR = 2.4; 95% CI, 1.3–4.4; trend *p* = 0.003), and carbaryl ( $\geq$  56 exposure days: OR = 1.7; 95% CI, 1.1–2.5; trend *p* = 0.013) (155). Other studies did not report results on these pesticides to allow examination of replication of results. One case-control study showed increased statistically significant risk between indoor pesticide exposure and melanoma whereas in the same study outdoors pesticide exposure was not associated with melanoma (106). The remaining studies on organochlorines showed heterogeneous results with few statistically significant results (Hexachlorobenzene (HCB), mirex), which do not provide evidence for an association between these pesticides and melanoma.

#### 8.7. Breast cancer

Overall, 14 studies (and 3 abstracts) after 2006 examined the relationship between pesticide exposure and breast cancer. The vast majority of studies and analyses concentrate on organochlorine pesticide, which they are assessed through biomarker analyses. Two previous meta-analyses on breast cancer and DDT exposure have been published (Khanjani 2007, López-Cervantes 2004). Overall, previous meta-analyses did not show a significant association between any cyclodiene chemical and breast cancer except for heptachlor, but that was based on only two studies. Meta-analysis on identified studies in this systematic review on Dichlorodiphenyldichloroethylene (DDE) and breast cancer (5 studies) also shows no evidence for association. We have also performed a meta-analysis across all identified studies on breast cancer, selecting each time the analysis within each study with the largest sample size. Studies ID CAN 019 and ID CAN 023 were excluded from synthesis, as effect sizes and confidence interval to allow synthesis were not provided and study ID CAN 022 was excluded as it reported very tight confidence intervals which did not were assumed to be reported incorrectly. The synthesis here involves the pooled effect of many different pesticides definitions and biomarkers (DDE, lindane, and broad pesticide definition) and is difficult to be interpreted. The pooled effect shows a statistically significant increased risk of breast cancer (1.07 (0.87 to 1.31)) but this result need cautious interpretation. The meta-analysis combines very different categories of pesticides and is largely dominated by one study (ID CAN 022), which assessed pesticide exposure by self-reported residential pesticide use and is therefore of modest quality compared to the rest of the studies which assessed pesticides via biomarkers.

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### 8.6. 皮膚がん

17件の研究では、メラノーマと農薬ばく露との関連が調査された。大半の研究は有機塩素系農薬を 対象としたものであった。ここでも、メラノーマに関する17件の研究のうち14件がAHSの結果であり、 各論文では異なる農薬のカテゴリーやばく露の定義が異なっており、いくつかの結果が提示されてい た。AHSの26の異なる分析のうち、8つは統計的に有意であり、すべて同じ出版物(ID CAN\_085)に由来 するもので、50種類の農薬と皮膚メラノーマとの量反応関係が報告されている。この研究では、皮膚メ ラノーマとmaneb/mancozeb(63日以上のばく露日数:OR = 2.4;95%CI、1.2-4.9;トレンドp = 0.006)、 parathion(56日以上のばく露日数:OR = 2.4;95%CI、1.3-4.4;トレンドp = 0.003)及びcarbary1 (56日以上のばく露日数:OR = 1.7;95%CI、1.1-2.5;トレンドp = 0.013)との間の有意な関連が報 告されている(155)。他の研究では、これらの農薬に関して再現性を調査できるような結果を報告し ていない。1件の症例対照研究では、屋内での農薬ばく露とメラノーマとの間に統計的に有意なリスク の増加が示されたが、同じ研究では気を外での農薬ばく露はメラノーマとは関連していなかった(106)。 有機塩素系農薬に関する残りの研究では、統計的に有意な結果がほとんど得られない不均質な結果が 得られており(ハキサクロロベンゼン(HCB)、マイレックス)、これらの農薬とメラノーマとの関連 を示すエビデンスは得られてない。

### 8.7. 乳がん

全体では、2006年以降の14件の研究(うち3件は要約)が農薬ばく露と乳がんとの関係を調査した。 大半の研究と分析は有機塩素系農薬に焦点を当てており、バイオマーカー分析によって評価されてい る。乳がんとDDTばく露に関する2つの過去のメタアナリシスが発表されている(Khanjani 2007, López-Cervantes 2004)。全体的に、以前のメタアナリシスでは、ヘプタクロルを除き、シクロジエン系化学 物質と乳がんとの間に有意な関連は示されなかったが、それはわずか2件の研究に基づくものであった。 ジクロロジフェニルジクロロエチレン(DDE)と乳がんに関するシステマティックレビューで同定され た研究(5研究)のメタアナリシスでも、関連を示すエビデンスは示されなかった。我々はまた、乳が んに関する同定されたすべての研究でメタアナリシスを実施し、サンプルサイズが最も大きい各研究 内の分析を毎回選択した。研究ID CAN 019とID CAN 023は、統合を可能にする効果量と信頼区間が提 供されていなかったため、統合から除外された。また、研究ID CAN\_022は、誤って報告されたとは想定 されていない非常に狭い信頼区間を報告していたため除外された。ここでの統合には、多くの異なる 農薬の定義とバイオマーカー(DDE、リンデン、広義の農薬定義)の統合効果が含まれており、解釈が 難しい。統合効果は統計的に有意な乳がんリスクの増加(1.07(0.87~1.31))を示したが、この結果 は慎重な解釈が必要である。メタアナリシスでは、非常に異なるカテゴリーの農薬を組み合わせてお り、主に1つの研究(ID CAN 022)が占めているが、これは住居用農薬の自己申告による農薬ばく露を 評価したもので、バイオマーカーを介して農薬を評価した他の研究に比べて質が低いものである。

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#### 8.8. Bladder cancer

Sixteen studies examining bladder cancer in relation to pesticide exposure were identified; however, 13 were studies from the same population the AHS as previously observed for other cancer outcomes. Among the 25 different analyses presented, only one provided statistically significant results for occupational exposure to imazethapyr in the AHS. However, due to multiple testing the results need cautious interpretation and based on the evidence reviewed in this report there is no suggestion for an association between pesticide exposure and bladder cancer.

#### 8.9. Kidney cancer

Ten studies examined kidney cancer in relation to pesticide exposure; however, data from two populations only, the AHS and the BC (British Columbia) sawmill workers cohort study. Results form the BC sawmill workers cohort study (ID CAN\_129 and ID CAN\_125) were both on occupational exposure to pentachlorophenol and tetrachlorophenol but examining different approaches to statistical analyses. Results from the AHS were on different pesticide classes. Overall, no statistically significant results were observed and the limited number of contributing populations (n=2) does not allow further quantitative synthesis.

#### 8.10. Pancreatic cancer

Seven studies examined pancreatic cancer in relation to pesticide exposure; 4 were reports from the AHS. The overwhelming majority of analyses considered organochlorine pesticides. In a small casecontrol study of modest quality significantly increased concentrations of hexachlorobenzene (HCB), sum of chlordanes and polybrominated diphenylethers (PBDEs) were found in the pancreatic cancer cases compared to healthy controls (ID CAN\_090). In the AHS, among 46 different analyses, significant associations were reported for Pendimethalin and S-Ethyl dipropylthiocarbamate (EPTC). Applicators in the top half of lifetime pendimethalin use had a 3.0-fold (95% CI 1.3–7.2, p-trend 5 0.01) risk compared with never users, and those in the top half of lifetime EPTC use had a 2.56-fold (95% CI 5 1.1–5.4, p-trend=0.01) risk compared with never users. Organochlorines were not associated with an excess risk of pancreatic cancer in the AHS. These findings suggest that herbicides may be associated with pancreatic cancer but require replication by future studies as they all come from a single population without adjustments for multiple testing.

#### 8.11. Testicular cancer

Overall, 8 studies examined testicular cancer. Two studies also reported outcomes for seminoma cancer. All but one study assessed biomarker levels and concentrated on organochlorine pesticides with a range of different biomarkers assessed and studies showing a weak effect for an association with testicular cancer. However, information on more than 4 studies was available for p-p'DDE only and quantitative synthesis showed a non-significant effect and modest heterogeneity (Figure 20). Quantitative synthesis across any pesticide was not performed due to heterogeneity of biomarkers assessed in each study. Overall, there is no evidence to support an association between pesticide exposure and testicular cancer based on evidence reviewed herein.

#### 8.12. Stomach cancer

Six studies examined association between pesticide exposure and stomach cancer. All studies examined occupational exposure to pesticides, a range of pesticide classes was studies; 2 studies had a prospective design but all had modest to small sample sizes. In agreement with previous meta-analysis

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#### 8.8. 膀胱がん

農薬ばく露に関連して膀胱がんを調査した16件の研究が同定されたが、13件は他の発がん影響について以前に観察されたのと同様にAHSの同じ集団からの研究であった。25の異なる分析結果のうち、AHS におけるイマゼタビルへの職業ばく露について統計的に有意な結果を示したのは1件のみであった。しかし、複数の試験を行っているため、結果は慎重に解釈する必要があり、報告書で調査されたエビデンスに基づいても、農薬ばく露と膀胱がんとの関連は示唆されていない。

### 8.9. 腎臓がん

10件の研究が農薬ばく露に関連して腎臓がんを調査したが、データはAHSとBC(ブリティッシュコロ ンビア州)製材所労働者コホート研究の2つの集団からのみであった。BC州製材所労働者コホート研究 (ID CAN\_129及びID CAN\_125)の結果は、いずれもペンタクロロフェノール及びテトラクロロフェノ ールへの職業ばく露に関するものであったが、統計分析のアプローチが異なっていた。AHSの結果は、 異なる農薬クラスに関するものであった。全体的に、統計的に有意な結果は観察されず、寄与した集団 の数が限られているため(n=2)、これ以上の定量的な統合はできなかった。

### 8.10. 膵臓がん

農薬ばく露に関連して膵臓がんを調査した研究は7件、AHSからの報告は4件であった。分析の圧倒的 多数は有機塩素系農薬を対象としたものであった。質の低い小規模な症例対照研究では、ヘキサクロ ロベンゼン(HCB)、クロルデンの和及びポリ臭化ジフェニルエーテル(PBDE)の濃度が健常対照者と 比較して膵臓がん症例で有意に上昇していた(ID CAN\_090)。AHSでは、46種類の分析のうち、 PendimethalinとS-Ethyl dipropylthiocarbamate (EPTC)について有意な関連が報告されている。ペン ディメタリンの生涯使用量の上位半分の散布者は、非使用者と比較して3.0倍(95%CI 1.3-7.2、pトレ ンド5 0.01)のリスクを有し、EPTCの生涯使用量の上位半分の散布者は、非使用者と比較して2.56倍 (95%CI 5 1.1-5.4、pトレンド=0.01)のリスクを有していた。有機塩素系薬剤は、AHSにおける膵臓 がんの過剰リスクとは関連していなかった。これらの知見は、除草剤が膵臓がんと関連している可能 性を示唆しているが、これらはすべて単一の集団から得られたものであり、多重検定調整を行ってい ないため、今後の研究で反復する必要がある。

### 8.11. 精巣がん

全体では8件の研究で精巣がんが調査された。2件の研究では精上皮腫への影響も報告された。1件を 除くすべての研究がバイオマーカーレベルを評価し、有機塩素系殺虫剤に集中しており、さまざまな バイオマーカーが評価され、精巣がんとの関連には弱い影響を示した研究もあった。しかし、p-p'DDE のみについては4件以上の研究の情報が得られ、定量的統合では有意ではない効果と適度な不均一性が 示された(図20)。各研究で評価されたバイオマーカーの不均一性のため、どの農薬についても定量的 統合は行われなかった。全体的に、ここで調査されたエビデンスに基づいて、農薬ばく露と精巣がんと の関連を支持するエビデンスはない。

### 8.12. 胃がん

6件の研究で、農薬ばく露と胃がんとの関連が調査された。すべての研究は農薬への職業ばく露を調 査したもので、農薬のクラスは多岐にわたっていた;2件の研究は前向きデザインであったが、すべて の研究でサンプルサイズは中等度から低度であった。農家に関する以前のメタアナリシス(Saphir 1998)と一致するように、研究は不十分で、主に有意でない結果を報告していた。United Farm Workers of America (UFW) コホートにおける胃がんのコホート内症例対照研究(ID CAN\_028)では、有意な関 連が報告された。フェノキシ酢酸除草剤2,4-ジクロロフェノキシ酢酸(2,4-D)の使用量が多い地域で

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on farmers (Saphir 1998), studies reported weak and mainly non-significant results. A nested casecontrol study (ID CAN 028) of gastric cancer embedded in the United Farm Workers of America (UFW) cohort reported significant associations: working in areas with high use of the phenoxyacetic acid herbicide 2.4-dichlorophenoxyacetic acid (2.4-D) was associated with gastric cancer (OR 1.85; 95% CI 1.05-3.25); use of the organochlorine insecticide chlordane was also associated with the disease (OR 2.96; 95% CI 1.48-5.94). Gastric cancer was associated with use of the acaricide propargite (OR 2.86; 95% CI 1.56-5.23). Nonetheless, the study is limited by a relatively small number of cases and controls, multiple testing and exposure misclassification, as assessment was ecological in nature. In the AHS, based on 15 exposed cases, stomach cancer risk increased monotonically with increasing methyl bromide use (RR = 3.13; 95 % CI, 1.25–7.80 for high use compared with no use; p for trend = 0.02). However, again the associations suffer from multiple testing as all other cancer subtypes have been associated with methyl bromide use in this study (ID CAN 147). Meta-analysis selecting the analysis with largest sample size is shown in Figure 21 but results require careful consideration. Despite a statistical significant pooled large effect size, this is dominated by two studies (ID CAN 125, ID CAN 147), which examine pentachlorophenol and methyl bromide; two compounds that are not approved in the European Union.

Pesticide epidemiology

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#### 8.13. Liver cancer

Five studies (including 11 separate analyses) and one conference abstract examined associations between pesticide exposure and liver cancer. The majority of analyses examined exposure to organochlorine pesticides and all studies examined occupational exposure to pesticides. Both studies on DDT (IDs CAN\_076 and ID CAN\_079) reported statistically significant associations with liver cancer; the remaining analyses were non-statistically significant. These two studies largely dominate the meta-analysis on liver cancer, which shows a statistically significant pooled result largely driven by the DDT studies.

#### 8.14. Cancer subgroups with few studies

As illustrated in Table 6, for a large number of individual cancers only very few studies are available to allow synthesis of evidence for each cancer subgroup. Our systematic review did not identify any previously published meta-analyses on these cancer subtypes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these cancer subtypes were of small effect and not statistically significant with few exceptions concerning occupational exposure only. Given the large number of analyses within each study, these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these cancer subtypes.

There were also a large number of studies examining all cancers (composite cancer outcome) in relation to pesticide. Cancers represent a very heterogeneous group of disorders and simultaneous examination of all cancer subtypes may introduce bias in the associations. Overall, 30 analyses examining "all cancers" were identified and 28 of them were analyses of the same cohort, the AHS, not allowing further synthesis of the results. Only 4 results out of 31 were statistically significant were associated with poor quality of studies and therefore do not merit interpretation at this stage.

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働くことは、胃がんと関連していた(OR 1.85;95%CI 1.05-3.25)。有機塩素系殺虫剤クロルデンの 使用もこの疾患と関連していた(OR 2.96;95%CI 1.48-5.94)。胃がんは、殺ダニ剤プロパルギトの 使用と関連していた(OR 2.86;95%CI 1.56-5.23)。にもかかわらず、評価が生態学的なものであっ たため、この研究は症例数と対照数が比較的少ないこと、多重検定及びばく露の誤分類によって制限 されている。AHSでは、15例のばく露例に基づいて、胃がんリスクは臭化メチルの使用量の増加ととも に単調に増加した(RR = 3.13;95%CI、非使用と比較して高頻度使用では1.25-7.80;トレンドのp = 0.02)。しかし、この研究では他のすべてのがんのサブタイプが臭化メチル使用と関連していたため、 やはり多重検定の問題がある(ID CAN\_147)。最大のサンプルサイズで分析を選択したメタアナリシス は図21に示されているが、結果は慎重に調査する必要がある。統計的に有意な統合された大きな効果 量にもかかわらず、この結果は、欧州連合で承認されていない2つの化合物であるペンタクロロフェノ ールと臭化メチルを調査している2つの研究(ID CAN\_125, ID CAN\_147)の影響が大きい。

### 8.13. 肝臓がん

5件の研究(11件の個別の分析を含む)と1件の学会発表の要約で、農薬ばく露と肝臓がんとの関連が 調査された。分析の大部分は有機塩素系農薬へのばく露を対象としており、すべての研究は農薬への 職業ばく露を対象としていた。DDTに関する両研究(ID:CAN\_076及びID:CAN\_079)では、肝がんとの 統計的に有意な関連が報告されたが、残りの分析では統計的に有意ではなかった。これら2つの研究が 肝臓がんに関するメタアナリシスの大部分を占めており、統合された結果は統計学的に有意であり、 主にDDT研究に牽引されている。

### 8.14. 研究数が少ないがんサブグループ

表6に示されているように、多数のがんについては、各がんサブグループのエビデンスを総合的に判 断できる研究は非常に限られている。我々のシステマティックレビューでは、過去に発表されたエビ デンス(2006年以前)との比較を可能にするために、これらのがんサブタイプに関する過去に発表され たメタアナリシスを確認しなかった。一般的に、これらのがんサブタイプに関する結果では影響は小 さく、職業ばく露のみに関する少数の例外を除いて統計的に有意ではなかった。各研究内の分析数が 多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農薬ば く露とこれらのがんサブタイプとの間の関連を示唆するエビデンスはない。

また、農薬に関連してすべてのがん(複合発がん影響)を調査した研究も多数あった。がんは非常に 異質な疾患群であり、すべてのがんサプタイプを同時に調査すると、関連にバイアスがかかる可能性 がある。全体では、「すべてのがん」を対象とした30の分析が同定されたが、そのうち28の分析は同じ コホート(AHS)を対象としたものであり、結果をさらに統合することはできなかった。31件のうち統 計的に有意な結果が得られたのは4件のみで、質の低い研究と関連していたため、現段階では解釈に値 しない。

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### Table 6: Summary of eligible studies identified per cancer subgroup

Cancers	N studies	Meta-analysis recommended	Previous meta- analysis identified
Haematological neoplasms	88	Yes	Yes
Prostate cancer	39	No	Yes
Lung cancer	30	Yes	No
All cancers	30	No	No
Childhood cancer	45	Yes	Yes
Colorectal cancer	26	No	No
Skin cancer	17	Yes	No
Bladder cancer	16	Yes	No
Breast cancer	14	Yes	Yes
Kidney cancer	10	No	No
Pancreatic cancer	7	No	No
Testicular cancer	8	No	No
Lip, oral cavity and pharynx cancer	5	No	No
Stomach cancer	6	No	No
Liver cancer	5	No	No
Brain cancer	6	No	No
Bone cancer	5	No	No
Oesophageal cancer	5	No	No
Larynx cancer	3	No	No
Biliary tract cancer	2	No	No
Soft-tissue	2	No	No
Female reproductive system cancer	2	No	No
Other	9	No	No

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# 表6: がんサブグループごとに同定された調査対象研究の要約

がん	研究数	メタアナリシス推奨	以前のメタアナリシス
造血器新生物	88	Yes	Yes
前立腺がん	39	No	Yes
肺がん	30	Yes	No
すべてのがん	30	No	No
小児がん	45	Yes	Yes
大腸がん	26	No	No
皮膚がん	17	Yes	No
膀胱がん	16	Yes	No
乳がん	14	Yes	Yes
腎臓がん	10	No	No
膵臓がん	7	No	No
精巣がん	8	No	No
口唇・口腔・咽頭のがん	5	No	No
胃がん	6	No	No
肝臓がん	5	No	No
脳腫瘍	6	No	No
骨がん	5	No	No
食道がん	5	No	No
喉頭がん	3	No	No
胆道がん	2	No	No
軟組織	2	No	No
女性生殖器系がん	2	No	No
その他	9	No	No

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 Table 7:
 Summary results across eligible studies that reported information on the same pesticide class and risk of leukaemia (DDE: Dichlorodiphenyldichloroethylene)

a	Publication					0.5			Level of
Study ID	Date	Class	Pesticide Type	Outcome	N	OR	LCI	UCI	Adjustment
DDE									
CAN_064	2010	p,p'-DDE	Biomarker	Chronic Lymphocytic Leukemia	210	0.78	0.28	2.21	+++
CAN_063	2010	p,p'-DDE	Questionnaire	Chronic Lymphocytic Leukemia	148	0.62	0.29	1.3	++
CAN_056	2008	p,p'-DDE	Biomarker	Chronic Lymphocytic Leukemia	71	1	0.4	2.5	+
Insecticides									
CAN_072	2006	Insecticides	Questionnaire	All leukemias	1304	1	0.7	1.4	+
CAN_049	2009	Insecticides	Questionnaire	Chronic Lymphocytic Leukemia	37	0.8	0.3	2.1	+
CAN_024	2010	Insecticides	Questionnaire	Acute Myeloid Leukemia	158	1.52	0.16	2.04	+++
Herbicides									
CAN_072	2006	Herbicides	Questionnaire	All leukemias	1260	1.4	0.8	2.3	++
CAN_049	2009	Herbicides	Questionnaire	Chronic Lymphocytic Leukemia	39	0.5	0.2	1.3	+
CAN_024	2010	Herbicides	Questionnaire	Acute Myeloid Leukemia	45	1.83	0.99	3.38	+++
CAN_058	2008	Herbicides	Questionnaire	Chronic Lymphocytic Leukemia	523	1.15	0.76	1.74	++

 Table 8:
 Examples of identified studies from the Agricultural Health Study (AHS) that

 evaluated the same biomarkers of pesticide exposure in relation to colorectal cancer (DDVP:
 2,2-dichlorovinyl dimethyl phosphate)

				Sample	Effect	Lower	Upper	Adjust
Study ID	Pesticide	Outcome	Comparison	size	Estimate (OR)	95% CI	95% CI	ments
			Highest					
			tertile of					
	Dichlorvos/		exposure vs					
CAN_122	DDVP	Colon cancer	no	202	1.48	0.78	2.8	+
	Dichlorvos/							
CAN_024	DDVP	Colon cancer	Ever vs. never	56813	1.5	0.9	2.4	++
	Fonofos		-					
CAN_024	Fonoros	Colon cancer	Ever vs. never	56813	1.5	1	2.2	++
			Highest					
			tertile of					
			exposure vs					
CAN_119	Fonofos	Colon cancer	no	126	1.66	0.92	3.03	++
-								
		Colorectal						
CAN_024	Malathion	cancer	Ever vs. never	56813	0.8	0.6	1.1	++
			Highest					
			tertile of					
		Colorectal	exposure vs					
CAN_121	Malathion	cancer	no	58	0.84	0.48	1.48	++
		Rectum						
CAN_118	Toxaphene	cancer	Yes vs. no	75	2	1.1	3.5	+++
		Rectum						
CAN_024	Toxaphene	cancer	Ever vs. never	56813	2.1	1.2	3.6	++ ,

# ン)についての情報を報告した調査対象試験全体の要約結果

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Study ID	掲載 日	殺虫剤クラス	殺虫剤の種類	影響	N	OR	95% LCI	95% UCI	Level of Adjustment	
DDE										
CAN_064	2010	p, p'-DDE	バイオマーカー	慢性リンパ性白血病	210	0.78	0.28	2.21	+++	
CAN_063	2010	p, p'-DDE	質問紙	慢性リンパ性白血病	148	0.62	0.29	1.3	++	
CAN_056	2008	p, p'-DDE	バイオマーカー	慢性リンパ性白血病	71	1	0.4	2.5	+	
Insectic	Insecticides									
CAN_072	2006	Insecticides	質問紙	すべての白血病	1304	1	0.7	1.4	+	
CAN_049	2009	Insecticides	質問紙	慢性リンパ性白血病	37	0.8	0.3	2.1	+	
CAN_024	2010	Insecticides	質問紙	急性骨髓性白血病	158	1.52	0.16	2.04	+++	
Herbicid	Herbicides									
CAN_072	2006	Herbicides	質問紙	すべての白血病	1260	1.4	0.8	2.3	++	
CAN_049	2009	Herbicides	質問紙	慢性リンパ性白血病	39	0.5	0.2	1.3	+	
CAN_024	2010	Herbicides	質問紙	急性骨髓性白血病	45	1.83	0.99	3.38	+++	
CAN_058	2008	Herbicides	質問紙	慢性リンパ性白血病	523	1.15	0.76	1.74	++	

# 表8:大腸がん(DDVP:2,2-ジクロロビニルジメチルリン酸塩)に関連して農薬ば く露の同じバイオマーカーを評価した農業健康調査(Agricultural Health Study:AHS)から同定された研究の例

Study ID	殺虫剤	影響	比較	サンプル サイズ	効果推定 値(OR)	下位 95% CI	上位 95% CI	調整
bludy 1b			Highest tertile			30% 01	30,001	HAND THEY
	ジクロロボス		of exposure vs					
CAN_122	/DDVP	大腸がん	no	202	1.48	0.78	2.8	+
	ジクロロボス							
CAN_024	/DDVP	大腸がん	Ever vs. never	56813	1.5	0.9	2.4	++
CAN_024	フォノフォス	大腸がん	Ever vs. never	56813	1.5	1	2.2	++
			Highest tertile					
			of exposure vs					
CAN_119	フォノフォス	大腸がん	no	126	1.66	0.92	3.03	++
CAN_024	マラチオン	直腸がん	Ever vs. never	56813	0.8	0.6	1.1	++
			Highest tertile					
			of exposure vs					
CAN_121	マラチオン	直腸がん	no	58	0.84	0.48	1.48	++
CAN_118	トキサフェン	直腸がん	Yes vs. no	75	2	1.1	3.5	+++
CAN_024	トキサフェン	直腸がん	Ever vs. never	56813	2.1	1.2	3.6	++

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Table 9:Studies on biomarkers of pesticide exposure and testicular cancer with morethan >2studies per biomarker (DDE: Dichlorodiphenyldichloroethylene; HCB:Hexachlorobenzene)

		Effect estimate					
Study ID	Pesticide	type	Comparison level	Total N	Effect estimate	95% LCI	95% UCI Adjustments
CAN_111	Dieldrin	OR	high tertile vs low	418	0.79	0.44	1.41 ++
CAN_115	Dieldrin	OR	high tertile vs low	60	2.1	0.5	9.5 +
CAN_113	HCB	OR	yes/no	57	4.4	1.7	12 +
CAN_115	HCB	OR	high tertile vs low	70	2.9	0.5	15.2 +
CAN_111	Heptachlor epoxide	OR	high tertile vs low	407	0.67	0.35	1.29 ++
CAN_115	Heptachlor epoxide	OR	high tertile vs low	68	2.4	0.6	9.1 +
CAN_111	Mirex	OR	high tertile vs low	557	0.87	0.5	1.53 ++
CAN_112	Mirex	RR	high tertile vs low	1333	0.24	0.9	1.74 +++
CAN_115	Mirex	OR	high tertile vs low	66	1.2	0.4	3 +
CAN_111	o,p-DDT	OR	high tertile vs low	514	1.3	0.67	2.53 ++
CAN_115	o,p'-DDT	OR	high tertile vs low	71	1.4	0.4	4.5 +
CAN_116	o,p'-DDT	Mean difference	unit increase	60	0.46	n/a	n/a n/a
CAN_111	p,p'-DDT	OR	high tertile vs low	533	1.17	0.68	2 ++
CAN_112	p,p'-DDT	RR	high tertile vs low	1493	1.13	0.71	1.82 +++
CAN_115	p,p'-DDT	OR	high tertile vs low	63	2.1	0.6	7.2 +
CAN_116	p,p'-DDT	Mean difference	unit increase	60	-1.2	n/a	n/a n/a
CAN_111	p,p'-DDE	OR	high tertile vs low	554	0.61	0.32	1.14 ++
CAN_112	p,p'-DDE	RR	high tertile vs low	884	1.71	1.23	2.38 +++
CAN_113	p,p'-DDE	OR	yes/no	44	1.3	0.5	3 +
CAN_115	p,p'-DDE	OR	high tertile vs low	65	2.2	0.7	6.5 +
CAN_116	p,p'-DDE	Mean difference	unit increase	60	-15.29		n/a n/a
CAN_117	p,p'-DDE	OR	high tertile vs low	98	3.21	0.77	13.3 +
							,
CAN_111	Oxychlordane	OR	high tertile vs low	538	0.93	0.5	1.73 ++
CAN_112	Oxychlordane	RR	high tertile vs low	841	1.27	0.92	1.76 +++
CAN_115	Oxychlordane	OR	high tertile vs low	68	3.2	0.6	16.8 +
CAN_111	Total chlordanes	OR	high tertile vs low	562	0.93	0.51	1.68 ++
CAN_112	Total chlordanes	RR	high tertile vs low	842	1.51	1.09	2.1 +++
CAN_113	Sum of chlordanes	OR	yes/no	49	1.9	0.7	5 +
CAN_115	Total chlordanes	OR	high tertile vs low	70	2.3	0.6	7.2 +
	1	<sup>1</sup> -  -	· · · · · · · · · · · ·	1			
CAN_111	Trans -nonachlor	OR	high tertile vs low	564	0.89	0.49	1.61 ++
CAN_112	Trans -nonachlor	RR	high tertile vs low	875	1.46	1.07	2 +++
CAN_115	Trans -nonachlor	OR	high tertile vs low	62	2.6	0.7	8.9 +

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# 表9: 農薬ばく露のバイオマーカーと精巣がんについて、1 つのバイオマーカーに つき2件以上の研究を行った研究(DDE: ジクロロジフェニルジクロロエチレ ン、HCB: ヘキサクロロベンゼン)

Study ID	殺虫剤	効果推定型	比較レベル	合計数	効果の 推定	95% LCI	95% UCI	調整
CAN_111	ディルドリン	OR	high tertile vs low	418	0.79	0.44	1.41	
CAN_115	ディルドリン	OR	high tertile vs low	60	2.1	0.5	9.5	+
CAN_113	HCB	OR	yes/no	57	4.4	1.7	12	+
CAN_115	HCB	OR	high tertile vs low	70	2.9	0.5	15.2	+
CAN_111	ヘプタクロルエ ポキシド	OR	high tertile vs low	407	0.67	0.35	1.29	++
CAN_115	ヘプタクロルエ ポキシド	OR	high tertile vs low	68	2.4	0.6	9.1	+
CAN_111	ミレックス	OR	high tertile vs low	557	0.87	0.5	1.53	++
CAN_112	ミレックス	RR	high tertile vs low	1333	0.24	0.9	1.74	+++
CAN_115	ミレックス	OR	high tertile vs low	66	1.2	0.4	3	+
CAN_111	o, p-DDT	OR	high tertile vs low	514	1.3	0.67	2.53	++
CAN_115	o,p'-DDT	OR	high tertile vs low	71	1.4	0.4	4.5	+
CAN_116	o,p'-DDT	Mean difference	unit increase	60	0.46	n/a	n/a	n/a
CAN_111	p, p'-DDT	OR	high tertile vs low	533	1.17	0.68	2	++
CAN_112	p, p'-DDT	RR	high tertile vs low	1493	1.13	0.71	1.82	+++
CAN_115	p, p'-DDT	OR	high tertile vs low	63	2.1	0.6	7.2	+
CAN_116	p, p'-DDT	Mean difference	unit increase	60	-1.2	n/a	n/a	n/a
CAN_111	p, p'-DDE	OR	high tertile vs low	554	0.61	0.32	1.14	++
CAN_112	p, p'-DDE	RR	high tertile vs low	884	1.71	1.23	2.38	+++
CAN_113	p, p'-DDE	OR	yes/no	44	1.3	0.5	3	+
CAN_115	p, p'-DDE	OR	high tertile vs low	65	2.2	0.7	6.5	+
CAN_116	p,p'-DDE	Mean difference		60	-15.29	n/a	n/a	n/a
CAN_117	p,p'-DDE	OR	high tertile vs low	98	3.21	0.77	13.3	+
CAN_111	オキシクロルデ ン	OR	high tertile vs low	538	0.93	0.5	1.73	++
CAN_112	オキシクロルデ ン	RR	high tertile vs low	841	1.27	0.92	1.76	+++
CAN_115	オキシクロルデ ン	OR	high tertile vs low	68	3.2	0.6	16.8	+
CAN_111	全クロルデン	OR	high tertile vs low	562	0.93	0.51	1.68	
CAN_112	全クロルデン	RR	high tertile vs low	842	1.51	1.09	2.1	
CAN_113	クロルデンの和	OR	yes/no	49	1.9	0.7	5	
CAN_115	全クロルデン	OR	high tertile vs low	70	2.3	0.6	7.2	+
CAN_111	トランス-ノナ クロル	OR	high tertile vs low	564	0.89	0.49	1.61	++
CAN_112	トランス-ノナ クロル	RR	high tertile vs low	875	1.46	1.07	2	+++
CAN_115	トランス-ノナ クロル	OR	high tertile vs low	62	2.6	0.7	8.9	+

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Pesticide epidemiology

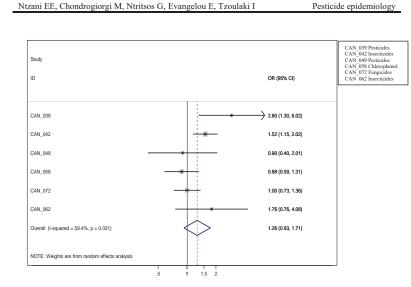


Figure 8: Random effects meta-analysis of the association between exposure to pesticides and Leukemia

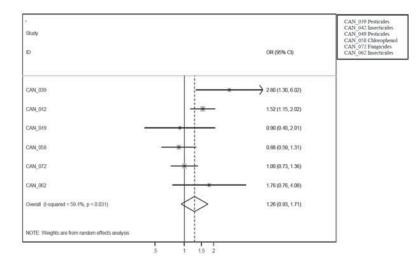
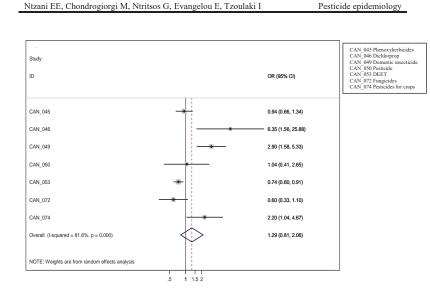


図8:農薬ばく露と白血病との関連のランダム効果メタアナリシス

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**Figure 9:** Random effects meta-analysis of the association between exposure to pesticides and Hodgkin Lymphoma

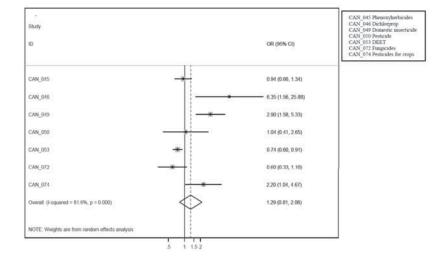


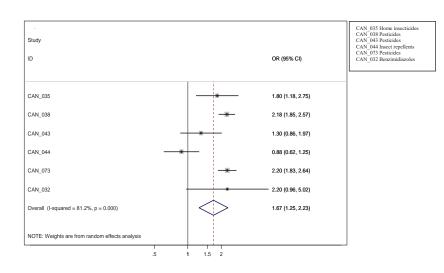
図9:農薬ばく露とホジキンリンパ腫との関連のランダム効果メタアナリシス



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**Figure 10:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Any exposure to pesticide during pregnancy and childhood leukemia)

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Pesticide epidemiology

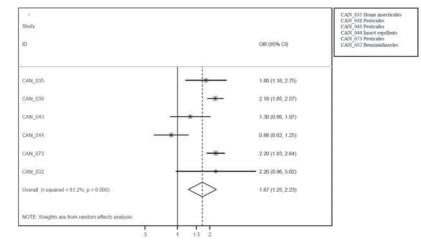


図10:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期のあらゆる農薬ばく露と小児白血病)

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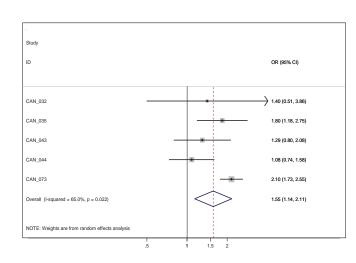


Figure 11: Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Exposure to insecticides during pregnancy and childhood leukemia)



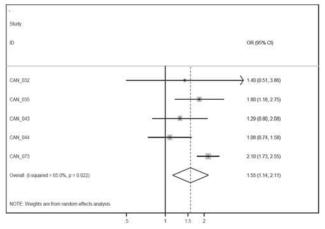
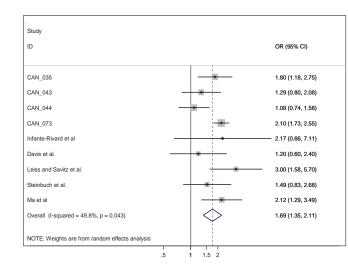


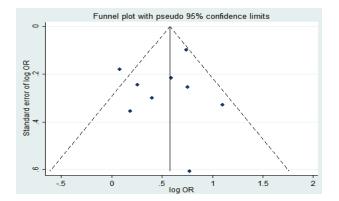
図11:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期の殺虫剤ばく露と小児白血病)

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**Figure 12:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Residential exposure to insecticide during pregnancy and childhood leukemia) (update to meta-analysis 2010 using published effect sizes; Turner 2010) and associated funnel plot

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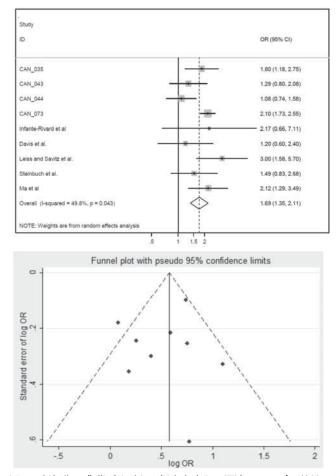


図12:妊娠期の農薬ばく露と小児白血病との関連のランダム効果メタアナリシス (妊娠期の殺虫剤への住居内ばく露と小児白血病(公表されている効果量を用 いたメタアナリシス2010の更新;Turner 2010)、ならびに関連するファンネル プロット)

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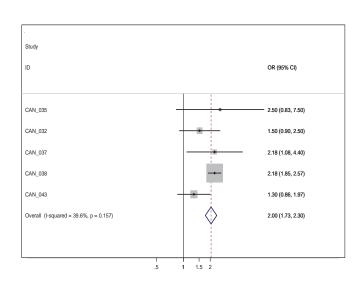


Figure 13: Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Exposure to unspecified pesticides during pregnancy and childhood leukemia)

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Pesticide epidemiology

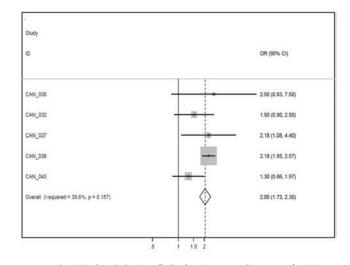
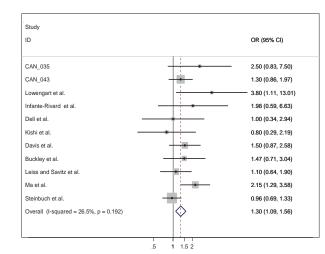


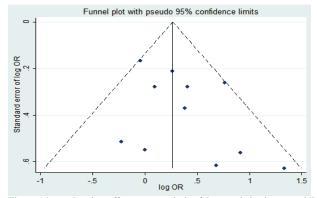
図13:小児白血病と妊娠期の農薬ばく露との関連のランダム効果メタアナリシス (妊娠期の不特定の農薬ばく露と小児白血病)

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**Figure 14:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during pregnancy (Residential exposure to unspecified pesticides during pregnancy and childhood leukemia (update to meta-analysis 2010, Turner 2010) and associated funnel plot)

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Pesticide epidemiology

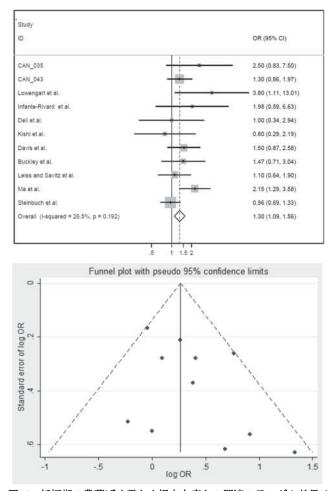
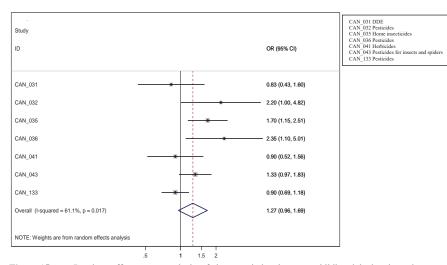


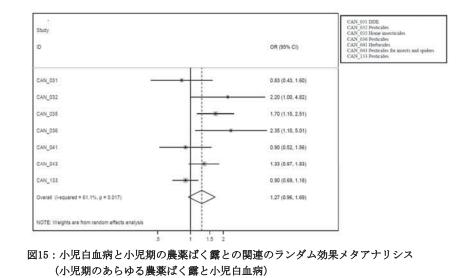
図14:妊娠期の農薬ばく露と小児白血病との関連のランダム効果メタアナリシス (妊娠期の不特定の農薬への住居内ばく露と小児白血病(メタアナリシス2010、 Turner 2010への更新)、ならびに関連するファンネルプロット)





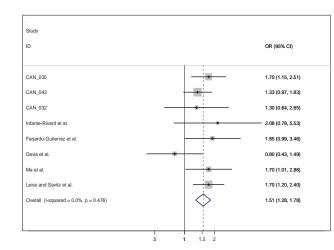
**Figure 15:** Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Any exposure to pesticide during childhood and childhood leukemia)

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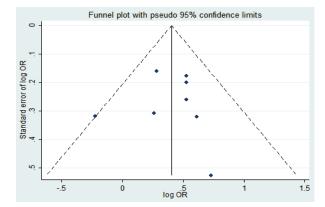


Figure 16: Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Residential exposure to insecticide during childhood and childhood leukemia (update to meta-analysis 2010 using published effect sizes, Turner 2010) and associated funnel plot)

Pesticide epidemiology

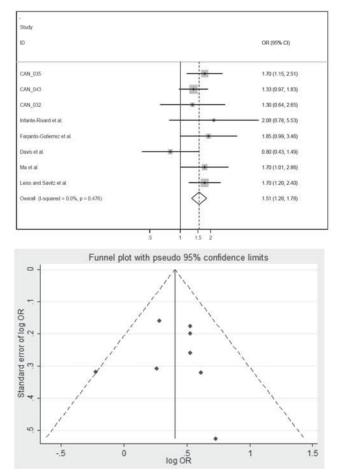


図16:小児白血病と小児期の農薬ばく露との関連のランダム効果メタアナリシス (小児期の殺虫剤への住居内ばく露と小児白血病(公表されている効果量を用 いたメタアナリシス2010の更新、Turner 2010)、ならびに関連するファンネル プロット)

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Study

CAN 035

CAN 043

CAN\_032

Kishi et al.

Fdell et al.

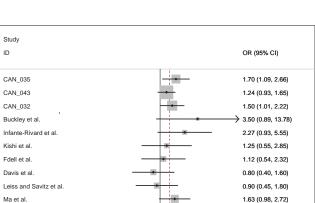
Davis et al.

Ma et al.

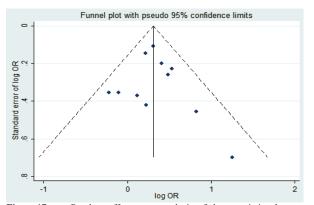
Steinbuch et al.

Overall (I-squared = 0.0%, p = 0.524)

ID



1.36 (1.10, 1.68) 1.36 (1.19, 1.55)



.5 1 1.5 2

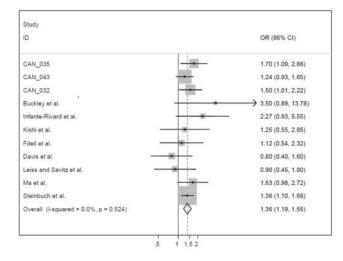
Figure 17: Random effects meta-analysis of the association between childhood leukemia and exposure to pesticides during childhood (Residential exposure to unspecified pesticides during childhood and childhood leukemia (update to meta-analysis 2010 using published effect sizes, Turner 2010) and associated funnel plot)

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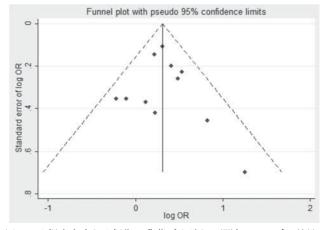
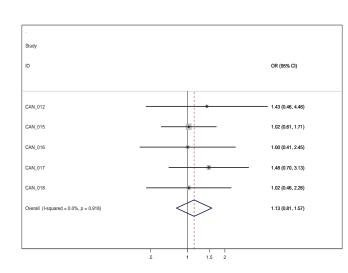


図17:小児白血病と小児期の農薬ばく露との関連のランダム効果メタアナリシス (小児期の不特定の農薬への住居内ばく露と小児白血病(公表されている効果 量を用いたメタアナリシス2010の更新、Turner 2010)、ならびに関連するファ ンネルプロット)

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**Figure 18:** Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and breast cancer on studies that examined DDE exposure to pesticide with breast cancer



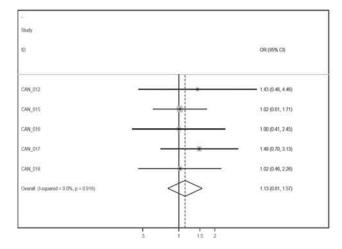
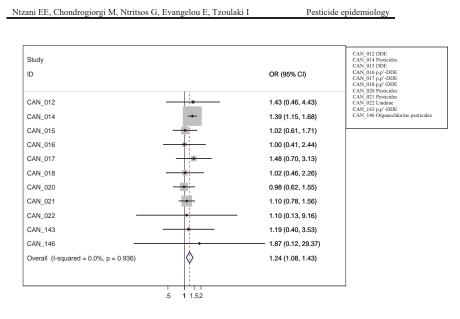


図18:農薬のジクロロジフェニルジクロロエチレン (DDE) ばく露と乳がんを調査し た研究でのDDEの情報と乳がんのランダム効果メタアナリシス

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**Figure 19:** Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and breast cancer selecting analyses with the largest sample size within each study (pesticides assessed in each study are shown in on the right key).

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Pesticide epidemiology

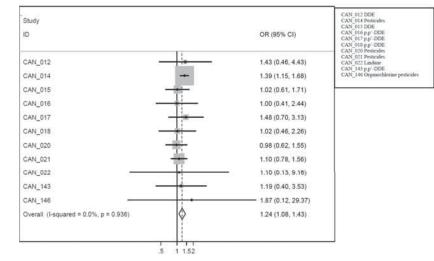


図19:各研究内で最大のサンプルサイズでの分析を選択したジクロロジフェニルジ クロロエチレン(DDE)の情報と乳がんのランダム効果メタアナリシス(各研究で 評価された農薬は右に示されている)

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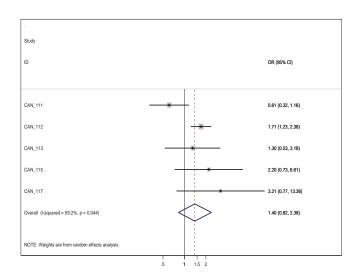


Figure 20: Random effects meta-analysis for studies with information on Dichlorodiphenyldichloroethylene (DDE) and testicular cancer



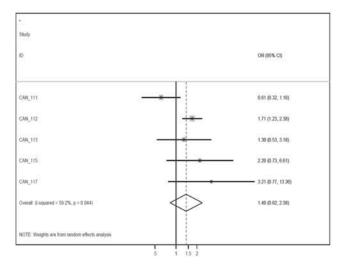


図20:ジクロロジフェニルジクロロエチレン (DDE) に関する情報と精巣がんのラン ダム効果メタアナリシス

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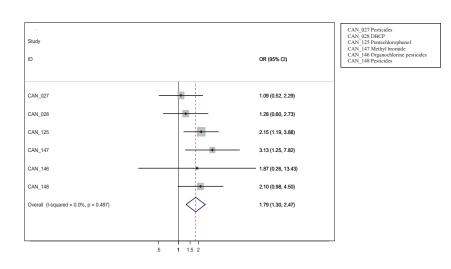


Figure 21: Random effects meta-analysis for studies that examined any exposure to pesticide with stomach cancer selecting analyses with the largest sample size within each study

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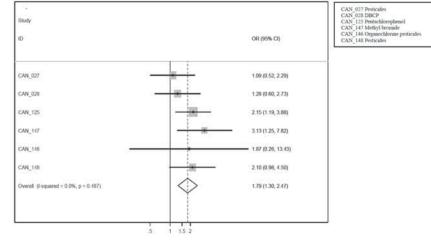


図21:あらゆる農薬ばく露と胃がんを調査した研究のうち、各研究内で最大のサンプ ルサイズでの分析を用いた研究のランダム効果メタアナリシス

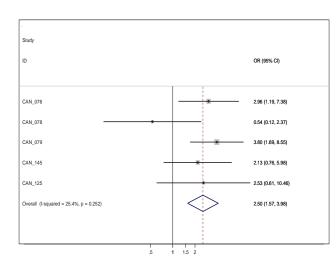
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**Figure 22:** Random effects meta-analysis for studies that examined any exposure to pesticide with liver cancer selecting analyses with the largest sample size within each study (pesticides assessed in each study are shown on the right key)

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

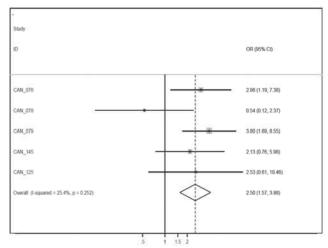


図22:あらゆる農薬ばく露と肝臓がんを調査した研究のうち、各研究内で最大のサ ンプルサイズの分析を用いた研究のランダム効果メタアナリシス(各研究で評 価された農薬は右に示されている)

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## 9. Child health

Overall, 84 individual studies examined the effect of pesticide exposure on child health outcomes (median sample size: 267; IOR 119-811), contributing 821 separate analyses in the data extraction database. More than 120 health-related outcomes were assessed with a large proportion focusing on congenital malformations and developmental parameters including but not restricting to somatometrics (Table 10). As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 38 out of the 84 were prospective cohort studies and other 5 were nested case-controls; the majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 23 and 183,313 participants (median 267) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we observed no large clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS), while 26 studies assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was more prominent here  $(n=49, \dots, n=4)$ 58%) while 3 studies assessed occupational exposure through JEM. The different outcome categories examined are presented in Table 10 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis (Table 11). Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for urological malformations only.

## 9.1. Prematurity

Fifteen studies assessed the association between pesticide exposure during pregnancy and prematurity with a median sample size of 193 (IQR 87-469), contributing 54 separate extracted comparisons in the database. More than half of the studies were retrospective and in more than three-fourths of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (8 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 091) assessed a Dutch population of greenhouse workers and reported a decreased risk of preterm birth among male greenhouse workers (OR= 0.47; 95%CI= 0.35–0.65) while the observed increased risk in women was not statistically significant (OR= 1.14, 95%CI= 0.57–2.31). The remaining studies reported statistically non-significant results with effect estimates pointing towards a positive association. Moreover, no meta-analysis of published studies was identified. Based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and prematurity in general.

#### 9.2. Restricted fetal growth

Twelve studies assessed the association between pesticide exposure during pregnancy and restricted fetal growth and/or small for gestational age neonates with a median sample size of 422 (IQR 178-1,630), contributing 44 separate extracted comparisons in the database. Sixty percent of the studies were prospective, three assessed occupational exposure and in more than two-thirds of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (4 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a

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#### 9. 小児の健康

全体では 84 件の個別研究が農薬ばく露の小児への健康影響を調査し(サンプルサイズ中央値:267、 IQR 119-811)、データ抽出データベースでは 821 件の分析が行われた。120 以上の健康関連影響が 評価されており、その大部分は先天性奇形と身体測定を含むがそれに限定されない発生パラメータに 焦点を当てている(表 10)。他の影響に見られるように、ばく露の定義の多様性には目を見張るもの があり、データ統合に特別な問題を与えている。84 件のうち 38 件のみが前向きコホート研究であり、 他の 5 件はコホート内症例対照研究であった。エビデンスの大部分は後ろ向き症例対照分析に由来し ており、これはばく露量測定においてリコールバイアスがかかりやすい。報告された分析におけるサ ンプルサイズはしばしば小さく、23~183,313人(中央値267人)であり、この領域の最大の研究は、が ん分野で評価された最大の研究よりも小さい。ここでは、農業健康調査(Agricultural Health Study: AHS)のような、この分野の大規模でよく知られた研究に由来する多数の出版物まとまっては観察され なかったが、26件の研究が職業ばく露を評価していた。さらに、ばく露のバイオマーカーに関する情報 を有する研究の存在は、ここではより多かった(n=49、58%)が、3つの研究はJEMによる職業ばく露を 評価した。調査した異なるカテゴリーに属する影響を、各カテゴリーに寄与した研究数と定量的統合 の決定とともに表 10 に示す(表 11)。データの不均一性と同定された研究数が少なかったため、デ ータの統計的統合(メタアナリシス)は泌尿器の奇形のみを対象に実施した。

## 9.1. 未熟児

妊娠期の農薬ばく露と未熟児との関連を評価した研究は 15 件あり、サンプルサイズの中央値は 193 件 (IQR 87-469) で、データベースには 54 件の比較が抽出されている。半数以上の研究が後ろ 向きで、4分の3以上の研究ではバイオマーカーを用いてばく露が評価されていた。個々の農薬の評価 は多種多様で、DDT代謝物の評価がより頻繁に行われていた(8件の研究)。それにもかかわらず、同じ 比較単位を使用した 4 件以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われず、 定量的な統合は行われなかった。最大の前向き研究 (ID CH 091) では、オランダの温室労働者を対象 とし、温室労働者の男性における早産リスクの減少 (OR= 0.47; 95%CI= 0.35-0.65) が報告されたが、 女性におけるリスクの増加は統計的に有意ではなかった (OR= 1.14, 95%CI= 0.57-2.31)。残りの研 究では、正の関連を示す推定効果が得られたが、統計的に有意な結果はなかった。さらに、発表された 研究のメタアナリシスは確認されなかった。これらのデータに基づいて、農薬ばく露と未熟児との間 に臨床的に有意な関連を示唆する最近のエビデンスはない。

## 9.2. 胎児の発育制限

12の研究では、妊娠期の農薬ばく露と胎児の発育制限及び/または在胎不当過小児との関連を評価し ており、サンプルサイズの中央値は422(IQR 178-1,630)で、データベースには44件の個別の比較が抽 出されていた。研究の60%は前向きで、3つの研究では職業ばく露を評価し、3分の2以上の研究ではバ イオマーカーを用いてばく露を評価している。個々の農薬の評価は多種多様で、DDT代謝物の評価がよ り頻繁に行われていた(4件の研究)。それにもかかわらず、同じ比較単位を使用した 4 件以上の研 究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は行われなかった。最 大の研究(ID RPD 26)では、後ろ向きコホートを対象に、飲料水中のアトラジンが在胎不当過小(SGA) 及び早産の罹患率の増加と関連しているかどうかを評価した。著者らの報告によると、妊娠第 3 期及 び全妊娠期間の飲料水中のアトラジンは、SGA(Small for Gestational Age)の罹患率の有意な増加

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quantitative synthesis was not performed. The largest study (ID RPD 26) assessed in a retrospective cohort whether atrazine in drinking water is associated with increased prevalence of small-forgestational age and preterm birth. The authors reported that atrazine in drinking water during the third trimester and the entire pregnancy was associated with a significant increase in the prevalence of SGA (Small for Gestational Age); atrazine in drinking water > 0.1 µg/L during the third trimester resulted in a 17–19% increase in the prevalence of SGA compared with the control group (< 0.1 µg/L). All the remaining studies reported statistically non-significant results without a consistent pattern regarding the effect direction of the effect magnitude. Moreover, no meta-analysis of published studies was identified. Based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and prematurity in general.

#### 9.3. Somatometrics (Body size metrics)

Numerous studies examined the association between pesticide exposure and growth.

#### 9.3.1. Birth length / Height

Length at birth and height was assessed in 13 and 8 studies, respectively, contributing 78 separate comparisons in the database. In the vast majority of the studies, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently; nevertheless no single pesticide and related biomarker was assessed in more than 4 studies, thus a quantitative synthesis was not performed.

The largest prospective study (ID CH 073) assessing a North American population born before 1980, reported that only the highest prenatal concentrations of p,p'-DDE (>60 mg/l), as compared with the lowest (<15 mg/l), were statistically significantly associated with decreased height at age 7 years [adjusted coefficient (SE) -2.21 cm (0.67)]. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis was identified. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and birth length or height in general.

#### 9.3.2. Body weight

Twenty-six studies assessed the association between pesticide exposure during pregnancy and birth weight, contributing 134 separate extracted comparisons in the database. Another 5 studies assessed the association between pesticide and ponderal index. In a large number of comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (11 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 014) was a Agricultural Health Study (AHS) publication and reported that first-trimester pesticide-related tasks were not associated with birth weight and that, after multiple analyses, ever use of the pesticide carbaryl was associated with decreased birth weight (-82 g, 95% CI = -132, -31). The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis of published studies was identified. We identified though a meta-analysis of individual participants data from European cohorts which reported that a  $1-\mu g/L$  increase in p.p'-

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と関連しており、妊娠第3期に飲料水中のアトラジンが0.1 µg/Lを超えると、対照群(0.1 µg/L 未満)と比較してSGAの罹患率が17~19%増加した。残りのすべての研究では、効果量の方向性に 関する一貫したパターンがなく、統計的に有意ではない結果が報告された。さらに、発表された研究の メタアナリシスは確認されなかった。これらのデータに基づいて、農薬ばく露と未熟児との間に臨床 的に有意な関連を示唆する最近のエビデンスはない。

## 9.3. 身体測定(体格計測)

農薬ばく露と発育の関連を調査した研究は数多くある。

## 9.3.1. 出生時の体長/身長

出生時の体長と身長はそれぞれ13件と8件の研究で評価され、データベースでは78件の比較が行われた。大半の研究では、パイオマーカーを用いてばく露が評価されている。個々の農薬の評価は多種多様で、DDT代謝物の評価がより頻繁に行われていたが、4件以上の研究では単一の農薬と関連するパイオマーカーの評価は行われておらず、定量的な統合は行われていない。

1980 年以前に生まれた北米の集団を対象とした最大の前向き研究(ID CH 073)では、出生前の p,p'-DDE の最高濃度(>60 mg/1)のみが、最低濃度(<15 mg/1)と比較して、7 歳時の身長低下と統 計的に有意に関連していることが報告された[調整係数(SE) -2.21 cm (0.67)]。残りの研究では、 効果の方向性または効果量に一貫したパターンがなく、相反する結果が報告された。さらに、メタアナ リシスは確認されなかった。多数の分析を考慮すると、これらの結果は慎重に解釈する必要があり、こ れらのデータに基づいて、農薬ばく露と出生時の体長または身長の間に、妥当で臨床的に有意な関連 を示唆する最近のエビデンスはない。

#### 9.3.2. 体重

妊娠期の農薬ばく露と出生時体重との関連を評価した研究は 26 件あり、データベースには 134 件 の個別抽出比較が掲載されている。他の 5 件の研究では、農薬とポンデラル指数との関連を評価した。 多くの比較では、バイオマーカーを用いてばく露が評価された。個々の農薬の評価は多種多様で、DDT の代謝物の評価がより頻繁に行われた(11 研究)。しかし、同じ比較単位を使用した 4 件以上の研 究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は行われなかった。最 大の前向き研究(ID CH 014)は農業健康研究(Agricultural Health Study: AHS)の論文で、妊娠3ヶ 月までの農薬関連作業は出生時体重とは関連しておらず、複数回の分析の結果、カルバリル農薬の使 用があった場合は出生時体重の減少と関連していた(-82 g、95% CI = -132, -31)と報告された。残 りの研究では、効果の方向性や効果量に一貫したパターンがなく、相反する結果が報告された。さら に、発表された研究のメタアナリシスは確認されなかった。しかし、我々はp,p<sup>´</sup>-DDEの1µg/Lの増加は 出生時体重の7gの減少(95% CI = -18, 4g)と関連していた(Govarts E 2012)ことを報告したヨーロ ッパのコホートの個々の参加者のデータのメタアナリシスを同定した。分析の数が多いことを考える と、これらの結果は慎重な解釈が必要であり、これらのデータに基づくと、農薬ばく露と出生体重との 間の妥当で臨床的に有意な関連を示唆する最近のエビデンスはない。

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DDE was associated with a 7-g decrease in birth weight (95% CI= -18, 4 g) (Govarts E 2012). Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and birth weight in general.

Twenty-six studies assessed the association between pesticide exposure and body weight at various time-points after birth, contributing 68 separate extracted comparisons in the database. In almost 85% of the assessed comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with DDT metabolites being assessed more frequently (10 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same outcome definition, the same time-point for the outcome assessment, the same pesticide, and the same comparison unit, thus a quantitative synthesis was not performed. The largest study (ID CH 074) assessing DDT exposure in a Mexican population of boys born in 2002 and 2003, reported that, overall, associations between prenatal DDE level and Body Mass Index (BMI) at any given age were not observed and that the predicted values showed that children with the highest exposure (DDE: 49.00 mg/g) compared to those least exposed (DDE: <3.01 mg/g) grew similarly and they had a BMI similar to the referent group. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis was identified. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and body weight in general.

## 9.3.3. Head circumference

Fourteen and three studies assessed the association between pesticide exposure during pregnancy and head circumference at birth and after birth, respectively, contributing 85 separate extracted comparisons in the database. In more than two-thirds of the comparisons, the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed for birth head circumference, with DDT metabolites being assessed more frequently (7 studies). Nevertheless no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed. The largest prospective study (ID CH 026) was a Generation R study publication which explored associations between maternal occupational exposure to various chemicals and fetal growth in 4,680 pregnant women participating in this population-based prospective cohort study in the Netherlands (2002-2006). For fetal head circumference, only maternal occupational exposure to alkylphenolic compounds showed a statistically significant lower growth rate (-0.01752 SD per gestational week) compared with nonexposed mothers, adjusted for potential confounders. The remaining studies reported conflicting results without a consistent pattern either towards the effect direction or the effect magnitude. Moreover, no meta-analysis of published studies was identified. Given the large number of analyses the reported study results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between pesticide exposure and head circumference in general.

#### 9.3.4. Congenital malformations

Five studies examined the association between pesticide exposure and congenital malformations in general. The largest study (ID CH 002) assessed a Canadian farm population, reported 146 potential associations, did not yield statistically significant results in the primary analysis and proposed that pre-

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26件の研究が、出生後の様々な時点での農薬ばく露と体重との関連を評価しており、データベース には68件の個別の比較が抽出された。評価された比較のほぼ85%では、バイオマーカーを介してばく 露が評価された。個々の農薬の評価は多岐にわたり、DDT代謝物の評価がより頻繁に行われた(10研究)。 しかし、同じ定義の影響、同じタイムポイントでの影響評価、同じ農薬、同じ比較単位を使用した4件 以上の研究において評価された単一の農薬とそれに関連するバイオマーカーはなく、定量的な統合は 行われなかった。2002年と2003年に生まれたメキシコの男子集団におけるDDTばく露を評価した 最大の研究(ID CH 074)では、全体的に、出生前のDDEレベルと任意の年齢におけるBMI(Body Mass Index)との間の関連は観察されず、予測値は、最も高いばく露量(DDE: 49.00 mg/g)の小児と最も低 いばく露量(DDE: <3.01 mg/g)の小児とでは発育が似通っており、BMIは参照グループと同程度であ ったことを示した。残りの研究では、効果の方向性や効果量に一貫したパターンがなく、相反する結果 が報告された。さらに、メタアナリシスは確認されなかった。分析数が多いことから、これらの結果は 慎重な解釈が必要であり、これらのデータに基づいて、農薬ばく露と体重との間に臨床的に有意な関 連を示唆する最近のエビデンスはない。

## 9.3.3. 頭囲

妊娠期の農薬ばく露と出生時及び出生後の頭囲との関連を評価した研究はそれぞれ 14 件と 3 件 あり、データベースには 85 件の個別比較が抽出されている。3 分の 2 以上の比較では、バイオマー カーを用いてばく露が評価されていた。出生時の頭囲については、多種多様な農薬が評価されており、 中でも DDT 代謝物がより頻繁に評価されていた (7 研究)。それにもかかわらず、同じ比較単位を用 いた 4 件以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われず、定量的な統合は 行われていない。最大の前向き研究 (ID CH 026) は、オランダの集団ベースの前向きコホート研究 (2002-2006) に参加した 4,680 人の妊婦を対象に、様々な化学物質への母親の職業ばく露と胎児の 発育との関連を調査したジェネレーション R 研究の出版物である。胎児頭囲については、アルキルフ ェノール化合物への母親の職業ばく露のみが、潜在的な交絡因子を調整した上でばく露していない母 親と比較して統計的に有意に低い成長率(妊娠週あたり-0.01752 SD)を示した。残りの研究では、効 果の方向性や効果量に一貫したパターンがなく、相反する結果が報告されている。さらに、発表された 研究のメタアナリシスは確認されなかった。分析数が多いことを考えると、報告された研究結果は慎 重に解釈する必要があり、これらのデータに基づいて、農薬ばく露と頭囲との間に臨床的に有意な関 連を示唆する最近のエビデンスはない。

## 9.3.4. 先天性奇形

5件の研究では、農薬ばく露と先天性奇形全般との関連が調査された。最大の研究(ID CH 002) はカ ナダの農場の集団を評価し、146の潜在的な関連を報告したが、主要分析では統計的に有意な結果は得 られず、シアナジン(OR = 4.99、95%CI:1.63-15.27) 及びジカンバ(OR = 2.42、95%CI:1.06-5.53) への妊娠前のばく露が、男児の先天性奇形のリスクの増加と関連していることが提案された。それに もかかわらず、利用可能な比較研究の数及び本研究におけるばく露と影響が自己報告であることを考 慮すると、今回の知見は慎重に調査されるべきである。残りの4件の後ろ向き研究では、相反する結果 が報告された(ID CH 043、職業ばく露(父親)、OR: 3.42、95% CI:1.97-5.92; ID CH 035、少な くとも片方の親の職業ばく露、OR = 1.3、95%CI = 0.4-3.9; ID CH 008、母体の尿中メトラクロルの HR、95% 0.4-1.4)。)多数の分析を考慮すると、これらの結果は慎重な解釈が必要であり、これらの

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conception exposure to cyanazine (OR = 4.99, 95% CI: 1.63–15.27) and dicamba (OR = 2.42, 95% CI: 1.06–5.53) were associated with increased risk of birth defects in male offspring. Nevertheless, given the number of the available comparisons and the self-reported nature of the exposure and outcomes in this study, the present findings should be considered with caution. The remaining four retrospective studies reported conflicting results (ID CH 043, occupational exposure (father), OR: 3.42, 95% CI: 1.97–5.92; ID CH 035, at least one parent exposed, OR = 1.3, 95%CI = 0.4 - 3.9; ID CH 008, HR for maternal urine metolachor, 95% 0.4-1.4). Given the large number of analyses these results need cautious interpretation and, based on these data, there is no recent evidence to suggest association between pesticide exposure and congenital malformations in general.

## 9.3.5. Neural tube defects

Identified studies examined associations between pesticide exposure and neural tube defects (N=4 studies), including anencephaly and spina bifida and providing a very large number of reported analyses between different pesticides and neural tube defects, anencephaly and spina bifida with no adjustments for multiple testing (average 27 analyses per paper). Out of the 134 extracted analyses, 43 were statistically significantly positively associated with the outcome (of which 14 borderline significant) but need to be interpreted with caution due to high false positive probability. The range of different pesticides analysed by each of the 5 studies as well as the varying definitions of pesticide exposure do not allow for a meaningful quantitative synthesis of the results even using the "any pesticide" exposure definition since there is also considerable heterogeneity between studies regarding the exposure period as well as the parent analysed; three studies assessed maternal exposure, one study assessed paternal exposure and one study both. Previous meta-analyses on neural tube defects and pesticide exposure have not been identified. Overall, the evidence for pesticides and neural tube defects is limited and the current state of the most recent literature does not support a robust association. Of note, the largest study in the field (ID CH 044) investigated whether maternal residential proximity to applications of specific pesticides or physicochemical groups of pesticides during early gestation increases the risk of these malformations, included 731 cases and 940 controls and after reporting 107 different analyses for individual pesticides, pesticide physicochemical categories and any exposure, no exposure and multiple exposure definitions yielded 15 statistically significant results without correction for multiple testing and without a particular pattern with regards to a pesticide category or an additive effect.

#### 9.3.6. Urogenital malformations

Overall, 19 studies examined urogenital malformations, namely cryptorchidism (n=9) and hypospadias (n=9).

Cryptorchidism was assessed in nine mostly retrospective studies, of a median sample size of 199 (IQR 136-710). Four studies assessed DDT levels; hexachorobenzene (HCB) and chrordane were assessed in one study each, while general pesticide exposure was assessed in 2 studies. When we attempted to investigate the association between exposure to any pesticide and cryptorchidism across all assessed studies, the observed effect was not statistically significant (OR 1.19, 95% CI 0.96 - 1.49,  $I^2$  24%) (Figure 23). Moreover, when we assessed the potential association between DDT exposure and cryptorchism, we again observed a statistically non-significant association (OR 1.47, 95% CI 0.98 - 2.2,  $I^2$  51%) (Figure 24). Given the large number of analyses, these results need cautious interpretation and, based on these data, there is no recent evidence to suggest a robust, clinically significant association between any pesticide exposure and cryptorchidism.

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## 9.3.5. 神経管閉鎖障害

同定された研究では、農薬ばく露と無脳症や二分脊椎を含む神経管閉鎖障害との関連を調査しており(N=4研究)、種々の農薬と神経管閉鎖障害、無脳症、二分脊椎との間の分析が非常に多く報告されており、多重検定の調整は行われていない(1論文あたり平均27分析)。抽出された134の分析のうち、43の分析は結果と統計的に有意に関連していたが(うち14の分析は境界線上で有意であった)、偽陽性の確率が高いので注意して解釈する必要がある。5つの研究のそれぞれで分析された農薬の種類の多さと、農薬ばく露の定義の違いにより、ばく露期間や対象とした親に関しても研究間でかなりの不均一性があるため、「何らかの農薬」ばく露の定義を用いても、結果の意味のある定量的な統合を行うことはできない。神経管閉鎖障害と農薬ばく露に関するこれまでのメタアナリシスは確認されていない。 全体的に、農薬と神経管閉鎖障害に関するエビデンスは限られており、最新の文献の現状では、妥当な関連を支持するものではない。注目すべきは、この分野で最大の研究(ID CH 044)では、妊娠初期に特定の農薬または農薬の物理化学的グループの使用に母親が居住している場所に近いことが、これらの奇形のリスクを増加させるかどうかが調査され、731例と940例の対照が含まれ、個々の農薬、農薬の物理化学的カテゴリー、任意のばく露、ばく露なし、多重はく露の定義について107の異なる分析を報告した後、多重試験の補正なして、農薬のカテゴリーまたは相加効果に関して特定のパターンを持たない15の統計的に有意な結果が得られたことである。

## 9.3.6. 泌尿生殖器の奇形

全体では、19件の研究で泌尿生殖器の奇形、すなわち停留精巣(n=9)と尿道下裂(n=9)が調査された。

停留精巣は9件の研究で評価されたが、そのうちの9件はほとんどが後ろ向き研究であり、サンプルサ イズの中央値は199件(IQR 136-710)であった。4件の研究ではDDTレベルが評価され、ヘキサクロロベ ンゼン(HCB)とクロルデンはそれぞれ1件ずつ評価され、一般的な農薬はく露は2件の研究で評価され た。評価したすべての研究で、いずれかの農薬へのばく露と停留精巣との関連を調査しようとしたと ころ、観察された影響は統計的に有意ではなかった(OR 1.19、95%CI 0.96~1.49、I2 24%)(図 23)。 さらに、DDTばく露と停留精巣との間の潜在的な関連を評価したところ、再び統計的に有意ではない関 連が観察された(OR 1.47、95% CI 0.98~2.2、I2 51%)(図 24)。多数の分析を考慮すると、これら の結果は慎重に解釈する必要があり、これらのデータに基づいて、あらゆる農薬ばく露と停留精巣と の間に、臨床的に有意な関連を示唆する最近のエビデンスはない。

尿道下裂は主に9件の後ろ向き研究で評価され、サンプルサイズの中央値は784人(IQR 200 - 861) であった。2件の研究ではDDTレベルが評価され、6件の研究では農薬はく露一般が評価された。評価さ れたすべての研究において、何らかの農薬への母親のばく露(妊娠前及び妊娠期)と尿道下裂との関連 を調査しようとしたところ、観察された影響は統計的に有意ではなかった(OR 1.02、95%CI 0.74-1.39、I2 72%)(図25)。特定の農薬を評価した3つの研究(DDT、n=2;クロルデン、n=1)を分析 に含めると、再び統計的に有意でない関連が観察された(OR 1.00、95%CI 0.84-1.16、I2 66%)(図 26)。我々のシステマティックレビューでは、1966年1月から2008年3月までに英語で出版され、PubMed に索引付けされたオリジナルの研究を含む1つのメタアナリシスを検索した(Rocheleau CM, 2009)。 2007年以前に発表された9件の研究がすべての研究の包含基準を満たしており、著者らは、尿道下裂の

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Hypospadias was assessed in 9 mostly retrospective studies, of a median sample size of 784 (IQR 200

- 861). Two studies assessed DDT levels, while general pesticide exposure was assessed in 6 studies. When we attempted to investigate the association between maternal exposure to any pesticide (during preconception and pregnancy) and hypospadias across all assessed studies, the observed effect was not statistically significant (OR 1.02, 95% CI 0.74 – 1.39,  $I^2$  72%) (Figure 25). When we included in the analysis the three studies that assessed a specific pesticide (DDT, n=2; chrordane, n=1), we again observed a statistically non-significant association (OR 1.00, 95% CI 0.84-1.16, I<sup>2</sup> 66%) (Figure 26). Our systematic review retrieved one meta-analysis including original research published in English and indexed in PubMed from January 1966 through March 2008 (Rocheleau CM, 2009). Nine studies published before 2007 met all study inclusion criteria and the authors reported that elevated but marginally significant risks of hypospadias were associated with maternal occupational exposure (PRR of 1.36, CI=1.04-1.77), and paternal occupational exposure (PRR of 1.19, CI=1.00-1.41). Due to the different time-periods for the literature assessment and the resulting minimal overlap between our review and the published meta-analysis, we were able to synthesize the two efforts and again we retrieved a statistically non-significant result (OR 1.14, 95% CI 0.84 – 1.55, I<sup>2</sup> 73%). Thus, there is no recent evidence to suggest a robust, clinically significant association between any pesticide exposure and cryptorchidism.

#### 9.4. Child health outcomes with few studies

For all the assessed outcomes not included in Table 10, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a vast variety of captured information ranging from well-defined clinical entities yet with too few studies, such as gastroschisis, cardiac birth defects, diaphragmatic hernia, and esophageal atresia, as well as a large numbers of metrics pertaining to broad clinical entities but with a prominent lack of harmonization and standardization in the outcome definition. For example, outcomes related to neurodevelopment were assessed extensively; nevertheless the metrics used, ranging from IQ measurement to perceptual reasoning, deemed any further attempt towards a quantitative synthesis impossible. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

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リスクの上昇は、母親の職業ばく露(PRR1.36、CI=1.04-1.77)及び父親の職業ばく露(PRR1.19、CI=1.00-1.41)と関連していたが、わずかに有意であったと報告している。異なる期間に文献評価した結果とし て、我々のレビューと発表されたメタアナリシスとの重複が最小限であったために、我々は2つの成果 を統合することができ、再び我々は統計的に有意ではない結果を取得した(OR 1.14、95%CI 0.84 -1.55、I2 73%)。このように、いかなる農薬ばく露と停留精巣との間に、妥当で臨床的に有意な関連 を示唆する最近のエビデンスはない。

## 9.4. 研究が少ない小児健康影響

Table 10にはない健康影響には、個々の健康影響のエビデンスの統合には研究数が少なすぎるもの がある。すなわち、これらの健康影響には、腹壁裂、心臓先天異常、横隔膜ヘルニア、食道閉鎖症など のような明確に定義されてはいるが研究数が少ない臨床所見から、広範な臨床所見に関連する多数の 指標からなるが健康影響の定義の一致と標準化が著しく欠如しているものまで、多種多様な情報が収 集されている。例えば、神経発達に関連した健康影響は広範囲に評価されているが、IQ測定から知覚推 論に至るまで使用されている指標は、定量的な統合を目指したさらなる試みは不可能であると考えら れた。我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を 可能にするために、これらの健康影響に関する以前に発表されたメタアナリシスは確認されなかった。 一般的に、これらの健康影響に関する結果は効果量が小さく、統計的に有意ではなかった(少数の例外 を除く)。分析数が多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータ に基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

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 Table 10:
 Assessed outcomes in the field of child health as defined by eligible studies

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Health outcome		
Abnormal urogenital distance	Body mass index (BMI) Z-score	Increased serum prolactin levels
Abnormal body mass index (BMI)	Body fat percentages (log transformed)	Increased serum total testosterone leve
Abnormal bone age	Chordee	IQ
Abnormal breast size	Coarctation of the aorta	LH dysregulation
Abnormal change of body mass index	Congenital diaphragmatic hernia (CDH)	Low annual height velocity
Abnormal change of height	Congenital heart defects	Major congenital anomalies
Abnormal chest circumference	Congenital malformations	Male genital malformations
Abnormal gestational age	Cretinism	Maternal age
Abnormal head circumference-for-age	Crown-Heel Length	Maternal weight gain
Abnormal height	Cryptorchidism	Miscarriage or stillbirth
Abnormal hip circumference	Decreased inhibin B levels	Musculoskeletal defects
Abnormal length	Decreased serum FSH levels	Neural tube defects
Abnormal ovarian measurements	Decreased serum inhibin B levels	Obesity
Abnormal penis length (stretched)	Decreased serum SHBG levels	Oestradiol dysregulation
Abnormal penis width	Decreased testicular volume	Perceptual Reasoning
Abnormal serum DHT levels	Decreased testosterone levels	Performance IQ
Abnormal sitting height	Decresed serum LH levels	Ventricular septal defect
Abnormal standing height	Duration of lactation	Placental weight
Abnormal Tanner stage	Esophageal atresia	Placental weight
Abnormal upper arm circumference	Fetal death	Ponderal Index
Abnormal upper arm fold circumference	Fetal head circumference	Ponderal index
Abnormal uterine measurements	Fetal length	Precocious puberty
Abnormal waist circumference	Fetal weight	Preeclampsia
Abnormal weight	FGR	Premature breast development
Abnormal weight-for-length	Freedom from distractability	Premature oestradiol secretion
Affected breast development	FSH dysregulation	Premature puberty onset (pubic hair)
Anal position index	Gastroschisis	Prematurity
Androstendione dysregulation	Gestational age	Processing speed
Anencephaly	Gynecomastia	Rapid infant weight gain
Anti-mullerian hormone dysregulation	Head Circumference	SGA
APGAR 1-minute score	Hypospadias	SHC
APGAR 5-minute score	Idiopathic precocious puberty	Spina bifida
Atrioventricular septal defect	Increased FSH levels	Sum of four skin folds
Birth head circumference	Increased levels of SHBG	Testosterone dysregulation

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# 表10:対象となる研究で定義された小児健康分野で評価された影響

アンドロステンジオンの調節障害     在胎月齢     処理速度       無脳症     女性化乳房     乳幼児の急激な体重増加       抗ミュラーホルモンの調節障害     頭囲     SGA       APGAR 1分間のスコア     尿道下裂     SHC	健康影響		
American (Mathematican)         American (Mathematican)           異常なボディマス指数(BMI)         体脂肪草(対数変換)         血清トータルデストステロン値の上 昇           角中かうこうの異常         尿道菜         10           脚の大きこの異常         大助脈の動脈硬化         11 調節障害           肥満度指数の異常変化         先天性機陽膜ヘルニア(CDH)         年間の高さ速度が低い           身長の異常な変化         先天性心疾患         重篤な先天性異常           胸間の異常         た天性や疾患         重篤な先天性異常           鼻窩な高さ         停留特単         液産・死産           段間節周囲の異常         インビビンB 値の低下         船骨格系の次陥           身長の異常調定         血清 FSH 値の低下         肥満           ペニスの長さの異常(伸びる)         血清 SHEG 値の低下         エストラジオールの調節障害           ペニスの長の異常         焼泉 宿倉 成少         知覚推論           ムニスの幅の異常         ケストステロン値の低下         ボンゴラルの両調障害           ペニスの毎回異常         焼泉 宿倉 成少         知覚推論           ムニスの細胞の異常         ケストステロン値の低下         ベストラジオールの調節障害           ペニスの長の異常(伸びる)         血清 FHE の低下         形成           異常な座高         血清 ILI 値の低下         水ご テルーンフ目Q           異常な座高         血清 ILI 値の低下         ベシェ 「アーベンゴ Q           異常な座高         血清 ILI 値の低下         小室中脳大通           異常な座高         上満田町         防量重量           上師朋の見常         胎児の死亡         ビンデクルインデックス <th></th> <th></th> <th></th>			
月月骨年齢の異常尿道索10胸の大きさの異常大動脈の動脈硬化11 調節障害肥減度指数の異常変化先天性核隔膜ヘルニア (CDH)年間の高き速度が低い身長の異常な変化先天性な疾患重篤な先天性異常胸囲の異常先天性の疾患更体性器奇形クレチン症母体の体重増加異常な高さ伊留特巣流産・死産腹間筋周囲の異常インビビンB 値の低下静僧客気の気筋身長の異常期定血清 FSH 値の低下把満中経管障害二中振の異常潮定血清 AンビビンB 値の低下エストラジオールの調節障害ペニスの長さの異常精影容積の成少知覚推論ペニスの幅の異常方ストステロン値の低下パフォーマンス 10異常な症の高さ長乳期間胎盤重量異常な症の高さ長乳期間胎盤重量異常な位の高さ長見期間影盤重量異常な体重鮎児の死亡ボンデラルインデックス夏常な小重の異常鮎児の死亡ボンデラルインデックス乳房の発達への影響FGR早期な1万の光具家な小する重要の異常注意散漫でない早期末ストラジオール分泌乳房の発達への影響FSH の調筋障害現田36Aアンドロステンジオン両筋障害須囲5GA和lopition index腹壁裂未熟児無脳症女性化乳房乳幼児の急激な体重増加北ミュラーホルモンの調筋障害頭囲SGAAPA大性化乳房35HCAPASHDのデ用助計測損生時の調用FSH の具用デの管轄第二FSH 位の上昇女子市の調筋障害第回のSASHG和国のSASHG和国のSASHG和国のSASHG和国のSASHG和国のSHESHE新路SHESHE第回のSHESHE第回のSHE第回の	尿路性器距離の異常	体格指数 (BMI) Z スコア	血清プロラクチン値の上昇
中国         中国           南の大きさの異常         尿道索         10           胸の大きさの異常         大動脈の動脈硬化         13 調節障害           肥満皮指数の異常変化         先天性横隔膜ペルニア(CBH)         年間の高き速度が低い           身長の異常な変化         先天性心疾患         重篤な先天性異常           胸囲の異常         人レチン症         母体年齢           年齢に応じた頭囲の異常         勇曜長         母体の体重増加           異常な高さ         停留精果         流産・死産           股関筋周囲の異常         インビビンB 値の低下         筋骨格系の次陥           身長の異常激定         血清7とH 位の低下         熱骨体系の気障           タ長の異常潮定         血清7とH どンB 値の低下         米福等障害           の異常激定         血清7とH どンB 値の低下         肥満           ペニスの長さの異常(伸びる)         血清8KBG 値の低下         光気トラジオールの調節障害           ペニスの幅の異常         方ストステロン値の低下         肥満           ムニスの長さの異常(伸びる)         血清8KBG 値の低下         水ストラジオールの調節障害           ペニスの長さの異常         精巣な前値の低下         ンストラジオールの調節障害           ペニスの長さの異常         精巣な配         知業           奥賞な空価の異常         大気方ロン         知業           奥山湾の長常         たるの低下         パンドフィーンス10           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         新生           鼻痛な立位の高さ         脱児期間         新生 <td>異常なボディマス指数 (BMI)</td> <td>体脂肪率 (対数変換)</td> <td>血清トータルテストステロン値の上</td>	異常なボディマス指数 (BMI)	体脂肪率 (対数変換)	血清トータルテストステロン値の上
胸の大きさの異常大動脈の動脈硬化LI 副節障害肥満皮指数の異常変化先天性検陽線ヘルニア (CDH)年間の高さ速皮が低い身長の異常な変化先天性心疾患重篤な先天性異常胸囲の異常先天性心疾患更傷な先天性異常胸囲の異常クレチン症母体年齢年齢に応じた頭囲の異常頭瘫長母体の体重増加異常な高さ停留精巣洗産・死産股関節周囲の異常インヒビンB 値の低下神経管障害卵巣の異常測定血清 FSH 値の低下二ペニスの長さの異常(伸びる)血清 SHG 値の低下エストラジオールの調節障害ペニスの幅の異常精巣容積の減少知覚推論血清 DHT 値の累常た入トステロン値の低下パフォーマンス IQ異常な空高血清 LH 値の低下心室中隔欠損異常な空高血清 LH 値の低下応室 FML異常な空向高さ授乳期間胎盤重量上腕囲の異常胎児の死亡ポンデラルインデックス眉曲上腕囲の異常胎児の死亡ポンデラルインデックス眉面上腕囲の異常胎児の死亡ポンデラルインデックス星な食FGR早熱な乳房の発達長さに対する重量の異常注意散漫でない早期完異方な発量た意し愛でない早期の発遣(陰毛)Anal position index腹壁裂未熟児ケンドロステンジオンの調筋障害頑囲SGAArCAR 1 分開のスコア探道下裂SHCArGAR 5 分間のスコアFISH 値の上昇欠下貼防計測出生時の頭囲SHBG の増加デストステロンの調筋障害			昇
肥満度指数の異常変化先天性横隔膜ヘルニア (CDH)年間の高き速度が低い身長の異常な変化先天性心疾患重篤な先天性異常胸囲の異常た天性奇形男性性器奇形在胎月齡の異常クレチン症母体年齡年齢に応じた頭囲の異常頭踵長母体の体重増加異常な高さ停留精巣流産・死産股関節周囲の異常インヒビンB値の低下筋骨格系の欠陥身長の異常血清 FSH 値の低下脚満ポニスの長さの異常(伸びる)血清 SHG 値の低下エストラジオールの調節障害ペニスの長さの異常持巣容積の減少知覚推論血清 DHT 値の異常テストステロン値の低下パフォーマンス IQ異常な座高血清 LH 値の低下心室中隔欠損異常な位の高さ授乳期間胎盤重量異常な少十一段階食道閉厳症胎盤重量上腕囲の異常胎児の死亡ポンデラルインデックス目曲上腕囲の異常胎児の死亡ポンデラルインデックス写の測定値の異常胎児の保重要期早発実名の風索ト房早期二系トラジオール分泌乳房の発達への影響FSH の調節障害思春期早餐気気の強重の異常注意散漫でない早期二ストラジオール分泌乳房の発達の影響FSH の調節障害思春期早発症(陰毛)和1 position index腹壁裂未熟児大ドコステンジオンの調節障害妊胎月齡処理速度無脳症女性化乳房乳幼児の急激な体重増加抗ミュラーホルモンの調節障害頭囲SGAAFGAR 1分間のスコア房差性早熟性思春期二分脊椎房室中隔欠損症FSH 60%加子方キンコンの調節障害出生時の頭囲SHGの増加デンキマコンの調節障害	骨年齢の異常	尿道索	IQ
身長の異常な変化先天性心疾患重篤な先天性異常胸囲の異常た天性奇形男性性器奇形在胎月齡の異常クレチン症母体年齡年齡に応じた頭囲の異常頭踵長母体の体重増加異常な高さ停留精巣流産・死産股関節周囲の異常インヒビンB値の低下筋骨格系の欠陥身長の異常血清 75H 値の低下神経管障害卵巣の異常測定血清 75H 値の低下肥満ペニスの長さの異常(伸びる)血清 75B 値の低下エストラジオールの調節障害ペニスの幅の異常持巣容積の減少知覚推論血清 DHT 値の異常テストステロン値の低下パフォーマンス IQ異常な座高血清 LH 値の低下心室中隔欠損異常な位の高さ授乳期間胎盤重量異常な立位の高さ授乳期間胎盤重量異常な少ナー段階食道閉鎖症胎盤重量上腕囲の異常胎児の死亡ポンデラルインデックス耳面山脏間の異常胎児の死亡ポンデラルインデックス子宮の測定値の異常胎児の長さ思春期早発ウェスト周りの異常胎児の存重平期前症異常な体重FGR早熟な乳房の発達長言に対する重量の異常FSH の調節障害思春期早発症(陰毛)和1 position index腹壁裂未熟児アンドロステンジオンの調節障害妊胎月齡処理速度無脳症女性化乳房乳幼児の急激な体重増加抗ミュラーホルモンの調節障害頭囲SGAAFGAR 1分間のスコプ房道 作型長田訪計測出時の頭囲SHG の増加デストステロンの調節障害	胸の大きさの異常	大動脈の動脈硬化	LH 調節障害
胸囲の異常         先天性奇形         男性性器奇形           在貼月齡の異常         クレチン症         母体年齡           年齡に応じた頭囲の異常         頭麗長         母体の体重増加           異常な高さ         停留精巣         流産・死産           股関節周囲の異常         インヒビンB値の低下         節骨格系の欠陥           身長の異常         血清 VSH 値の低下         神経管障害           卵巣の異常測定         血清 VSH 値の低下         把満           ペニスの長さの異常(伸びる)         血清 SHBG 値の低下         エストラジオールの調節障害           ペニスの幅の異常         デストステロン値の低下         パフォーマンス 10           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な空白の高さ         授乳期間         胎盤重量           異常な空白の高さ         授乳期間         胎盤重量           異常な空白の高さ         授乳期間         胎盤重量           異常な少す一段階         食道閉鎖症         胎盤重量           鼻痛な少す一段階         食道閉鎖症         胎盤重量           異常な少す一段階         食道閉鎖症         胎盤重量           異常な少す一段階         食道閉鎖症         影響重量           異常な少す         免防         食道閉鎖症         胎量電量           異常な少す         白鳥         知見の死亡         ボンデラルインデックス           写っ調定         胎児の長さ         思春期早発         ウェンデックス           ウススト間の異常         胎児の体重         子嘯前症           真恋教学を施	肥満度指数の異常変化	先天性横隔膜ヘルニア (CDH)	年間の高さ速度が低い
内・レデン症         日本日本           在船月齡の異常         グレチン症         日本体の体重増加           異常な高さ         伊留精巣         洗産・死産           股関節周囲の異常         インヒビンB値の低下         新骨格系の欠陥           身長の異常         血清 FSH 値の低下         神経管障害           卵巣の異常測定         血清 インヒビンB値の低下         肥満           ペニスの長さの異常(伸びる)         血清 SHBG 値の低下         エストラジオールの調節障害           ペニスの幅の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         デストステロン値の低下         パフォーマンス 10           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な空白の高さ         授乳期間         胎盤重量           異常な少しの高さ         授乳期間         胎盤重量           異常な少しつ高さ         授乳期間         胎盤重量           異常な少しつ高さ         授乳期間         胎盤重量           異常な少しの高さ         授乳期間         胎室重量           異常な少しつ長さ         見定期         おどブラルインビックス           国由上腕囲の異常         胎児の死亡         ポンデラルインデックス           「日本         「おいて         ボンデラルインデックス           日本         胎児の異常         胎児の長さ         思奉期早発           ウススト周りの異常         胎児の体重         ア増前定           真正の異常な単の異常         ドGR         早期エストラジオール分泌           見たの調節障害         三素期見         二素期見	身長の異常な変化	先天性心疾患	重篤な先天性異常
一日         日本日本           年齢に応じた頭囲の異常         頭踵長         母体の体重増加           異常な高さ         停留精巣         流産・死産           股関節周囲の異常         インヒビンB値の低下         肺経管障害           卵巣の異常測定         血清 FSH 値の低下         肥満           タ長の異常         血清 FSH 値の低下         肥満           パロスの長さの異常(伸びる)         血清 SHBG 値の低下         肥満           ムニスの幅の異常         テストステロン値の低下         ボストラジオールの調節障害           ペニスの幅の異常         ケストステロン値の低下         パフォーマンス IQ           異常な座高         血清 IH 値の低下         小管推論           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         胎盤重量           異常ななシナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           居由上腕囲の異常         胎児の死亡         ポンデラルインデックス           日本         階層の長常         陸害 期早発           シロッスト局りの異常         胎児の悪い         デ備の金           ウェスト局りの異常         上児の体重         子癇前症           異常な体重         FGR         早期なれ見の見楽         大参加           シンドコの事件         陸鏖戦力         大戦加         アレール分泌	胸囲の異常	先天性奇形	男性性器奇形
異常な高さ         停留積巣         流産・死産           股関節周囲の異常         インヒビンB値の低下         筋骨格系の欠陥           身長の異常         血清 FSH 値の低下         神経管障害           卵巣の異常測定         血清 FSH 値の低下         肥満           ベニスの長さの異常(伸びる)         血清 SHB 値の低下         エストラジオールの調節障害           ベニスの幅の異常         デストステロン値の低下         エストラジオールの調節障害           本湾 FSH 値の低下         エストラジオールの調節障害           本ニスの幅の異常         ケストステロン値の低下         パフォーマンス IQ           異常な空高         血清 IH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         胎盤重量           異常な立位の高さ         授乳期間         胎盤重量           異常なな近の異常         胎児の死亡         ポンデラルインデックス           星鹿山院囲の異常         胎児の死亡         ポンデラルインデックス           星市協囲の異常         胎児の死亡         ポンデラルインデックス           子宿前鹿         光デラルインデックス         アジー           日本上範囲の異常         胎児の保査         早期主要           ウェン周りの異常         胎児の長         思春期早発           東京な体重         FGR         早期な乳房の発達         見たに対した           真容は夏素での影響         FSH の調節障害         思春期早発         (除毛)           和目のさい面の具常         夏嘘裂         未熟児         アンドロステジジオール分泌	在胎月齢の異常	クレチン症	母体年齢
股関節周囲の異常         インヒビンB値の低下         筋骨格系の欠陥           身長の異常         血清 FSH 値の低下         神経管障害           卵巣の異常測定         血清 FSH 値の低下         肥満           ベニスの長さの異常(伸びる)         血清 SHB6 値の低下         エストラジオールの調節障害           ベニスの転の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         デストステロン値の低下         パフォーマンス IQ           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な空高         血清 LH 値の低下         公室中隔欠損           異常な空高         血清 LH 値の低下         小室車下隔欠損           異常な空高         血清 LH 値の低下         小室車「隔欠損           異常な空高         血清 LH 値の低下         小室車「隔欠損           異常な空高         魚川         胎盤重量           異常な空の高さ         授乳期間         胎盤重量           異常な空の高さ         授乳期間         胎盤重量           異常な空の高さ         授乳期間         脂盤重量           異常な空の高さ         授乳期間         ボンデラルインデックス           尾鹿山田の異常         胎児の死亡         ボンデラルインデックス           白油目の現常         胎児の死亡         ボンデラルインデックス           ターの影響         胎児の体重         早期なる見房の発達           見な体重         FGR         早期なる見方の発売           見方の発電         内容         外見の急激な体重増加           プレドステンジオンの調節障害         妊胎月齢	年齢に応じた頭囲の異常	頭踵長	母体の体重増加
中国         中国         中国         中国           身長の異常         血清 FSH 値の低下         神経管障害           卵巣の異常測定         血清 FSH 値の低下         肥満           ベニスの長さの異常(伸びる)         血清 SHB6 値の低下         エストラジオールの調節障害           ベニスの幅の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         デストステロン値の低下         パフォーマンス IQ           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常なな立の高さ         授乳期間         胎盤重量           異常なな方一段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ボンデラルインデックス           腐血上腕囲の異常         胎児の死亡         ボンデラルインデックス           居由上腕囲の異常         胎児の死亡         ボンデラルインデックス           星の調定値の異常         胎児の死亡         ボンデラルインデックス           子宮の測定値の異常         胎児の長さ         思春期早発           ウェスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達         「協用           見房の発売への影響         FSH の調節障害         思春期早発         「知会           乳房の発達への影響         FSH の調節障害         処理速度            乳目         psition index         腹墜裂         未熟児           アンドロステンジオンの調節障害         項囲	異常な高さ	停留精巣	流産・死産
加速した         加速した           卵巣の異常測定         血清くどとビンB値の低下         肥満           ベニスの長さの異常(伸びる)         血清 SHBG 値の低下         エストラジオールの調節障害           ベニスの幅の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         テストステロン値の低下         パフォーマンス 1Q           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常な女ナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の死亡         ポンデラルインデックス           尾筋の異常         胎児の長さ         児奉期早発           ウエスト周りの異常         胎児の長さ         思奉期早発           貴な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思奉期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         標道下裂         SHC           APGAR 5 分間のスコア         特発性早熟性思報         二分脊椎           房室中隔欠損症         FSH 値の上昇         <	股関節周囲の異常	インヒビン B 値の低下	筋骨格系の欠陥
ベニスの長きの異常(伸びる)         血清 SHBG 値の低下         エストラジオールの調節障害           ベニスの幅の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         テストステロン値の低下         パフォーマンス IQ           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な空の高さ         授乳期間         胎盤重量           異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の死亡         ポンデラルインデックス           石油力         胎児の死亡         ポンデラルインデックス           石油上腕囲の異常         胎児の死亡         ポンデラルインデックス           マゴの測定値の異常         胎児の長さ         思春期早発           ウェスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           見たがする重量の異常         ド島児の体重         子癇前症           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           乳房の発達への影響         FSH の調節障害         と作用前           パンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児のの急激な体重増加           ガンドロステンジョン         尿道下裂         SHC           APGAR 1分間のスコア	身長の異常	血清 FSH 値の低下	神経管障害
ペニスの幅の異常         精巣容積の減少         知覚推論           血清 DHT 値の異常         テストステロン値の低下         パフォーマンス IQ           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の死亡         ポンデラルインデックス           子宮の測定値の異常         胎児の長さ         思春期早発           ウエスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         路児の体重         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           和目 position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5 分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           開生時の頭囲         ShG の増加 <td>卵巣の異常測定</td> <td>血清インヒビンB値の低下</td> <td>肥満</td>	卵巣の異常測定	血清インヒビンB値の低下	肥満
血清 BHT 値の異常         デストステロン値の低下         パフォーマンス IQ           異常な座高         血清 LH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常な女ナー段階         食道閉鎖症         胎盤重量           異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の長さ         思春期早発           ウエスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         ドSH の調節障害         思春期早発症(陰毛)           和al position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脇症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         探道作裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHG の増加         デストステロンの調節障害	ペニスの長さの異常(伸びる)	血清 SHBG 値の低下	エストラジオールの調節障害
異常な座高         血清 LH 値の低下         心室中隔欠損           異常な立位の高さ         授乳期間         胎盤重量           異常な女亡の段階         食道閉鎖症         胎盤重量           異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の死亡         ポンデラルインデックス           宮面山を随の異常         胎児の長さ         思春期早発           ウエスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5 分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHG の増加         デストステロンの調節障害	ペニスの幅の異常	精巣容積の減少	知覚推論
内田 日         内田 田         内田 田           異常な立位の高さ         授乳期間         胎盤重量           異常な女しの高さ         長道閉鎖症         胎盤重量           異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児の長さ         思春期早発           ウェスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         治児の体重         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           男生時の頭囲         欧増加         デストステロンの調節障害	血清 DHT 値の異常	テストステロン値の低下	パフォーマンス IQ
異常なタナー段階         食道閉鎖症         胎盤重量           上腕囲の異常         胎児の死亡         ボンデラルインデックス           屈曲上腕囲の異常         胎児の長さ         思春期早発           ウェスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 5 分間のスコア         尿道下裂         SHC           APGAR 5 分間のスコア         SHG の増加         二分脊椎           出生時の頭囲         SHG の増加         テストステロンの調節障害	異常な座高	血清 LH 値の低下	心室中隔欠損
上腕囲の異常         胎児の死亡         ポンデラルインデックス           屈曲上腕囲の異常         胎児頭囲         ポンデラルインデックス           子宮の測定値の異常         胎児の長さ         思春期早発           ウエスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         BUGの増加         テストステロンの調節障害	異常な立位の高さ	授乳期間	胎盤重量
屈曲上腕囲の異常         胎児頭囲         ポンデラルインデックス           子宮の測定値の異常         胎児の長さ         思春期早発           ウエスト周りの異常         胎児の体重         子癇前症           異常な体重         FGR         早熟な乳房の発達           異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         BUG の増加         テストステロンの調節障害	異常なタナー段階	食道閉鎖症	胎盤重量
子宮の測定値の異常     胎児の長さ     思春期早発       ウエスト周りの異常     胎児の体重     子癇前症       異常な体重     FGR     早熟な乳房の発達       長さに対する重量の異常     注意散漫でない     早期エストラジオール分泌       乳房の発達への影響     FSH の調節障害     思春期早発症(陰毛)       和al position index     腹壁裂     未熟児       アンドロステンジオンの調節障害     在胎月齢     処理速度       無脳症     女性化乳房     乳幼児の急激な体重増加       抗ミュラーホルモンの調節障害     頭囲     SGA       APGAR 1分間のスコア     尿道下裂     SHC       APGAR 5分間のスコア     特発性早熟性思春期     二分脊椎       房室中隔欠損症     FSH 値の上昇     皮下脂肪計測       出生時の頭囲     SHG の増加     テストステロンの調節障害	上腕囲の異常	胎児の死亡	ポンデラルインデックス
ウエスト周りの異常         胎児の体重         子癇前症           奥常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHGの増加         テストステロンの調節障害	屈曲上腕囲の異常	胎児頭囲	ポンデラルインデックス
異常な体重         FGR         早熟な乳房の発達           長さに対する重量の異常         注意散漫でない         早期エストラジオール分泌           乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHGの増加         テストステロンの調節障害	子宮の測定値の異常	胎児の長さ	思春期早発
長さに対する重量の異常     注意散漫でない     早期エストラジオール分泌       乳房の発達への影響     FSHの調節障害     思春期早発症(陰毛)       Anal position index     腹壁裂     未熟児       アンドロステンジオンの調節障害     在胎月齢     処理速度       無脳症     女性化乳房     乳幼児の急激な体重増加       抗ミュラーホルモンの調節障害     頭囲     SGA       APGAR 1分間のスコア     尿道下裂     SHC       APGAR 5分間のスコア     特発性早熟性思春期     二分脊椎       房室中隔欠損症     FSH 値の上昇     皮下脂肪計測       出生時の頭囲     SHGの増加     テストステロンの調節障害	ウエスト周りの異常	胎児の体重	子癇前症
乳房の発達への影響         FSH の調節障害         思春期早発症(陰毛)           Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齢         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         BLGの増加         テストステロンの調節障害	異常な体重	FGR	早熟な乳房の発達
Anal position index         腹壁裂         未熟児           アンドロステンジオンの調節障害         在胎月齡         処理速度           無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         BHGの増加         テストステロンの調節障害	長さに対する重量の異常	注意散漫でない	早期エストラジオール分泌
アンドロステンジオンの調節障害     在胎月齢     処理速度       無脳症     女性化乳房     乳幼児の急激な体重増加       抗ミュラーホルモンの調節障害     頭囲     SGA       APGAR 1分間のスコア     尿道下裂     S H C       APGAR 5分間のスコア     特発性早熟性思春期     二分脊椎       房室中隔欠損症     FSH 値の上昇     皮下脂肪計測       出生時の頭囲     6     アムトステロンの調節障害	乳房の発達への影響	FSH の調節障害	思春期早発症(陰毛)
無脳症         女性化乳房         乳幼児の急激な体重増加           抗ミュラーホルモンの調節障害         頭囲         SGA           APGAR 1分間のスコア         尿道下裂         S H C           APGAR 5分間のスコア         特発性早熟性思春期         二分育椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         6         アムトステロンの調節障害	Anal position index	腹壁裂	未熟児
抗ミュラーホルモンの調節障害     頭囲     SGA       APGAR 1 分間のスコア     尿道下裂     S H C       APGAR 5 分間のスコア     特発性早熟性思泰期     二分脊椎       房室中隔欠損症     FSH 値の上昇     皮下脂肪計測       出生時の頭囲     SHG の増加     テストステロンの調節障害	アンドロステンジオンの調節障害	在胎月齡	処理速度
APGAR 1分間のスコア         尿道下裂         SHC           APGAR 5分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHBG の増加         テストステロンの調節障害	無脳症	女性化乳房	乳幼児の急激な体重増加
APGAR 5 分間のスコア         特発性早熟性思春期         二分脊椎           房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHBG の増加         テストステロンの調節障害	抗ミュラーホルモンの調節障害	頭囲	SGA
房室中隔欠損症         FSH 値の上昇         皮下脂肪計測           出生時の頭囲         SHBG の増加         テストステロンの調節障害	APGAR 1 分間のスコア	尿道下裂	SHC
出生時の頭囲 SHBG の増加 テストステロンの調節障害 (1)	APGAR 5分間のスコア	特発性早熟性思春期	二分脊椎
	房室中隔欠損症	FSH 値の上昇	皮下脂肪計測
出生時の体長 LH/テストステロン比率の増加 ファロー四徴症	出生時の頭囲	SHBG の増加	テストステロンの調節障害
	出生時の体長	LH/テストステロン比率の増加	ファロー四徴症

Birth height	Increased ratio LH/testosterone	Tetralogy of Fallot
Birth Weight	Increased serum AMH levels	Transposition of the great arteries
Birth weight, adjusted for gestational age	Increased serum androstenedione levels	Verbal comprehension
BMI	Increased serum DHEAS levels	Verbal IQ
BMI at delivery	Increased serum free testosterone level	Working memory

Pesticide epidemiology

出生時体重	血清 AMH 値の上昇	大動脈の転換
出生時体重、在胎月齢で調整	血清アンドロステンジオン値	言語理解
	の上昇	
BMI	血清 DHEAS 値の上昇	言語性 IQ
出産時の BMI	血清遊離テストステロン値の	ワーキングメモリ
	上昇	
妊娠前の BMI		

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 Table 11:
 Summary of studies identified per outcome subgroup with more than 4 studies (NA: not available)

Outcome	N studies	Meta-analysis done	Previous meta-analysis result
Congenital malformations			
General	5	No	NA
Neural tube defects	4	No	NA
Urogenital malformations	19	Yes	Hypospadias: maternal occupational exposure (RR 1.36; 95% Cl 1.04–1.77), and paternal occupational exposure (RR 1.19; 95% Cl 1.00–1.41)
Development	40	No	NA
Growth			
Height/Birth Iength	21	No	NA
Weight	26	No	Birth weight (individual participants' data meta-analysis of 12 European cohorts): A 1- μg/L increase in p.p <sup>-1</sup> DDE was associated with a 7-g decrease in birth weight (95% CI: -18, 4 g).
Head circumference	17	No	<u>NA</u>
Sexual maturation	9	No	NA

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

# 表 11:4 件以上の研究がある影響のサブグループごとに同定された研究の要約 (NA:利用できない)

	(101 • 1-1/1)			
影響		研究数	メタアナリシス実施	前回のメタアナリシス結果
先天的	生奇形			
	全身	5	No	NA
	神経管閉鎖障害	4	No	NA
	泌尿生殖器の奇形	19	Yes	尿道下裂:母方の職業ばく露(RR 1.36; 95%CI 1.04-1.77)及び父方の職業ばく露 (RR 1.19;95%CI 1.00-1.41)
発生		40	No	NA
発育				
	身長/出産時長	21	No	NA
	体重	26	No	<u>出生時体重(ヨーロッパの12のコホートを 対象とした個々の参加者のデータメタアナリ シス)</u> : p,p <sup>-</sup> -DDEの1µg/L増加は、出生 時体重の7g減少と関連していた(95%CI:- 18.4g)
	頭囲	17	No	NA
	性的成熟	9	No	NA

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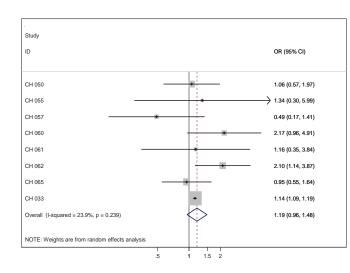


Figure 23: Random effects meta-analysis for studies with information on pesticide exposure and risk of cryptorchidism



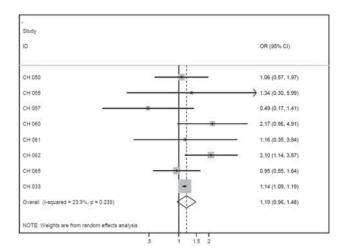


図23:農薬ばく露に関する情報と停留精巣のリスクのランダム効果メタアナリシス

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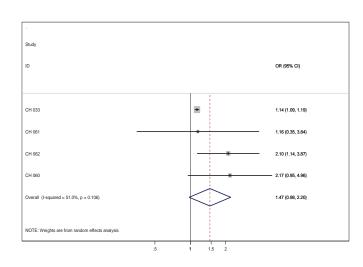


Figure 24: Random effects meta-analysis for studies with information on DDT exposure and risk of cryptorchidism



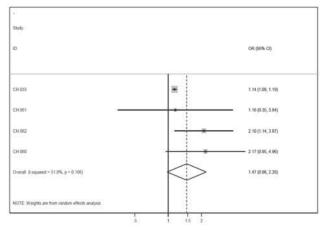


図24: DDTばく露に関する情報と停留精巣のリスクのランダム効果メタアナリシス

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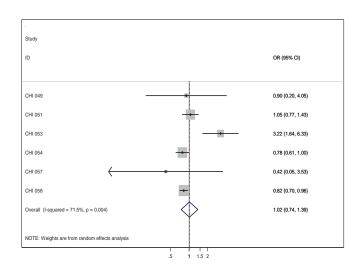


Figure 25: Random effects meta-analysis for studies with information on general pesticide exposure and risk of hypospadias

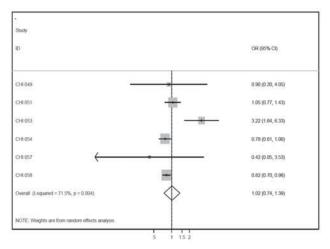


図25:農薬ばく露一般に関する情報と尿道下裂のリスクのランダム効果メタアナリ シス

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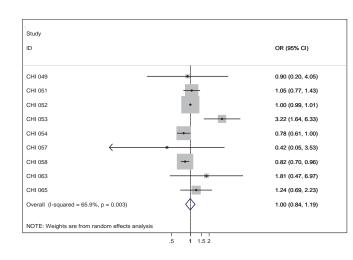


Figure 26: Random effects meta-analysis for studies with information on general pesticide exposure and risk of hypospadias, including studies on specific pesticides

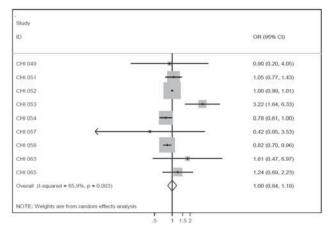


図26:特定の農薬に関する研究を含む農薬ばく露一般に関する情報と尿道下裂のリ スクのランダム効果メタアナリシス

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## 10. Reproductive diseases

Overall, 63 publications examined the effect of pesticide exposure on child health outcomes (median sample size: 299; IOR 111-544), contributing 578 separate analyses in the data extraction database. More than one third of the analyses (n=217, 38%) assess the sperm/semen quality, whereas other cluster of studies/analyses examine among others reproductive related hormones, infertily and spontaneous abortion As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 4 out of the 64 were prospective cohort studies whereas the vast majority of the studies were cross sectional (n=45, 70%). The sample size in the reported analyses was rather small; it ranged between 41 and 29,649 participants (median 161) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we observed a cluster of publications coming from INUENDO (INUit-ENDOcrine) research group (n=8), a project that has been established in three European countries together with a population of Inuits from Greenland and aims to enlighten the impact of Persistent Organic Pollutants (POPs) on human reproductive function. Almost 2/3 of the studies were conducted in Europe and America (n=22 and 20 respectively). Twenty-two studies assessed occupational exposures and, in addition, more than half of the studies had information on biomarkers of exposure (n=38, 59%), 3 studies assessed occupational exposure through Job Exposure Matrix (JEM), whereas 2 studies used both questionnaires and biomarkers. The different outcome categories examined are presented in Table 12 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data, statistical synthesis of the data (meta-analysis) was only performed for abortion.

#### 10.1. Impaired sperm parameters

Twenty-five studies (median 189: IQR 87-336) assessed the association of pesticides on sperm/semen quality using a variety of outcomes. The total analyses conducted for these outcomes are 217 and the sample size of the conducted analyses is small ranging from 41 to 763. The largest study is a European cross-sectional study from INDUENDO research group (ID RPD 009) and assess the impact of p,p'-DDE to sperm concentration, sperm motility and sperm morphology and showed that the sperm motility was negatively associated with p,p'-DDE across the four populations under study. Another large study from the same group (ID RPD 012) did not provide evidence that Persistent Organic Pollutants (POPs) may interfere with male reproductive function. Even though a large number analyses have been conducted no single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit and analysis, thus a quantitative synthesis was not performed.

#### 10.2. Fecundability disorders

Eight studies including 30 different analyses assess the effect of pesticides on low fecundability. The sample sizes are rather small ranging from 41 to 2,365 participants. Different effect sizes and analyses are used for the assessment of potential associations therefore the synthesis of the results through meta-analysis is not feasible. The largest study (ID RPD 038) that examined pesticide exposure of female greenhouse farm workers reported a reduced fecundability (OR=0.68, 95% CI=0.49-0.94). However the second largest study in the field (ID RPD 034) on female greenhouse farm workers did not shown a significant association (OR=1.11, 95%CI=0.96-1.29). Fourteen additional analyses did not report significant findings; therefore the evidence is contradictory in the field.

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#### 10. 生殖疾患

全体では、63の論文が農薬ばく露の小児健康影響を調査しており(サンプルサイズ中央値:299、IQR 111-544)、データ抽出データベースには578の個別の分析が含まれていた。分析の3分の1以上(n=217、 38%)が精子/精液の質を評価しているのに対し、他の研究/分析では生殖関連ホルモン、不妊、自然流 産などを評価している。他の影響に見られるように、ばく露の定義の多様性は顕著であり、データ統合 に特別な問題を与えている。64件のうち4件のみが前向きコホート研究であり、大多数の研究は横断研 究であった(n=45、70%)。報告されている分析のサンプルサイズはかなり小さく、41人から29,649人 の範囲であった(中央値161)。この領域での最大の研究は、がん分野で評価された最大の研究よりも 小規模である。ここでは、グリーンランドのイヌイットの集団とともにヨーロッパ3カ国で設立された プロジェクトであるINUEND0(INUIt-ENDOcrine)研究グループ(n=8)からの出版物を調査した。研究 のほぼ3分の2がヨーロッパとアメリカで実施された(それぞれn=22と20)。22件の研究が職業ばく露を 評価し、さらに半数以上の研究がばく露のバイオマーカーに関する情報を有し(n=38,59%)、3件の研 究が職業ばく露を職業ばく露マトリックス(JEM)で評価し、2件の研究が質問紙とバイオマーカーの両 方を使用した。調査した異なる影響カテゴリーを、各カテゴリーに寄与した研究の数と定量的統合の 決定とともに表12に示した。データの不均一性のため、データの統計的統合(メタアナリシス)は流産 についてのみ実施された。

## 10.1 障害のある精子パラメータ

25件の研究(中央値189件:IQR 87-336)が、様々な影響を用いて農薬と精子/精液の質との関連を 評価した。これらの結果について実施された分析の総数は217件で、実施された分析のサンプルサイズ は41~763件と小規模である。最大の研究はINDUENDO研究グループによるヨーロッパの横断研究(ID RPD 009)で、p,p'-DDEの精子濃度、精子運動性、精子形態への影響を評価し、精子運動性は研究対象 の4つの集団においてp,p'-DDEと負の関係があることが示された。同じグループによる別の大規模研 究(ID RPD 012)では、残留性有機汚染物質(POPs)が男性の生殖機能を阻害する可能性のエビデンス は示されていない。多数の分析が行われたにもかかわらず、同じ比較単位と分析を用いた4件以上の 研究では、単一の農薬と関連するバイオマーカーは評価されていないため、定量的な統合は行われな かった。

## 10.2 生殖能障害

30種類の分析を含む 8件の研究が、低生殖能に対する農薬の影響を評価している。サンプルサイズ は 41 から2,365 人とかなり小さい。潜在的な関連の評価には異なる効果量と分析が使用されている ため、メタアナリシスによる結果の統合は不可能である。温室栽培農場の女性労働者の農薬ばく露を 調査した最大規模の研究(ID: RPD 038)では、生殖能の低下が報告された(OR=0.68、95% CI=0.49-0.94)。しかし、温室栽培農場の女性労働者を対象とした2番目に大きなフィールド研究(ID RPD 034) では、有意な関連は示されなかった(OR=1.11、95%CI=0.96-1.29)。14の追加分析では有意な結果は報 告されなかったため、フィールド調査ではエビデンスが矛盾している。

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#### 10.3. Spontaneous abortion

Ten studies of spontaneous abortion focused on occupational exposure. We were able to synthesize data from six studies that provided an effect estimate and a metric of its variation. The summary OR was 1.52 (95%: 1.09-2.13) using random effects models and large heterogeneity was observed (I<sup>2</sup>=63%) (Figure 27). However, the largest cross-sectional study on this outcome conducted by the INUENDO research group (ID RPD 003) did not shown any statistical effect (OR=1) between p,p'-DDE and abortion. One more study compared full-time vs. part time farming and did not report a significant association (p-value=0.99). Three other studies did not provide adequate information for their inclusion in the meta-analysis.

## 10.4. Reproductive hormones

Nineteen studies (median sample size 257: IQR 97-322) contributing with 250 analyses for various reproductive hormones were identified in this systematic review. The studies were comparable to the other large group of impaired sperm parameters sample size-wise; their range was from 62 to 887. The largest study is a European cross-sectional study that assess the effect of hexachlorobenzene on the levels of testosterone and estradiol. Hormonal status of 14- to 15- year-old male adolescents was studies in relation to internal exposure to pollutants. The study shows that the exposure is associated with substantial differences in hormone concentrations. Different patterns were observed in study conducted by the INUENDO research group where the overall analysis between DDE and reproductive related hormones did not reveal any significant results. However in center-specific analysis, gonadotropin levels and sex-hormone-binding globulin seem to be affected by exposure on p,p'-DDE supporting substantial variations between different populations. The large variety of outcomes and pesticides assessed did not allow for any quantitative synthesis of the data.

#### 10.5. Reproductive outcomes with few studies

For all the assessed outcomes not included in Table 12, assessment of menstrual cycles cannot allow synthesis of the available evidence. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Results on different menstrual outcomes showed that it is unlikely that exposure to DDE is a main cause of menstrual disturbances.

#### 10.3. 自然流産

自然流産に関する10件の研究では、職業ばく露に焦点が当てられていた。効果推定値とその変動の 指標を提供した6件の研究からデータを統合することができた。ランダム効果モデルを用いた要約0Rは 1.52 (95%:1.09-2.13) であり、大きな不均一性が観察された(I2=63%)(図27)。しかし、INUENDO 研究グループが実施したこの影響に関する最大の横断研究(ID RPD 003)では、p,p'-DDEの農業労働 と流産との間に統計的効果(OR=1)は示されなかった。さらに1つの研究では、フルタイムとパート タイムを比較したが、有意な関連は報告されていない(p値=0.99)。他の3つの研究では、メタア ナリシスに含めるのに十分な情報が得られなかった。

## 10.4 生殖ホルモン

このシステマティックレビューでは、様々な生殖ホルモンについて250の分析を行った19の研究(サ ンプルサイズ中央値257:IQR 97-322)が同定された。これらの研究は、精子障害パラメータに関する 他の大規模なグループのサンプルサイズに匹敵するものであり、その範囲は62から887までであった。 最大の研究は、テストステロンとエストラジオールのレベルに対するヘキサクロロベンゼンの影響を 評価したヨーロッパの横断研究である。14~15歳の男性青年のホルモン状態は、汚染物質への内部ば く露に関連して研究された。この研究では、ばく露はホルモン濃度の大きな差異と関連していること が示された。INUENDO研究グループが行った研究では、DDEと生殖関連ホルモンの全体的な分析では有 意な結果は得られなかったが、異なるパターンが観察された。しかし、センターごとの分析では、ゴナ ドトロピン濃度と性ホルモン結合グロブリンはp,p' -DDEばく露の影響を受けているようであり、母集 団間に大きなばらつきがあることが示唆された。評価された結果と農薬の種類が多いため、データの 定量的な統合はできなかった。

## 10.5.研究数が少ない生殖影響

表12に含まれていないすべての影響については、月経周期の評価では利用可能なエビデンスを統合 することができない。我々のシステマティックレビューでは、過去に発表されたエビデンス(2006年以 前)との比較を可能にするために、これらの影響に関する過去に発表されたメタアナリシスは確認し なかった。異なる月経影響に関する結果から、DDEへのばく露が月経障害の主な原因であるとは考えに くいことが示された。

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 Table 12:
 Summary of studies identified per outcome subgroup with more than 4 studies (NA: not available)

Outcome	N studies	Meta-analysis done	Previous meta-analysis result
Impaired sperm parameters	25	No	NA
Fecundability disorders	8	No	NA
Abortion	10	Yes	NA
Reproductive hormones	19	No	NA

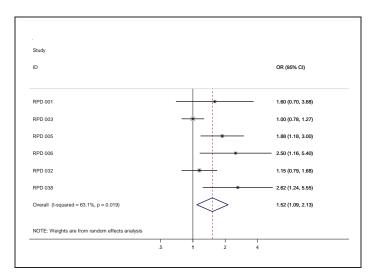
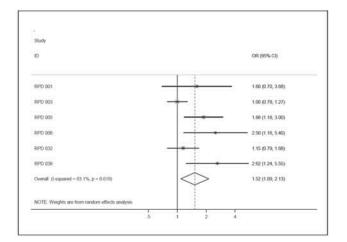


Figure 27: Random effects meta-analysis for studies with information on pesticide exposure and risk of abortion

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# 表 12:4 件以上の研究がある影響サブグループごとに同定された研究の要約(NA: 利用不可)

影響	研究数	メタアナリシス実施	前回のメタアナリシス結果
精子障害パラメータ	25	No	NA
生殖能障害	8	No	NA
流産	10	Yes	NA
生殖ホルモン	19	No	NA



# 図27:農薬ばく露に関する情報と流産リスクのランダム効果メタアナリシス

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#### 11. Neurological diseases

Overall, 60 publications examined the effect of pesticide exposure on neurological outcomes (median sample size: 390; IQR 246-781), contributing 573 separate analyses in the data extraction database. More than thirty health-related outcomes were assessed with the largest proportion focusing on Parkinson's disease with 32 studies (Table 13). As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Only 8 out of the 60 were prospective cohort studies and other 2 were nested case-controls; the majority of evidence comes from retrospective case-control analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 46 and 143,325 participants (median 390) and the largest studies in the domain are smaller than the largest studies assessed in the cancer field. Here, we also observed large clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS), while 43 studies assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was far less prominent here (n=7, 12%). The different outcome categories examined are presented in Table 13; due to the small number of studies identified per assessed outcome, statistical synthesis of the data (meta-analysis) was only performed for Parkinson's disease and amyotrophic lateral sclerosis

## 11.1. Parkinson's disease

Thirty-two studies assessed the association between pesticide exposure and Parkinson's disease with a median sample size of 399 (IQR 286-711), contributing 266 separate extracted comparisons in the database. Eighty percent of the retrieved studies assessed occupational exposures, only 10% were prospective and the exposure was assessed through a biomarker in a small number of studies (10%). A large variety of individual pesticides were assessed with the following pesticides being assessed more frequently: general pesticide (28 studies), as well as DDT (5 studies), paraquat (9 studies).

We initially assessed the association between general pesticide use and Parkinson's disease. The observed effect indicated a statistically significant association with the presence of considerable heterogeneity (random-effects OR 1.58, 95% CI 1.35 – 2.85, I<sup>2</sup> 61%) (Figure 28). With the exception of four studies where specific pesticides were assessed (e.g. paraquat), all the other studies assessed mainly occupational general pesticide use in mainly a retrospective fashion via a questionnaire. The results of the meta-analysis are in accordance with the largest studies on that research question.

We then proceeded to assess the association between DDT exposure and Parkinson's disease. The observed effect indicated a non-statistically significant association without the presence of heterogeneity (random-effects OR=1.01, 95% CI=0.78–1.30,  $I^2$ =0%) (Figure 29). Finally, we assessed the association between paraquat exposure and Parkinson's disease. The observed effect indicated a statistically significant association with the presence of moderate heterogeneity (random-effects OR=1.32, 95% CI=1.10–1.60,  $I^2$ =34%) (Figure 30). The results of the meta-analysis are in accordance with the largest studies on these research questions.

Our literature search yielded 7 systematic reviews and/or meta-analyses on the association between pesticide exposure and Parkinson's disease published from 2000 to 2013 (Pezzoli 2013, Van-Maele Fabry 2012, van der Mark 2012, Dick 2006, Priyadarshi 2001, Priyadarshi 2000, Allen 2013). Despite the considerable time interval between the oldest and most recent research synthesis effort and the different methodologies endorsed (prospective studies only assessed, methodological assessment of

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#### 11. 神経疾患

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全体では 60 の論文が農薬ばく露の神経学的影響に及ぼす効果を調査しており(サンプルサイズ中央 値:390、IQR 246-781)、データ抽出データベースでは 573 件の個別分析が行われた。30 以上の健康 関連影響が評価されており、パーキンソン病に焦点を当てた研究が最も多く 32 件あった(表 13)。他 の影響に見られるように、ばく露の定義の多様性には目を見張るものがあり、データ統合に特別な問 題を与えている。60 件のうち 8 件のみが前向きコホート研究で、残りの 2 件はコホート内症例対照研 究であった。報告された分析におけるサンプルサイズはしばしば小さく、参加者数は 46~143,325 人 (中央値 390 人)であり、この領域における最大の研究は、がん分野で評価された最大の研究よりも 小規模であった。ここでは、農業健康調査(Agricultural Health Study: AHS)のようなこの分野の大 規模でよく知られた研究からの多くの出版物のまとまりも観察されたが、一方で 43 件の研究が職業ば く露を評価していた。さらに、ばく露のバイオマーカーに関する情報を持つ研究の存在は、ここではあ まり目立たなかった(n=7,12%)。評価された影響カテゴリーを表 13 に示す。評価された影響ごとに 同定された研究の数が少ないため、データの統計的統合(メタアナリシス)はパーキンソン病と筋萎縮 性側索硬化症についてのみ実施された。

## 11.1 パーキンソン病

農薬ばく露とパーキンソン病との関連を調査した研究は32件あり、サンプルサイズの中央値は399件 (IQR 286-711)で、データベースには266件の比較が抽出されている。検索された研究の80%は職業ば く露を評価していたが、前向き研究はわずか10%で、ばく露をバイオマーカーを介して評価した研究 は少数であった(10%)。個々の農薬の評価は多岐にわたったが、一般的な農薬(28件)、DDT(5件)、 パラコート(9件)などの農薬がより頻繁に評価されていた。

まず、一般的な農薬の使用とパーキンソン病との関連を評価した。観察された効果は、かなりの不均 一性が存在し、統計的に有意な関連を示した(ランダム効果0R 1.58、95%CI 1.35~2.85、I2 61%) (図 28)。特定の農薬を評価した 4 つの研究(例:パラコート)を除いて、他のすべての研究では、 主に質問紙による後ろ向きな方法で、主に職業上の一般的な農薬使用を評価している。メタアナリシ スの結果は、この研究の課題に関する最大の研究と一致している。

次に、DDTばく露とパーキンソン病との関連を評価した。観察された効果は、不均一性の存在なしに 統計学的に有意ではないことが示された(ランダム効果0R=1.01、95%CI=0.78-1.30、I2=0%)(図29)。 最後に、パラコートばく露とパーキンソン病との関連を評価した。観察された効果は、中等度の不均一 性の存在下で統計的に有意な関連を示した(ランダム効果0R=1.32、95%CI=1.10-1.60、I2=34%)(図 30)。メタアナリシスの結果は、これらの研究課題に関する最大規模の研究と一致している。

我々の文献検索では、2000年から2013年までに発表された農薬ばく露とパーキンソン病との関連に 関する7つのシステマティックレビュー及び/またはメタアナリシスが得られた (Pezzoli 2013, Van-Maele Fabry 2012, van der Mark 2012, Dick 2006, Priyadarshi 2001, Priyadarshi 2000, Allen 2013)。研究統合の最も古い取り組みと最新の取り組みとのかなりの時間的間隔、また、方法論(前向 き研究のみの評価、対象研究の方法論的評価など)の違いにもかかわらず、結果はメタアナリシス全体 で一貫しており、2006年からの現在の取り組みとも一致している(表14)。

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the included studies, etc.), the results are consistent across the meta-analyses and are also consistent with the present effort spanning from 2006 (Table 14).

#### 11.2. Amyotrophic lateral sclerosis (ALS)

Seven studies assessed the association between pesticide exposure and amyotrophic lateral sclerosis with a median sample size of 356 (IQR 201-1156), contributing 11 separate extracted comparisons in the database. All the retrieved studies assessed occupational exposures, while 4 also assessed residential exposure. Only one study was prospective and the exposure was assessed through a questionnaire in most of the studies (n=6).

We assessed the association between general pesticide use and ALS. The observed effect indicated a statistically significant association with the presence of small heterogeneity (fixed-effects OR=1.58, 95% CI=1.31 – 1.90, I<sup>2</sup> 10%) (Figure 31) and the results of the meta-analysis are in accordance with the largest studies on that research question.

Our literature search yielded 2 systematic reviews and/or meta-analyses on the association between pesticide exposure and ALS published in 2012 (Kamel 2012, Malek 2012). Regarding these efforts, the results are consistent with our findings and the authors' report of evidence on an association of exposure to pesticides and risk of ALS in male cases compared to controls (OR=1.88, 95% CI: 1.36-2.61), although the chemical or class of pesticide was not specified by the majority of studies.

#### 11.3. Neurological outcomes with few studies

With the exception of Parkinson's disease and amyotrophic lateral sclerosis, for all the remaining neurological outcomes, too few studies are available after 2006 to allow synthesis of evidence for each outcome alone; these outcomes comprise a vast variety of captured information ranging from well-defined clinical entities yet with too few studies, such as hearing loss or diabetic neuropathy, as well as a large number of metrics pertaining to neurological endophenotypes but with a prominent lack of harmonization and standardization in the outcome definition. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

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## 11.2. 筋萎縮性側索硬化症(ALS)

7件の研究が農薬ばく露と筋萎縮性側索硬化症との関連を評価しており、サンプルサイズの中央値は 356 (IQR 201-1156)で、データベースには11件の個別の比較が抽出されている。検索されたすべての 研究は職業ばく露を評価しており、4つの研究は住居ばく露も評価していた。前向き研究は1件のみで、 ほとんどの研究では質問紙を用いてばく露を評価していた (n=6)。

我々は一般的な農薬使用とALSとの関連を評価した。観察された効果は、小さな不均一性(固定効果 OR=1.58、95%CI=1.31~1.90、I2 10%)の存在とともに統計的に有意な関連を示し(図31)、メタア ナリシスの結果は、その研究課題に関する最大の研究に沿ったものであった。

文献検索を行った結果、2012 年に発表された農薬ばく露と ALS の関連に関する 2 つのシステマティックレビュー及び/またはメタアナリシスが得られた(Kamel 2012, Malek 2012)。これらの結果によれば、大多数の研究では農薬の化学物質やクラスが特定されていなかったが、我々の知見や男性で対照と比較して農薬へのばく露とALSの関連を示すエビデンス(OR=1.88、95% CI: 1.36-2.61) についての著者らの報告と一致していた。

## 11.3. 研究数の少ない神経学的影響

パーキンソン病と筋萎縮性側索硬化症を除いて、残りのすべての神経学的影響については、2006年 以降の研究が少なすぎて、それぞれの影響だけでエビデンスを統合することができない。我々のシス テマティックレビューでは、2006年以前に発表されたエビデンスとの比較を可能にするために、これ らの影響に関する以前に発表されたメタアナリシスは確認されなかった。一般的に、これらの影響に 関する結果は効果が小さく、統計的に有意ではなかったが、少数の例外を除いては有意であった。分析 数が多いことを考えると、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農 薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

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Assessed outcomes in the field of neurology

Table 13:

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Health outcome		
Abnormal alternating hand movements	Alzheimer's disease	Narcolepsy with cataple
Abnormal ankle reflex	Amyotrophic lateral sclerosis	Neurological symptoms
Abnormal distal motor amplitude	Cryptogenic polyneuropathy	Parkinson's disease
Abnormal distal motor latency	Decline in hand-grip strength	Parkinsonism
abnormal facial expression	Delayed memory impairment	Peripheral neuropathy

vith cataplexy

Progressive supranuclear palsy Restless legs syndrome Romberg sign Sporadic Motor Neuron Disease Subclinical neuropathy

Essential tremor Gait disorder Hearing loss Multiple System Atrophy

abnormal nerve conduction velocity Abnormal postural tremor Abnormal short F-wave latency Abnormal toe proprioception Abnormal toe vibration perception

Dementia

Tandem gait abnormality

Narcolepsy (with and without cataplexy)

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# 表13:神経学分野で評価された影響

& 10·种植于力封 (		
健康影響		
手の交代運動の異常	アルツハイマー病	カタプレキシーを伴うナルコレプシー
足首反射の異常	筋萎縮性側索硬化症	神経症状
遠位運動振幅の異常	原因不明の多発神経障害	パーキンソン病
遠位運動潜時の異常	握力の低下	パーキンソン症候群
表情の異常	遅延記憶障害	末梢神経障害
神経伝導速度異常	認知症	進行性核上性麻痺
異常な姿勢振戦	本態性振戦	レストレスレッグス症候群
異常な姿勢	歩行障害	ロンベルグ徴候
短 F 波潜時の異常	難聴	散発性運動ニューロン病
足尖の固有知覚の異常	多系統萎縮症	潜在的神経障害
足尖の振動知覚の異常	ナルコレプシー (カタプレキシー	つぎ足歩行異常
	の有無にかかわらず)	

Tzoulaki I	
Evangelou E,	
I, Ntritsos G	
Chondrogiorgi M	
Ntzani EE, 0	

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Characteristics of the studies assessing pesticide exposure and Parkinson's disease risk (n/a: not available) Table 14:

America Cohort Occupational Dir America Cose-control Occupational Dir America Case-control Occupational Dir America Case-control Occupational Dir Europe Nested case- Environmental Bio America Case-control Mixed Dir	Direct exposure questionnaire Direct exposure questionnaire Direct exposure questionnaire Biomarker Direct exposure questionnaire	ever/never ever/never ever vs. never ever vs. never per IOR increase	yes	size
CohortOccupationalCase-controlOccupationalCase-controlOccupationalNested case- controlEnvironmentalCase-controlMixed		ever/never ever/never ever vs. never per IOR increase	yes	
Cohort     Occupational       Case-control     Occupational       Case-control     Occupational       Nested case-     Environmental       control     Mixed       Case-control     Mixed		ever/never ever/never ever vs. never per IOR increase	yes	
Case-control Occupational Case-control Occupational Nested case- control Mixed Case-control Mixed		ever/never ever vs. never per IOR increase		8899
Case-control Occupational Nested case- Environmental control Mixed Case-control Mixed		ever vs. never per IOR increase	yes	808
Nested case- Environmental control Mixed Case-control Mixed		per IOR increase	yes	578
Case-control Mixed	ect exposure questionnaire		yes	292
		ever/never	ou	184
America Case-control Mixed Dir	Direct exposure questionnaire	ever/never	ou	184
America Cohort Occupational Dir	Direct exposure questionnaire	ever/never	yes	7393
America Case-control Environmental Res	Residential history	yes/no	yes	709
America Case-control Occupational Dir	Direct exposure questionnaire	ever/never	yes	1030
America Nested case- Occupational Dirr control	Direct exposure questionnaire	ever/never	yes	468
America Case-control Environmental Res	Residential history	yes/no	yes	709
America Case-control Occupational Dir	Direct exposure questionnaire	ever vs. never	yes	578
65 The meanst document has been conducted and advanced by the hodine identical above as author(c). This read has been conject out accludingly, by the outbod(c) in the context of a contrast harmone the	unthonica). This took has has comind ant evolution her t	the outhor(c) in the contax	t of a contract hat	65 the

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	年	場所	研究 デザイン	ばく 感 タイプ	はく露評価	比較単位	調整	サンプ サイズ
DDT								
NRD 027	2007	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	8899
					の質問紙			
NRD 025	2011	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	808
					の質問紙			
NRD 032	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	578
					の質問紙	never		
NRD 033	2010	ヨーロッパ	Nested case-	環境	バイオマーカー	per IQR	yes	292
			control			increase		
NRD 019	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	no	184
					の質問紙			
パラコー	ŀ							
NRD 019	2008	アメリカ	Case-control	混合	直接ばく露について	ever/never	no	184
					の質問紙			
NRD 027	2007	アメリカ	Cohort	職業	直接ばく露について	ever/never	yes	7393
					の質問紙			
NRD 023	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
NRD 030	2009	アメリカ	Case-control	職業	直接ばく露について	ever/never	yes	1030
					の質問紙			
NRD 037	2011	アメリカ	Nested case-	職業	直接ばく露について	ever/never	yes	468
			control		の質問紙			
NRD 020	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
NRD 022	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	578
					の質問紙	never		
NRD 038	2010	アメリカ	Case-control	職業	職歴	yes/no	yes	58
NRD 020	2009	アメリカ	Case-control	環境	居住履歴	yes/no	yes	709
農薬								
NRD 033	2010	ヨーロッパ	Nested case-	環境	バイオマーカー	per IQR	yes	292
			control.		(HCB)	increase		
NRD 058	2010	ヨーロッパ	Case-control	混合	直接ばく露について	yes/no	no	330

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調整 サンプル

# 表14:農薬ばく露とパーキンソン病リスクを評価した研究の特徴(n/a:なし)

Ntzani	EE, Chot	1drogiorgi M	Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I	lou E, Tzoulaki I	Pesticide epidemiology			
₽	Year	Location	Study design	Exposure type	Exposure assessment	Comparison unit	Adjustment	Sample
NRD 038	2010	America	Case-control	Occupational	Occupational history	yes/no	yes	58 58
NRD 020	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709
Pesticides	S							
NRD 033	2010	Europe	Nested case- control	Environmental	Biomarker (HCB)	per IQR increase	yes	292
NRD 058	2010	Europe	Case-control	Mixed	Direct exposure questionnaire (insecticides)	yes/no	оц	330
NRD 034	2010	Asia	Case-control	Occupational	Direct exposure questionnaire	ever/never	n/a	608
NRD 018	2008	Europe	Case-control	Occupational	Direct exposure questionnaire	ever/never	yes	233
NRD 017	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	yes	1666
NRD 014	2006	America	Cohort	Occupational	Direct exposure questionnaire	ever/never	yes	143325
NRD 029	2009	Europe	Case-control	Mixed	Direct exposure questionnaire and JEM	ever/never	QL	388
NRD 036	2011	Europe	Cohort	Occupational	JEM	JEM class		
NRD 015	2007	Asia	Case-control	Occupational	Direct exposure questionnaire	yes/no	yes	308
NRD 020	2009	America	Case-control	Occupational	Occupational history	yes/no	yes	709
NRD 028	2008	America	Case-control	Mixed	Direct exposure questionnaire	ever/never	yes	615
NRD 023	2009	America	Case-control	Environmental	Residential history	yes/no	yes	709
EFSA su	pporting	publication	EFSA supporting publication 2013:EN-497					99
The preser European F may not be present doc	tt documer. Food Safety considerer ument, wit	tt has been prov / Authority and d as an output a hout prejudice t	The present document has been produced and adopted by the discrepant Food Stery Authority and the analyon (s), wareded foil may not be considered as an output adopted by the Authority. present document, without prejudice to the rights of the authors.	e bodies identified abov Mowing a tender proced The European Food Saf	The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Stafey Authority and the author(s), and adopted by the Authority and a warded for the procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an onput adopted by the Authority and the transparency principle to which the Authority is subject. It may not be considered as an onput adopted by the Authority. The European Food Safety Authority and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.	y the author(s) in the conte ansparency principle to whit s the issues addressed and th	xt of a contract be ch the Authority is he conclusions reach	tween the subject. It ned in the

ばく 露 ばく露評価 場所 比較単位 調整サンプ デザイン の質問紙(殺虫剤) NRD 034 2010 アジア Case-control 職業 直接ばく露について ever/never n/a 608 の質問紙 NRD 018 2008 ヨーロッパ Case-control 職業 直接ばく露について ever/never yes 233 の質問紙 NRD 017 2008 アメリカ Case-control 混合 直接ばく露について ever/never yes 1666 の質問紙 NRD 014 2006 アメリカ Cohort 職業 直接ばく露について ever/never yes 143325 の質問紙 NRD 029 2009 ヨーロッパ Case-control 混合 直接ばく露について ever/never no 388 の質問紙と JEM NRD 036 2011 ヨーロッパ Cohort 職業 JEM JEM class NRD 015 2007 アジア Case-control 職業 直接ばく露について yes/no yes 308 の質問紙 NRD 020 2009 アメリカ Case-control 職業 職歴 yes/no yes 709 2008 アメリカ Case-control 混合 直接ばく露について ever/never yes 615 NRD 028 の質問紙 NRD 023 2009 アメリカ Case-control 環境 居住履歴 yes 709 yes/no NRD 016 2007 ヨーロッパ Case-control 混合 職歴・直接ばく露に high vs. no yes 2756 ついての質問紙 exposure 2010 ヨーロッパ Case-control 職業 NRD 024 職歴 yes/no no 387 NRD 025 2011 アメリカ Case-control 職業 直接ばく露について ever/never yes 808 の質問紙 NRD 058 2006 アメリカ Case-control 混合 直接ばく露について ever/never no 278 の質問紙 NRD 027 2007 アメリカ Cohort 職業 直接ばく露について ever/never yes 65183 の質問紙 Case-control 職業 職歴 NRD 035 2010 アジア yes/no no 525 NRD 030 2006 アメリカ Case-control 職業 直接ばく露について yes/no yes 430 の質問紙 NRD 030 2009 アメリカ Case-control 職業 直接ばく露について ever/never yes 1030

Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

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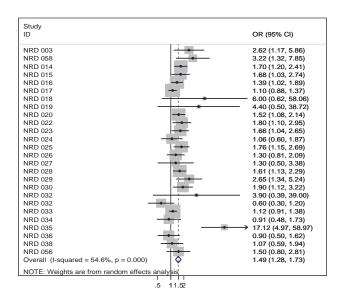
Sample	2756	387	808	278	65183	525	430	1030	781	184	352	578	264
Adjustment	yes	ои	yes	ou	yes	ou	yes	yes	yes	ou	yes	yes	yes
Comparison unit	high vs. no exposure	yes/no	ever/never	ever/never	ever/never	yes/no	yes/no	ever/never	ever/never	yes/no	ever vs. never	ever vs. never	yes/no
Exposure assessment	Occupational history and direct exposure questionnaire	Occupational history	Direct exposure questionnaire	Direct exposure questionnaire	Direct exposure questionnaire	Occupational history	Direct exposure questionnaire	n/a					
Exposure type	Mixed	Occupational	Occupational	Mixed	Occupational	Occupational	Occupational	Occupational	Occupational	Occupational	Occupational	Occupational	n/a
Study design	Case-control	Case-control	Case-control	Case-control	Cohort	Case-control	Case-control	Case-control	Case-control	Case-control	Case-control	Case-control	Case-control
Location	Europe	Europe	America	America	America	Asia	America	America	Europe	America	America	America	Europe
Year	2007	2010	2011	2006	2007	2010	2006	2009	2009	2008	2010	2010	2010
Q	NRD 016	NRD 024	NRD 025	NRD 058	NRD 027	NRD 035	NRD 026	NRD 030	NRD 022	NRD 019	NRD 032	NRD 032	NRD 003

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Ntza	ni EE	, Chondi	rogiorgi M, N	tritsos G, Evang	elou E, T	zoulaki I	Pest	ticide ep	idemiolog
		年	場所	研究	ばく	はく露評価	比較単位	調整	サンプル
				デザイン	タイプ				サイズ
						の質問紙			
NRD	022	2009	ヨーロッパ	Case-control	職業	直接ばく露について	ever/never	yes	781
						の質問紙			
NRD	019	2008	アメリカ	Case-control	職業	直接ばく露について	yes/no	no	184
						の質問紙			
NRD	032	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	352
						の質問紙	never		
NRD	032	2010	アメリカ	Case-control	職業	直接ばく露について	ever vs.	yes	578
						の質問紙	never		
NRD	003	2010	ヨーロッパ	Case-control	n/a	n/a	yes/no	yes	264

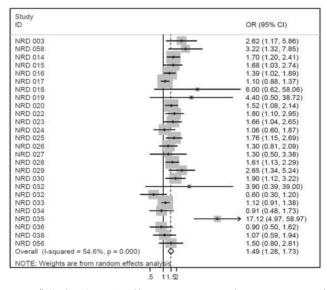
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**Figure 28:** Random effects meta-analysis for studies with information on any pesticide exposure and risk of Parkinson's disease (study with ID NRD 033, specifically assessed hexachlorobenzene)

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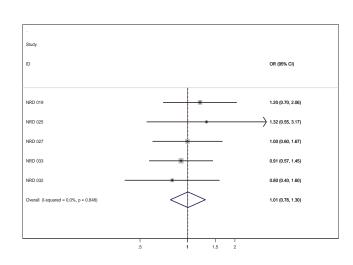
Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

図28: 農薬ばく露に関する情報とパーキンソン病のリスクのランダム効果メタアナ リシス (ID NRD 033の研究、特にヘキサクロロベンゼンを評価)

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Figure 29: Fixed-effects meta-analysis for studies with information on exposure and risk of Parkinson's disease

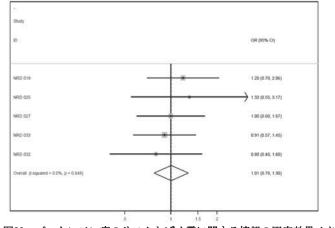


図29:パーキンソン病のリスクとばく露に関する情報の固定効果メタアナリシス

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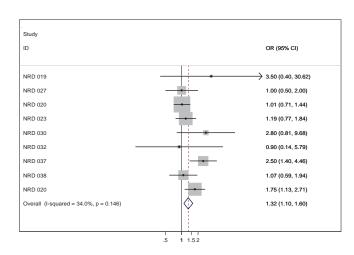
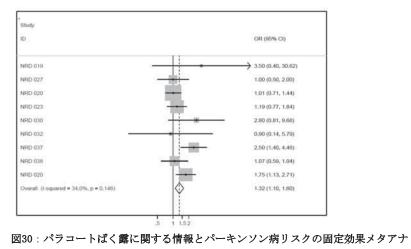


Figure 30: Fixed-effects meta-analysis for studies with information on paraquat exposure and risk of Parkinson's disease

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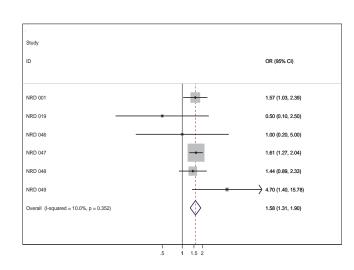
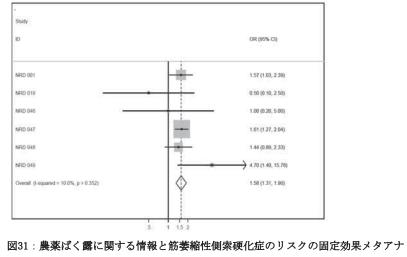


Figure 31: Fixed-effects meta-analysis for studies with information on general pesticide exposure and risk of amyotrophic lateral sclerosis

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### 12. Endocrine diseases

Overall, 35 publications examined the effect of pesticide exposure on thyroid hormone dysregulation (median sample size: 226; IOR 130-453), contributing 343 separate analyses in the data extraction database. The main outcomes assessed were thyroxin (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) levels. Only 3 prospective cohort studies were conducted in the field; the majority of evidence comes from retrospective case-control or cross-sectional analyses, which are prone to recall bias in exposure measurement. The sample size in the reported analyses was often small; it ranged between 27 and 16,529 participants (median 341). Here, we observed no large clusters of publications coming from large, well-known studies in the field, while the vast majority of the studies assessed environmental exposures (n=28, 80%). However, the presence of studies with information on biomarkers of exposure was more prominent here (n=29, 83%). Even though hypothyroidism, hyperthyroidism and other thyroid diseases contribute with more than 1/3 of the total analyses (n=123) the available evidence derives from Agricultural Health Study (AHS) which apparently is the largest in the field and examines the association between pesticide use and thyroid diseases in females. The study found an association between hypothyroidism and ever use of organochlorine insecticides (OR=1.2, 95% CI= 1.0-16) and fungicides (OR=1.4, 95% CI= 1.1-1.8). However, the results should be interpreted with caution due to borderline significance levels and absence of type-I error corrections due to multiple comparisons. Other studies in the field assessing several thyroid hormone levels are quite smaller and provide contradictory results. As seen with other outcomes, the diversity of the exposure definition is remarkable and poses special challenges to data synthesis. Due to heterogeneity of data and different analyses, effect sizes and metrics provided, statistical synthesis of the data (meta-analysis) was not performed.

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

#### 12. 内分泌疾患

全体では、35の出版物で農薬ばく露が甲状腺ホルモン調節障害に及ぼす影響が調査され(中央値サ ンプルサイズ226: IQR 130-453)、データ抽出データベースでは343の個別の分析が行われた。評価さ れた主な影響は、サイロキシン(T4)、トリヨードサイロニン(T3)、甲状腺刺激ホルモン(TSH)レ ベルであった。この分野で実施された前向きコホート研究は3件のみであり、エビデンスの大部分は後 ろ向き症例対照または横断分析によるものであるが、これらはばく露測定においてリコールバイアス がかかりやすい。報告されている分析におけるサンプルサイズはしばしば小さく、参加者数は27~ 16,529人(中央値341人)であった。ここでは、この分野でよく知られた大規模な研究からの出版物の 大きなクラスタは観察されず、大多数の研究は環境ばく露を評価していた(n=28、80%)。しかし、ば く露のバイオマーカーに関する情報を有する研究の存在は、ここではより多数であった(n=29、83%)。 甲状腺機能低下症、甲状腺機能亢進症、その他の甲状腺疾患が分析全体の1/3以上を占めているにもか かわらず(n=123)、利用可能なエビデンスは、この分野では明らかに最大規模で農薬使用と女性の甲 状腺疾患との関連を調査している農業健康調査 (Agricultural Health Study: AHS) に由来している。 この研究では、甲状腺機能低下症と有機塩素系殺虫剤(OR=1.2、95% CI=1.0-16)及び殺菌剤(OR=1.4、 95% CI=1.1-1.8)の使用歴との間に関連があることが明らかになった。しかし、この結果は、有意水準 の境界線上であったことと多重比較によるタイプIの誤差補正がないため、注意して解釈されるべきで ある。この分野でいくつかの甲状腺ホルモンレベルを評価している他の研究は非常に小規模であり、 矛盾する結果を示している。他の影響に見られるように、まちまちなばく露の定義に注目すべきであ り、データ統合に特別な問題を及ぼしている。データの不均一性と異なる分析、効果量及び指標の使用 のために、データの統計的統合(メタアナリシス)は行われていない。

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### 13. Mental and psychomotor development outcomes

Overall, 32 publications examined the effect of pesticide exposure on mental and psychomotor development outcomes in pediatric populations (median sample size: 238, IOR 109-305), contributing 462 separate analyses in the data extraction database. Only one study was performed in a population of non-European (Asian) ancestry, while seventeen health-related outcomes were assessed with a large proportion focusing on attention-deficit hyperactivity disorder (ADHD, 6 studies, 102 analyses). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. A large majority of the studies (23 publications, 72%) referred to prospective cohort studies, while the sample size in the reported analyses was often small; it ranged between 25 and 7,440 participants with the largest study assessing retrospectively maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Here, we also observed clusters of publications coming from large, well-known studies in the field, such as the CHAMACOS (The Center for Health Assessment of Mothers and Children of Salinas) (5 publications), while 84% of the studies assessed environmental exposures. In addition, the presence of studies with information on biomarkers of exposure was prominent here (n=28, 88%). The different outcome categories examined are presented in Table 15 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, no statistical synthesis of the data (meta-analysis) was performed for any outcome.

#### 13.1. Mental and psychomotor development outcomes with few studies

With the exception of mental and psychomotor development and Attention-deficit hyperactivity disorder (ADHD), for all the remaining assessed outcomes included in Table 15, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as autism, or pervasive developmental disorder, as well as a vast number of outcomes representing neurodevelopmental endo-phenotypes such as communication, fine and gross motor development or expressive language development. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the small number of studies and sample sizes, these results need cautious interpretation and, based on these data, there is no evidence to suggest a robust association between pesticide exposure and these outcomes.

#### 13.2. Attention-deficit hyperactivity disorder (ADHD)

Six studies assessed the association between pesticide exposure and ADHD with a sample size ranging from 278 to 2,539 participants, contributing 102 separate extracted comparisons in the database. Three studies were cohorts, all assessed environmental exposure and in all the exposure was assessed through a biomarker. General organophosphate exposure was assessed in the extudies, DDT exposure in two studies, while trans-nonachlor, hexachlorobenzene, and 2,4,6-Trichlorophenol (TCP) were assessed in one study each. Thus, no single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. The largest study in the field is a National Health and Nutrition Examination Survey (NHANES) report (ID 17) used data from the 1999-2004 NHANES to evaluate the association between urinary trichlorophenols (TCPs) and parent-reported ADHD among 2546 children aged 6-15 years. The authors report that children with low levels (<3.58 mg/g) and high levels EFSA supporting publication 2013:EN-497 73

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## 13. 精神及び精神運動発達影響

全体では、32の出版物が小児集団における精神及び精神運動発達影響に対する農薬ばく露の影響を調 査しており(サンプルサイズ中央値:238、IQR 109-305)、データ抽出データベースでは462の個別の 分析が行われた。非ヨーロッパ系(アジア系)の集団を対象とした研究は1件のみであり、注意欠陥多 動性障害(ADHD、6件、102件の分析)を中心に17件の健康関連影響が評価されていた。他の影響に見ら れるように、ばく露の定義はかなりまちまちであり、データ統合に特別な問題をもたらしている。大多 数の研究(23の論文、72%)は前向きコホート研究を参考にしているが、報告されている分析における サンプルサイズはしばしば小さく、25~7,440人の範囲で、最大のものはカリフォルニア・セントラル バレーの小児における農薬散布付近の母親の居住と自閉症スペクトラム障害の後ろ向き研究であった。 ここで、我々はまた、CHAMACOS(サリナスの母と小児の健康評価センター)(5出版物)などの大規模 な、この分野でよく知られた研究から来ている出版物のクラスタを観察したが、研究の84%が環境ば く露を評価していた。さらに、ばく露のバイオマーカーに関する情報を有する研究の存在がここでは 多数であった(n=28、88%)。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究の 数と定量的統合の決定とともに表15に示す。データの不均一性と同定された研究数が少なかったため、 どの影響についてもデータの統計的統合(メタアナリシス)は行われなかった。

## 13.1. 研究が少ない精神及び精神運動発達影響

精神及び精神運動発達影響と注意欠陥多動性障害(ADHD)を除いて、表15に記載の影響はすべて、そ れぞれの影響だけでエビデンスを総合的に判断するには、利用可能な研究が少なすぎる。これらの影 響は、自閉症や広汎性発達障害のように研究数が少ないが適切に定義された臨床所見から、コミュニ ケーション、微細及び粗大な運動発達、または表現的言語発達などの神経発達の中間形質を表す多数 の影響まで収集されたさまざまな情報で構成されている。我々のシステマティックレビューでは、2006 年以前に発表されたエビデンスとの比較を可能にするために、これらの影響に関する以前に発表され たメタアナリシスは確認しなかった。一般的に、これらの影響に関する結果は影響が小さく、統計的に 有意ではなかったが、少数の例外はあった。分析数が多く、研究数やサンプル数が少ないことを考える と、これらの結果は慎重に解釈する必要があり、これらのデータに基づいて、農薬ばく露とこれらの影 響との間に妥当な関連を示唆するエビデンスはない。

## 13.2. 注意欠陥多動性障害(ADHD)

6件の研究では、278人から2,539人の参加者のサンプルサイズで農薬ばく露とADHDとの関連を評価し、 データベースに102件の別個に抽出された比較を提供した。3つの研究はコホートであり、すべての研 究は環境ばく露を評価し、すべての研究でばく露はバイオマーカーを介して評価された。一般的な有 機リン剤へのばく露は3つの研究で、DDTへのばく露は2つの研究で評価され、trans-ノナクロル、ヘキ サクロロベンゼン、2,4,6-トリクロロフェノール (TCP) はそれぞれ1つの研究で評価された。このよう に、同等の影響の定義または同一の比較単位を用いた4 つ以上の研究では、単一の農薬と関連するバ イオマーカーは評価されておらず、定量的な統合は行われなかった。この分野で最大の研究は、国民健 康・栄養調査(NHANES)の報告書(ID 17)で、1999年から2004年のNHANESのデータを使用して、6歳か ら15歳の小児2546人の間で尿中トリクロロフェノール (TCPs)と親が報告したADHDとの関連を評価し

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(>3.58 mg/g) of urinary 2,4,6-Trichlorophenol (TCP) had a higher risk of parent-reported ADHD compared to children with levels below the limit of detection (OR 1.54, 95% CI 0.97 to 2.43 and OR 1.77, 95% CI 1.18 to 2.66, respectively; p for trend=0.006) after adjusting for covariates.

Our systematic review did not identify any previously published meta-analyses on ADHD to allow for comparisons with previously published evidence (prior to 2006). Generally the results on ADHD were of small effect and not statistically significant with few exceptions. Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and ADHD.

#### 13.3. Neurodevelopment

Thirty-one studies assessed the association between pesticide exposure and aspects of neurodevelopment with a sample size ranging from 25 to 1,041 contributing 325 separate extracted comparisons in the database. Only one study assessed neurodevelopmental aspects in Asian children; all the rest pertained to populations of European ancestry. Seventy-four percent of the studies were cohort studies and, in 27 studies the exposure was assessed through a biomarker. A large variety of individual pesticides were assessed with the general category of organophosphate pesticides being assessed more frequently (Table 16). No single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. Actually, the assessment of neurodevelopment, as seen for cognitive function, is another typical example of a general outcome category where the multiplicity and complexity of the 35 tools and sub-tools used (Table 17) renders the attempt to systematically and quantitatively synthesize the results of the published literature fruitless.

The largest study in the field is a Collaborative Perinatal Project report (ID MPD 029) assessing inutero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. The authors report that although levels of DDT and DDE were relatively high in this population (median DDT concentration, 8.9 g/L; DDE, 24.5 g/L), neither were related to Mental or Psychomotor Development scores on the Bayley Scales nor to Full-Scale Intelligence Quotient at 7 years of age.

Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on neurodevelopmental outcomes were of small effect and not statistically significant with few exceptions. Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

#### Table 15: Summary of studies and mental and psychomotor development outcomes

Outcome group	N analyses
Attention Deficit Hyperactivity Disorder (ADHD)	102
Autism	2

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ている。著者らの報告によると、尿中の2,4,6-トリクロロフェノール (TCP) の濃度が低値 (3.58 mg/g 未満)及び高値 (3.58 mg/g以上) の小児は、検出限界値未満の小児に比べて、親に報告されたADHDの リスクが高かった (OR 1.54、95%CI 0.97~2.43、OR 1.77、95%CI 1.18~2.66、それぞれpはトレン ド=0.006)。

我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を可能 にするために、ADHDに関する以前に発表されたメタアナリシスは確認しなかった。一般的にADHDに関 する結果は効果が小さく、少数の例外を除いて統計的に有意ではなかった。したがって、多数の分析を 考慮すると、これらの結果は慎重な解釈が必要であり、これらのデータに基づいて、農薬ばく露とADHD との関連を示唆するエビデンスはない。

## 13.3. 神経発達

31件の研究が農薬ばく露と神経発達の関連を評価しており、サンプルサイズは25~1,041で、データ ベースには325件の個別比較が掲載されている。アジア系の小児の神経発達を評価した研究は1件のみ であり、その他の研究はすべてヨーロッパ系の集団を対象としたものであった。研究の74%はコホー ト研究で、27の研究ではパイオマーカーを用いてばく露が評価されていた。個々の農薬の評価は多種 多様で、有機リン系農薬一般というカテゴリーがより頻繁に評価されている(表16)。比較可能な影響 の定義または同一の比較単位を用いた4件以上の研究では、単一の農薬と関連するバイオマーカーの 評価は行われていないため、定量的な統合は行われていない。実際、神経発達の評価は、認知機能に見 られるように一般的な影響カテゴリーのもう一つの典型的な例であり、使用されている35のツール とサブツールの多様性と複雑性(表17)は、公表されている文献の結果を体系的かつ定量的に統合し ようとする試みを無意味なものにしている。

この分野で最大の研究は、Collaborative Perinatal Projectの報告書(ID MPD 029)であり、ジク ロロジフェニルトリクロロエタンへの胎内ばく露と乳児及び学童期の小児の認知発達を評価している。 著者らの報告によると、この集団では DDT と DDE の濃度は比較的高かったが(DDT 濃度中央値 8.9 g/L、DDE 24.5 g/L)、7歳時のベイリー尺度の精神・精神運動発達スコアやフルスケール知能指数のい ずれにも関連していなかった。

我々のシステマティックレビューでは、2006年以前に発表されたエビデンスとの比較を可能にする ために、これらの結果に関する過去に発表されたメタアナリシスは確認しなかった。一般的に、神経発 達の影響に関する結果は、ほとんどの例外を除いて効果が小さく、統計的に有意ではなかった。したが って、多くの分析が行われたことを考えると、これらの結果は慎重に解釈する必要があり、これらのデ ータに基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

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Intelligence Quotient (IQ)	13
Learning disability	4
Cognitive disorders	20
Mental and psychomotor development	318
Pervasive developmental disorder	3

 Table 16:
 Pesticides assessed in neurodevelopmental aspects

Pesticide assessed	N analyses
DDT	81
Chlordecone	5
Chlorpyrifos	8
Hexachlorobenzene (HCB)	5
Insecticides	6
Malathion	8
Mirex	13
Organochlorine pesticides	2
Organophosphate and carbamate pesticide	7
Organophosphate pesticides	115
Pesticides	80
Piperonyl butoxide	1

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# 表15:研究の概要と精神及び精神運動発達影響

影響	分析数
注意欠陥多動性障害 (ADHD)	102
自閉症	2
知能指数	13
学習障害	4
認知障害	20
精神・精神運動発達	318
広汎性発達障害	3

# 表16:神経発達の観点で評価された農薬

評価した農薬	分析数
DDT	81
クロルデコン	5
クロルピリホス	8
ヘキサクロロベンゼン (HCB)	5
殺虫剤	6
マラチオン	8
マイレックス	13
有機塩素系農薬	2
有機リン系・カーバメート系殺虫剤	7
有機リン系農薬	115
農薬	80
ピペロニルブトキシド	1

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 Table 17:
 Outcome definitions and tools used in the 31 studies assessing neurodevelopment

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Outcome definition / Tool used
Accuracy, impulse control
Ages and Stages Questionnaire
Behavioral Assessment and Research System (BARS)
Bayley Psychomotor Development Index Scales for Infants
Bayley Mental Development Index Scales for Infants
Beery-Buktenica VMI developmental test
Benton Visual Retention Test (BVRT)
Box test
Brazelton neonatal behavioral assessment
Brunet-Lezine scale of psychomotor development
Children's Memory Scale
combining the Picture
Completion, Codin
Continuous Performance Test (CPT)
Digit Span
Fagan test of infant intelligence (FTII)
Finger Tapping Task
Gesell Developmental Schedules
Graham-Rosenblith test
Griffiths Mental Developmental Scale
Hit reaction time
Large-pellet test
McCarthy Scales of Children's Abilities
Mullen Scales of Early Learning: AGS Ed
Performance on Continuous Performance Test (CPT)
Raven Test
Santa Ana Form Board
Score in Lincoln-Oseretsky Motor
Small-pellet test
Stanford-Binet Copying Test
Teller visual Acuity Card (TAC) test
Trail Making
University of California Berkeley Preferential Looking Test
Wechsler Intelligence Scale for children
Wisconsin Card Sorting Test
wisconsin dara sorting fest

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影響の定義・使用ツ	
正確性、衝動制御〔』	Accuracy, impulse control]
年齢・段階別の質問	紙 [Ages and Stages Questionnaire]
行動評価研究システ	ム [Behavioral Assessment and Research System (BARS)]
乳幼児のためのベイ	リー精神運動発達指数尺度 [Bayley Psychomotor Development Index Scales for
Infants]	
乳幼児のためのベイ	リー精神発達指数尺度 [Bayley Mental Development Index Scales for Infants]
ベアリーブクテニカ	VMI 発達検査 [Beery-Buktenica VMI developmental test]
ベントン視覚記銘検	査 [Benton Visual Retention Test (BVRT)]
ボックステスト [Bo:	x test]
ブラゼルトン新生児	行動評価 [Brazelton neonatal behavioral assessmen]
ブルネ・レジン精神	運動発達尺度 [Brunet-Lezine scale of psychomotor development]
小児の記憶力尺度 [(	Children's Memory Scale]
イメージ連結 [Comb	ining the Picture]
完成、Codin [Comple	etion, Codin]
持続的パフォーマン	ステスト [Continuous Performance Test (CPT)]
数列暗唱 [Digit Spa	an]
ファーガンテスト(乳	L児知能)[Fagan test of infant intelligence (FTII)]
フィンガータッピン	グタスク [Finger Tapping Task]
ゲゼル発達スケジュ	ール [Gesell Developmental Schedules]
グラハムローゼンブ	リット検査 [Graham-Rosenblith test]
グリフィス精神発達	尺度 [Griffiths Mental Developmental Scale]
ヒット反応時間 [Hi	t reaction time]
大型ペレット検査 []	Large-pellet test]
マッカーシーの小児	能力尺度 [McCarthy Scales of Children's Abilities]
早期学習の Mullen ス	ドケール:AGS Ed [Mullen Scales of Early Learning: AGS Ed]
持続的パフォーマン	ス検査(CPT)のパフォーマンス[Performance on Continuous Performance Test]
レーヴン検査 [Raver	n Test]
Santa Ana Form Boa	rd
Lincoln-Oseretsky !	Motor スコア [Score in Lincoln-Oseretsky Motor]
小型ペレット検査 [	Small-pellet test]
スタンフォードビネ	ーコピー検査 [Stanford-Binet Copying Test]
	TAC) 検査 [Teller visual Acuity Card (TAC) test]
トレイルメイキング	
	バークレー校の選好注視テスト [University of California Berkeley Preferential
Looking Test]	
ウェクスラー小児知	能検査 [Wechsler Intelligence Scale for children]
	ド分類テスト [Wisconsin Card Sorting Test]

#### 14. Respiratory diseases

Overall, 29 publications examined the effect of pesticide exposure on respiratory outcomes (median sample size: 249, IQR 126-1728), contributing 399 separate analyses in the *data extraction database*. Sixty-seven percent came from Europe and America, while ten health-related outcomes were assessed with a large proportion focusing on asthma (N=9). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. Only 6 out of the 29 publications referred to prospective cohort studies and 12 were cross-sectional studies. The sample size in the reported analyses was often small; it ranged between 35 and 47,756 participants with the largest study being the Singapore Chinese Health Study. Here, we also observed large clusters of publications coming from large, well-known studies in the field, such as the AHS (6 publications), while 17 studies (68%) assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was less prominent here (N=8, 34%) while 1 study assessed occupational exposure through JEM. The different outcome categories examined are presented in Table 18 along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, statistical synthesis of the data (meta-analysis) was only performed for asthma.

#### 14.1. Respiratory outcomes with few studies

With the exception of asthma, for all the remaining assessed outcomes included in Table 18, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as idiopathic pulmonary fibrosis, or sarcoidosis, as well as a numbers of biomarkers such as forced expiratory volume (FEV). Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the fact that most of the results come from the Agricultural Health Study (AHS), these results need cautious interpretation and, based on these data, there is no evidence to suggest a robust association between pesticide exposure and these outcomes.

## 14.2. Asthma

Nine studies assessed the association between pesticide exposure and asthma with a median sample size of 402 (IQR 127-724), contributing 196 separate extracted comparisons in the database. More than half of the studies were cross-sectional and in more than two-thirds of the studies, the exposure was assessed through a questionnaire. A large variety of individual pesticides were assessed with DDT, paraquat and chlorpyrifos being assessed more frequently. With the exception of DDT, chlorpyrifos and paraquat (Table 19), no other single pesticide and related biomarker was assessed in more than 4 studies using the same comparison unit, thus a quantitative synthesis was not performed.

When we attempted to investigate the association between exposure to DDT and asthma across the 5 available studies, the observed effect was statistically significant without indications of heterogeneity (OR 1.29, 95% CI 1.14 - 1.45, I<sup>2</sup> 0%) (Figure 32). We then attempted to investigate the association between exposure to paraquat and asthma across the 6 available studies and the observed effect was not statistically significant with indications of heterogeneity (OR=1.40, 95%CI=0.95-2.06, I<sup>2</sup>=53%) (Figure 33). We finally attempted to investigate the association between exposure to chlorpyrifos and

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#### 14. 呼吸器疾患

全体では、29の出版物が呼吸器影響に対する農薬ばく露の効果を調査しており(サンプルサイズ中 央値:249、IQR 126-1728)、データ抽出データベースでは399の個別の分析が行われた。そのうち67% はヨーロッパとアメリカからのもので、10の健康関連影響が評価されており、その中でも特に喘息に 焦点を当てた影響が多かった(N=9)。他の影響に見られるように、ばく露の定義はかなりまちまちで あり、データ統合に特別な問題をもたらしている。29の出版物のうち、前向きコホート研究に言及して いるのは6件のみで、12件は横断研究であった。報告された分析におけるサンプルサイズはしばしば小 さく、35~47,756人の範囲であり、最大の研究はSingapore Chinese Health Studyであった。ここで は、AHS (6件)のようなこの分野でよく知られた大規模な研究からの出版物の大規模なクラスタも観察 され、17件(68%)の研究が職業的ばく露を評価していた。さらに、ばく露のバイオマーカーに関する 情報を持つ研究の存在は、ここではあまり目立たなかった(N=8、34%)が、1件の研究ではJEMによる 職業的ばく露を評価していた。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究 の数と定量的統合の決定とともに表 18 に示した。データの不均一性と同定された研究数が少ないた め、データの統計的統合(メタアナリシス)は喘息についてのみ実施した。

## 14.1. 研究数が少ない呼吸器影響

これらの影響は、特発性肺線維症やサルコイドーシス、努力性呼気量(FEV)などの多数のバイオマ ーカーに加えて、研究数が少なすぎるが、明確に定義された臨床所見まで、様々な情報を収集したもの で構成されている。我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以 前)との比較を可能にするために、これらの影響に関する以前に発表されたメタアナリシスは確認し なかった。一般的に、これらの影響に関する結果は効果が小さく、統計的に有意ではなかったが、少数 の例外を除いては有意であった。分析数が多く、結果のほとんどが農業健康調査(Agricultural Health Study: AHS)からのものであることを考えると、これらの結果は慎重に解釈する必要があり、これらの データに基づいて、農薬ばく露とこれらの結果との間に妥当な関連を示唆するエビデンスはない。

## 14.2. 喘息

9件の研究が農薬ばく露と喘息との関連を評価しており、サンプルサイズの中央値は402 (IQR 127-724) で、データベースには196件の比較が抽出されている。半数以上の研究が横断的に行われ、3分の 2以上の研究ではばく露は質問紙で評価されていた。個々の農薬の評価は多岐にわたり、DDT、パラコー ト、クロルピリホスがより頻繁に評価されている。DDT、クロルピリホス、パラコートを除いて(表 19)、 同じ比較単位を用いた 4 件以上の研究では、他の単一の農薬と関連するバイオマーカーは評価されて おらず、定量的な統合は行われていない。

利用可能な 5 つの研究で DDT へのばく露と喘息との関連を調査しようとしたところ、観察された 影響は不均一性を示すことなく統計的に有意であった (OR 1.29、95%CI 1.14~1.45、I2 0%) (図 32)。 次に、利用可能な6つの研究について、パラコートへのばく露と喘息との関連を調査しようとしたが、 観察された効果は、不均一性を示すもので統計的に有意ではなかった (OR=1.40、95%CI=0.95-2.06, I2=53%) (図33)。最後に、利用可能な5つの研究でクロルピリホスへのばく露と喘息との関連を調査 しようとしたが、観察された効果は、不均一性を示し統計的に有意ではなかった (OR=1.03、95% CI=0.82-1.28、I2=0%) (図34)。メタアナリシスの結果は主にAHSによるものであること、また、男 女別、アレルギー性・非アレルギー性喘息別に報告されているため、4つがAHSに属していることに注 意が必要である。また、メタアナリシスの結果は2006年以降に発表されたデータに限定されているこ とも認めている。したがって、DDTについては、これらの農薬へのばく露と喘息との間に統計的に有意 な中等度の関連を示唆する最近のエビデンスがあると結論付けたが、クロルピリホスとパラコートに ついてはそうではない。

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asthma across the 5 available studies and the observed effect was not statistically significant without indications of heterogeneity (OR= 1.03, 95% CI= 0.82–1.28,  $I^2$ =0%) (Figure 34). We caution that the meta-analyses results are largely driven by the AHS; in the meta-analyses 4 entries belong to the AHS as the results were separately reported for men and women and for allergic and non-allergic asthma. We also acknowledge that the results of the meta-analyses are restricted to data published after 2006. We thus conclude that for DDT, but not for chlorpyrifos and paraquat, there is recent evidence to suggest a statistically significant, moderate association between exposure to this pesticides and asthma.

 Table 18:
 Summary of studies and outcomes in the field of respiratory medicine (N/A: not available)

Outcome Group	N studies	Meta-analysis performed	Previous published meta-analysis
Cough	2	No	N/A
Breathlessness	1	No	N/A
Cough/Phlegm	2	No	N/A
Volume that has been exhaled at the end of the first second of forced expiration (FEV <sub>1)</sub>	1	No	N/A
FEV <sub>1</sub> / Forced vital capacity (FVC)	2	No	N/A
Asthma	9	Yes	N/A
Chronic bronchitis	5	No	N/A
Hypersensitivity pneumonitis	2	No	N/A
Lower respiratory tract infection	2	No	N/A
Sarcoidosis	1	No	N/A
Wheeze	2	No	N/A

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# 表 18:呼吸器医学の研究と影響のまとめ(N/A:なし)

影響	研究数	メタアナリシス実施	以前に発表されたメタア ナリシス
咳	2	No	N/A
息苦しさ	1	No	N/A
咳・痰	2	No	N/A
1 秒量(FEV1)	1	No	N/A
FEV1 / 努力性肺活量 (FVC)	2	No	N/A
喘息	9	Yes	N/A
慢性気管支炎	5	No	N/A
過敏性肺炎	2	No	N/A
下気道感染症	2	No	N/A
サルコイドーシス	1	No	N/A
異常呼吸音	2	No	N/A

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Characteristics of the associations eligible for meta-analysis

Table 19:

D		Location	Study design	Exposure type	Exposure assessment	Comparison	Adjustment	Sample size
DDT								
RESP_002	2006	Europe	Cohort	Environmental	Biomarker	Yes/no	++++	402
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++++	936
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	946
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++++	4391
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	4468
Paraquat								
RESP_019	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	134
RESP_022	2012	Asia	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	125
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	+++++++++++++++++++++++++++++++++++++++	292
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	294
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	3096
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	3108
Chlorpyrifos	S							
RESP_019	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	134
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	1017
RESP_004	2008	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	1019
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	2174
RESP_006	2009	America	Cross-sectional	Occupational	Questionnaire	Yes/no	++++	2199

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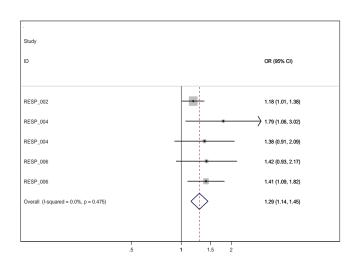
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表19:メタアナリシスの対象となる関連付けの特徴

ID	年	場所	研究 デザイン	ばく露 タイプ	ばく露評価	比較	調整	サンプル サイズ
DDT								
RESP_002	2006	ヨーロッパ	コホート	環境	バイオマーカー	Yes/no	+++	402
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	936
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	946
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	4391
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	4468
パラコート								
RESP_019	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	134
RESP_022	2012	アジア	横断	職業	質問紙	Yes/no	+++	125
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	292
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	294
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	++++	3096
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	3108
クロルピリ	ホス							
RESP_019	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	134
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	1017
RESP_004	2008	アメリカ	横断	職業	質問紙	Yes/no	+++	1019
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	2174
RESP_006	2009	アメリカ	横断	職業	質問紙	Yes/no	+++	2199

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**Figure 32**: Fixed-effects meta-analysis for studies with information on DDT exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

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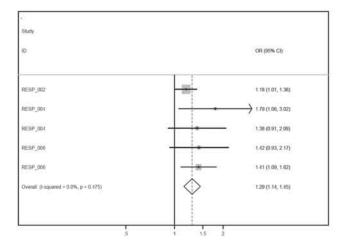


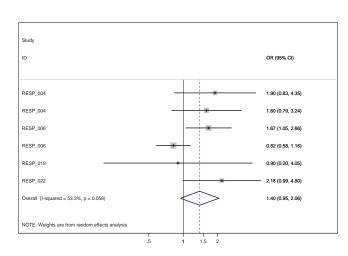
図 32: DDT ばく露に関する情報と喘息のリスクの固定効果メタアナリシス(研究 6 と 10 は農業健康研究の出版物を参照)

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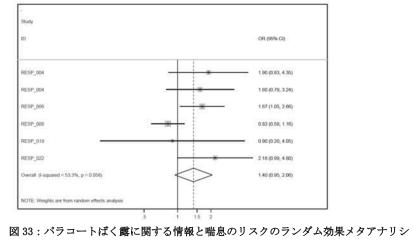
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**Figure 33:** Random-effects meta-analysis for studies with information on paraquat exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

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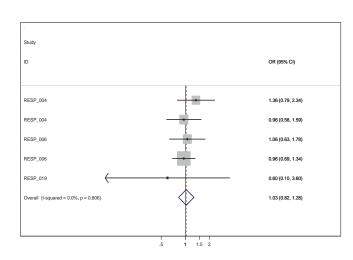
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**Figure 34**: Fixed-effects meta-analysis for studies with information on chlorpyrifos exposure and risk of any type of asthma (Studies 6 and 10 refer to Agricultural Health Study publications)

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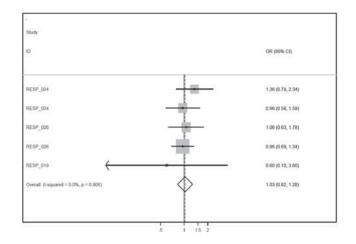


図 34: クロルピリホスばく露に関する情報と喘息のリスクの固定効果メタアナリシ ス(研究6と10は農業健康研究の出版物を参照)

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### 15. Neuropsychiatric diseases

Overall, 15 publications examined the effect of pesticide exposure on neuropsychiatric outcomes in adult populations (median sample size: 596, IOR 158-12,263), contributing 358 separate analyses in the data extraction database. Three-quarters came from Europe and America, while 17 health-related outcomes were assessed with a large proportion focusing on cognitive function (9 studies, 246 analyses). As seen with other outcomes, the diversity of the exposure definition is considerable and poses special challenges to data synthesis. Only 2 out of the 15 publications referred to prospective cohort studies and 60% of the publications were cross-sectional studies. The sample size in the reported analyses was often small; it ranged between 66 and 112,683 participants with the largest study being a retrospective American study. Here, we also observed clusters of publications coming from large, well-known studies in the field, such as the Agricultural Health Study (AHS) (4 publications), while all but one study assessed occupational exposures. In addition, the presence of studies with information on biomarkers of exposure was far less prominent here (n=2, 13%). The different outcome categories examined are presented in Table 20, along with the number of studies contributing to each outcome category and a decision on quantitative synthesis. Due to heterogeneity of data and small number of studies identified, no statistical synthesis of the data (meta-analysis) was performed for any outcome.

# 15.1. Cognitive function

Nine studies assessed the association between pesticide exposure and cognitive function with a median sample size of 80 (IQR 141-205), contributing 246 separate extracted comparisons in the database. All but one of the studies were cross-sectional and, in seven studies the exposure was assessed through a questionnaire. A large variety of individual pesticides were assessed with the general category of organophosphate pesticides being assessed more frequently. No single pesticide and related biomarker was assessed in more than 4 studies using comparable outcome definitions or the same comparison unit, thus a quantitative synthesis was not performed. Actually, the assessment of cognitive function is a typical example of a general outcome category where the multiplicity and complexity of the 62 tools and sub-tools used in the 15 available studies (Table 21) renders the attempt to systematically and quantitatively synthesize the results of the published literature fruitless.

The largest study in the field is an AHS report (ID NPD 014) assessing potential associations between long-term pesticide use and neurobehavioral function, with relevant tests administered to licensed pesticide applicators. The authors report that "test performance was associated with lifetime days of use of some pesticides". Ethoprop was significantly associated with reduced performance on a test of motor speed and visual scanning. Malathion was significantly associated with poor performance on a test of five organophosphate pesticides. Specifically, chlorpyrifos, coumaphos, parathion, phorate, and tetrachlorvinphos were associated with better verbal learning and memory; coumaphos was associated with better performance on a test of motor speed and visual scanning; and parathion was associated with better performance on a test of sustained attention. Overall, we found no consistent evidence of an association between organophosphate pesticide use and adverse test performance among this older sample of pesticide applicators. Potential reasons for these mostly null results include a true absence of effect as well as possible selective participation by healthier applicators.

Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on neuropsychiatric outcomes were of small effect and not statistically significant with few exceptions.

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### 15. 神経精神疾患

全体では、15の出版物が成人集団の神経精神影響に対する農薬ばく露の影響を調査しており(サン プルサイズ中央値:596、IQR158-12263)、データ抽出データベースでは358の個別の分析が行われてい る。4分の3はヨーロッパとアメリカの研究者であり、17の健康関連影響が評価され、大部分が認知機能 に焦点を当てていた(9研究、246の分析)。他の影響に見られるように、ばく露の定義はかなりまちま ちであり、データ統合に特別な問題を与えている。15の出版物のうち、前向きコホート研究に言及して いるのは2件のみで、出版物の60%は横断研究であった。報告された分析におけるサンプルサイズはし ばしば小さく、66~112,683人の範囲であり、最大の研究は米国の後ろ向き研究であった。ここでは、 農業健康調査(Agricultural Health Study、AHS)(4件)のようなこの分野でよく知られた大規模な 研究からの出版物のクラスタも観察されたが、1件を除いてすべての研究が職業ばく露を評価していた。 さらに、ばく露のバイオマーカーに関する情報を有する研究の存在は、ここではあまり目立たなかっ た(n=2、13%)。調査したさまざまな影響カテゴリーを、各カテゴリーに寄与した研究の数と定量的 統合の決定とともに表20に示した。データの不均一性と同定された研究数が少ないため、どの影響に ついてもデータの統計的統合(メタアナリシス)は行われていない。

# 15.1. 認知機能

9件の研究が農薬ばく露と認知機能との関連を評価しており、サンプルサイズの中央値は80(IQR 141-205)で、データベースには246件の比較が抽出されている。1件を除くすべての研究が横断的で、 7件の研究では質問紙でばく露が評価されていた。個々の農薬の評価は多種多様で、有機リン系農薬の 一般的なカテゴリーがより頻繁に評価されていた。比較可能な影響の定義や同じ比較単位を用いた4件 以上の研究では、単一の農薬と関連するバイオマーカーの評価は行われておらず、定量的な統合は行 われていない。実際、認知機能の評価は一般的な影響カテゴリーの典型的な例であり、利用可能な 15 の研究(表21)で使用された62のツールとサブツールの多様性と複雑性のため、公表されている文献の 結果を体系的かつ定量的に統合する試みは実りのないものとなっている。

この分野で最大の研究は、農薬の長期使用と神経行動機能との間の潜在的な関連を評価したAHSの報告書(ID NPD 014)であり、農薬散布者にこれらに関連する検査を行っている。著者らは、「検査結果は一部の農薬の生涯使用日数と関連していた」と報告している。エトプロプは、運動速度と視覚走査のテストのパフォーマンス低下と有意に関連していた。マラチオンは、視覚的走査と処理のテストのパフォーマンス低下と有意に関連していた。逆に、5種類の有機リン系農薬では、検査結果の有意な改善が観察された。具体的には、クロルビリホス、クマホス、パラチオン、ホレート、テトラクロルビンホスは言語学習と記憶力の向上と関連しており、クマホスは運動速度と視覚走査のテストの成績向上と関連しており、パラチオンは持続注意力のテストの成績向上と関連していました。全体的に、有機リン系農薬の使用とテスト成績低下との間には、この高齢の農薬使用者のサンプルでは一貫した関連のエビデンスは見られなかった。これらのほとんどが無効な結果となった理由としては、真の効果がないことや、より健康的な農薬使用者が選択的に参加している可能性が考えられる。

我々のシステマティックレビューでは、以前に発表されたエビデンス(2006年以前)との比較を可能 にするために、これらの結果に関する以前に発表されたメタアナリシスは確認しなかった。一般的に、 神経精神影響に関する結果は効果が小さく、統計的に有意ではなかったが、いくつかの例外を除いて

Thus, given the large number of analyses these results need cautious interpretation and, based on these data, there is no evidence to suggest association between pesticide exposure and these outcomes.

### 15.2. Neuropsychiatric outcomes with few studies

With the exception of cognitive function, for all the remaining assessed outcomes included in Table 20, too few studies are available to allow synthesis of evidence for each outcome alone; these outcomes comprise a variety of captured information ranging from well-defined clinical entities yet with too few studies, such as depression, or obsessive-compulsive disorder, as well as a numbers of outcomes representing neuropsychiatric endo-phenotypes such as hostility or orientation disorders. Our systematic review did not identify any previously published meta-analyses on these outcomes to allow for comparisons with previously published evidence (prior to 2006). Generally the results on these outcomes were of small effect and not statistically significant with few exceptions. Given the large number of analyses and the fact that a number of the results come from the AHS, these results need cautious interpretation and, based on these outcomes.

 Table 20:
 Summary of studies and neuropsychiatric outcomes

Outcome group	N studies
Anxiety	3
Attention and calculation disorders	1
Cognitive function	9
Depression	4
Electroencephalographic (EEG) state	1
Hostility	1
Interpersonal sensitivity diosrder	1
Learning disability	1
Nausea	1
Neuropsychiatric symptoms	3
Obsessive-compulsive disorder	1
Orientation disorders	1
Paranoid ideation	1
Psychotisism	1
Rapid Eye Movement (REM) Sleep Behavior	1
Disorders (RBD)	
Somatization	1
Suicide commitment	3

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# は有意であった。

したがって、多数の分析を考慮すると、これらの結果は慎重に解釈する必要があり、これらのデータ に基づいて、農薬ばく露とこれらの影響との関連を示唆するエビデンスはない。

# 15.2. 研究数が少ない神経精神影響

これらの影響は、うつや強迫性障害のような明確に定義されているが研究数が少なすぎる臨床所見 から、また敵意や見当識障害のような神経精神医学的な中間形質を表す影響も多数含まれている。我々 のシステマティックレビューでは、以前に発表されたエビデンス(2006 年以前)との比較を可能にす るために、これらの影響に関する以前に発表されたメタアナリシスは確認しなかった。一般的に、これ らの影響に関する結果は効果が小さく、統計的に有意ではなかった(少数の例外を除く)。分析の数が 多く、結果の多くが AHS からのものであることを考えると、これらの結果は慎重に解釈する必要があ り、これらのデータに基づいて、農薬ばく露とこれらの結果との間の妥当な関連を示唆するエビデン スはない。

# 表 20:研究と神経精神影響のまとめ

	研究数
不安	3
注意力・計算障害	1
認知機能	9
うつ	4
脳波 (EEG) の状態	1
敵意	1
対人感受性障害	1
学習障害	1
吐き気	1
神経精神症状	3
強迫性障害	1
見当識障害	1
被害妄想的なイデオロギー	1
精神病傾向	1
REM 睡眠行動障害(RBD)	1
身体化	1
自殺	3

Table 21:Outcome definitions and tools used in the 15 studies assessing cognitive function(BARS: Behavioral Assessment and Research System, AVLT:Auditory Verbal Learning Test, BVFT:Benton Visual Form Discrimination Test CALCALP: California Computerised Assessment PackageManual, WAIS: Wechsler Adult Intelligence Scale, WMS: Wechsler Memory Scale)

Outcome definition / Tool used			
% Correct rejects (BARS)	Selective attention latency (BARS)		
% Hits (BARS)	Selective attention trials (BARS)		
Recall (AVLT)	Sequences A test performance (seconds)		
Recognition (AVLT)	Sequences B test performance (seconds)		
Total recall (AVLT)	Serial digit learning task (BARS)		
Benton Visual Form Discrimination Test (BVFT)	Serial Digit Learning Test		
Block design test	Simple Reaction Time Test (ms)		
CALCAP choice test	Spatial span test		
Continuous Performance Test Score (m/s)	Stroop test		
Counting errors	Summary index (BARS)		
Digit span backward task (BARS)	Symbol Digit Substitution Test (s)		
Digit span forward task (BARS)	Symbol-digit latency task (BARS)		
Digit-Symbol test score (seconds)	Symptom Checklist 90 revised (SCL-90-R)		
False alarm latency (BARS)	Trails B test		
Fine motor control test	Verbal fluency test		
Finger tapping (preferred hand) (BARS)	WAIS-III picture arrangement test		
Finger tapping , dominant hand (BARS)	WAIS-III arithmetic test		
Finger tapping, (nonpreferred hand) (BARS)	WAIS-III comprehension test		
Finger tapping, alternating hand (BARS)	WAIS-III digit span test		
Graded naming test	WAIS-III digit symbol test		
Grooved pegboard, dominant hand score	WAIS-III full scale IQ		
Hit latency (BARS)	WAIS-III graded-naming test		
Match-Sample (BARS)	WAIS-III similarities test		
N100 latency (ms)	WAIS-III vocabulary test		
N200 latency (ms)	WMS-III auditory delayed memory test		
P200 latency (ms)	WMS-III auditory immediate memory test		
P300 amplitude (μν), Cz	WMS-III auditory recognition test		
P300 latency (ms)	WMS-III letter-number test		
Progressive ratio (BARS)	WMS-III visual delayed memory test		
Reaction time latency a (BARS)	WMS-III visual immediate test		
Reaction time latency a (BARS)	Selective attention interstimulus interval (BARS)		

表 21:認知機能を評価する 15 の研究で使用された影響の定義とツール (BARS. 行 動評価研究システム、AVLT:Auditory Verbal Learning Test、BVFT:Benton Visual Form Discrimination Test CALCALP:California Computerised Assessment Package Manual、WAIS:Wechsler Adult Intelligence Scale、 WMS:Wechsler Memory Scale)

影響の定義・使用ツール	
正解率 (BARS)[% Correct rejects]	遥択的注意潜時(BARS)[Selective attention latency]
ヒット数 (BARS) [% Hits]	遥択的注意試験(BARS)[Selective attention trials]
リコール (AVLT)	シーケンス A テスト性能 (秒)
[Recall]	[Sequences A test performance]
認識 (AVLT)	シーケンス B テスト性能 (秒)
[Recognition]	[Sequences B test performance]
トータルリコール (AVLT)	シリアルディジット学習タスク (BARS)
[Total recall]	[Serial digit learning task]
ベントン視覚形態判別テスト (BVFT)	シリアルデジット学習テスト
[Benton Visual Form Discrimination Test]	[Serial Digit Learning Test]
ブロックデザイン(積み木問題) [Block design test]	単純反応時間試験(ms)[Simple Reaction Time Test]
CALCAP選択テスト [CALCAP choice test]	空間スパンテスト [Spatial span test]
連続性能試験スコア (m/s)	ストループテスト
[Continuous Performance Test Score]	[Stroop test]
カウントエラー [Counting errors]	サマリーインデックス (BARS) [Summary index]
数列暗唱逆唱 (BARS) [Digit span backward task]	記号桁置換試験 [Symbol Digit Substitution Test]
数列暗唱順唱 (BARS) [Digit span forward task]	記号桁遅延タスク(BARS)[Symbol-digit latency task]
符号問題のスコア (秒)	症状チェックリスト 90 改訂版 (SCL-90-R)
[Digit-Symbol test score]	[Symptom Checklist 90 revised]
誤報待ち時間 (BARS) [False alarm latency]	トレイルズBテスト [Trails B test]
微細運動制御試験 [Fine motor control test]	言語流暢性テスト [Verbal fluency test]
フィンガータッピング (利き手) (BARS) [Finger tapping]	WAIS-Ⅲ絵画配列 [WAIS-III picture arrangement test]
フィンガータッピング、利き手 (BARS)	WAIS-Ⅲ計算問題
[Finger tapping, dominant hand]	[WAIS-III arithmetic test]
フィンガータッピング、(非利き手) (BARS) [Finger	WAIS-Ⅲ理解力 [WAIS-III comprehension test]
tapping]	
フィンガータッピング、交互に手を動かす (BARS)	WAIS-Ⅲ数列暗唱
[Finger tapping, alternating hand]	[WAIS-III digit span test]
グレーデッドネーミングテスト [Graded naming test]	WAIS-Ⅲ符号問題 [WAIS-III digit symbol test]
溝付きのペグボード、支配的な手のスコア	WAIS-III フルスケール IQ
[Grooved pegboard, dominant hand score]	[WAIS-III full scale IQ]
ヒット潜時 (BARS) [Hit latency]	WAIS-Ⅲ段階的命名試験 [WAIS-III graded-naming test]
マッチサンプル (BARS) [Match-Sample]	WAIS-Ⅲ類似 [WAIS-III similarities test]
N100 潜時 (ms) [N100 latency]	WAIS-Ⅲ語彙 [WAIS-III vocabulary test]
N200 潜時 (ms) [N200 latency]	WMS-Ⅲ 聴覚遅延記憶 [WMS-III auditory delayed memory test
P200 潜時 (ms)	WMS-Ⅲ聴覚即時記憶
[P200 latency]	[WMS-III auditory immediate memory test]
P300 振幅 (μv), Cz [P300 amplitude]	WMS-Ⅲ聴覚認識 [WMS-III auditory recognition test]
P300 の潜時 (ms) [P300 latency]	WMS-Ⅲ文字・数字配列決定 [WMS-III letter-number test]
進歩率 (BARS) [Progressive ratio]	WMS-Ⅲ視覚的遅延記憶 [WMS-III visual delayed memory test]
反応時間潜時 a (BARS) [Reaction time latency a]	WMS-Ⅲ視覚即時試験 [WMS-III visual immediate test]
反応時間潜時 a (BARS)	選択的注意間刺激間隔 (BARS)
[Reaction time latency a]	[Selective attention interstimulus interval]

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# 16. Diabetes

Overall, 23 publications examined the effect of pesticide exposure on diabetes related outcomes (median sample size: 430; IQR 192-1721), contributing 125 separate analyses in the data extraction database. Four health-related outcomes were assessed with a large proportion focusing on type 1 diabetes (n=93, 74%) whereas 18 analyses focused on type 2 diabetes. The rest of the outcomes assessed was prediabetes (n=10), gestational diabetes (n=2) and other glucose and insulin related outcomes (n=2). Only one prospective cohort study was performed; the large majority was crosssectional designs (n=15), whereas 3 studies were case-controls and 4 studies used a nested casecontrols. The large majority of the studies was conducted in America (n=15, 65%) whereas 7 studies where Europeans and only one Asian. Here, we did not observe large clusters of publications coming from large, well-known studies in the field, such as the AHS. Only three study assessed occupational exposures the rest examined environmental exposures (n=19) or both (n=1). In addition, the presence of studies with information on biomarkers of exposure was limited to 9 studies, whereas 10 studies included information both on questionnaire and biomarkers. The different outcome categories examined are presented in Table 22 along with the number of studies contributing to each outcome category. For the pesticides accessed meta-analysis was feasible for DDE and DDT exposure and type 1 diabetes and DDE exposure and type 2 diabetes.

### 16.1. Type 1 diabetes

Thirtheen studies assessed the effect of pesticides on type 1 diabetes (median sample size: 309, IQR: 159-398) and a meta-analysis of ORs was feasible for DDE and DDT exposure. For DDE, 9 studies contributed a median sample size of 202, IQR=142-334. We were not able to include a prospective study that reported a (significant) Incidence Rate Ratio (IRR) of 7.1 and compared the highest vs. the lowest tertile of exposure with DDE. The computed summary OR was 1.90 (95% CI: 1.25-2.86) for the DDE exposure using random effects models. Moderate heterogeneity was observed ( $I^2$ =49%). For DDT, 6 studies had available data for synthesis (median sample size: 577, IQR: 272-2163) providing a summary effect of 1.76 (95% CI: 1.20-2.59) with very large heterogeneity observed (( $I^2$ =76%). Main source of heterogeneity is the different exposure levels used for the calculations of the effect estimates. Even though there is evidence from the random effects meta-analysis that an increased risk for type 1 diabetes exists, however the findings should be interpreted with caution due to the heterogeneity that was observed.

### 16.2. Type 2 diabetes

Four studies were eligible for the assessment of the DDE exposure and risk for type 2 diabetes (median sample size: 471, IQR=292-642). The summary OR derived from those studies was 1.30 (95% CI: 1.13-1.48). No heterogeneity was observed, however the summary results is driven by a case-control study that reported an effect size OR=1.30 (95% CI=1.11-1.52). Even though, there is evidence suggesting that DDE exposure is a risk factor for developing type 2 diabetes, this is based on small studies.

### 16. 糖尿病

全体として、糖尿病関連影響に対する農薬ばく露の効果を調査した論文は23編(サンプルサイズ中 央値:430、IQR 192-1721)で、データ抽出データベースには125の個別の分析結果が掲載されていた。 4つの健康関連影響が評価されており、1型糖尿病(n=93、74%)に大きな割合を占めていたのに対し、 18の分析では2型糖尿病に焦点が当てられていた。評価されたその他の影響は、前糖尿病(n=10)、妊 娠糖尿病(n=2)、その他のグルコース及びインスリン関連影響(n=2)であった。前向きコホート研究 は1件のみで、大多数は横断的デザイン(n=15)であったが、3件は症例対照、4件はコホート内症例対 照を使用した研究であった。大多数の研究はアメリカで行われており(n=15、65%)、7研究はヨーロ ッパ人であり、アジア人は1研究のみであった。ここでは、AHSのような分野でよく知られた大規模な研 究からの出版物の大規模なクラスターは観察されなかった。3つの研究のみが職業ばく露を評価し、残 りは環境ばく露(n=19)またはその両方(n=1)を調査した。さらに、ばく露のバイオマーカーに関す る情報がある研究は9件に限られていたが、10件の研究では質問紙とバイオマーカーの両方の情報が含 まれていた。調査した異なる影響カテゴリーを、各カテゴリーに寄与した研究の数とともに表 22 に 示す。DDE と DDT ばく露と 1 型糖尿病、DDE ばく露と 2 型糖尿病についてはメタアナリシスが可能 であった。

# 16.1.1型糖尿病

1型糖尿病に対する農薬の影響を評価した研究は3件あり(サンプルサイズ中央値309、IQR:159-398)、DDE と DDT ばく露については OR のメタアナリシスが可能であった。DDEについては、9件の 研究がサンプルサイズ中央値202、IQR=142-334であった。我々は、(有意な)罹患率比(IRR)7.1を示 し、DDE被曝の最上位層と最下位層を比較した前向き研究を含めることができなかった。計算された要 約0Rは、ランダム効果モデルを用いたDDEばく露で1.90(95%CI:1.25-2.86)であった。中等度の不均 一性が観察された(I2=49%)。DDTに関しては、6件の研究が統合に利用可能なデータを持っていた(サ ンプルサイズ中央値:577、IQR:27-2-2163)ため、1.76(95%CI:1.20-2.59)の要約効果が得られ、 非常に大きな不均一性が観察された(I2=76%)。不均一性の主な原因は、効果推定値の計算に使用さ れた異なるばく露レベルである。ランダム効果メタアナリシスでは、1型糖尿病リスクの増加が存在す るというエビデンスがあるとはいえ、観察された不均一性のため、この結果は慎重に解釈されるべき である。

# 16.2.2型糖尿病

DDEばく露と2型糖尿病リスクを評価するために4件の研究が対象となった(サンプルサイズ中央値: 471、IQR=292-642)。これらの研究から得られた要約0Rは1.30(95%CI:1.13-1.48)であった。不均 一性は観察されなかったが、要約結果は、効果量0R=1.30(95%CI=1.11-1.52)を報告した症例対照研 究に牽引されている。DDEばく露が2型糖尿病発症のリスク因子であることを示唆するエビデンスはあ るが、これは小規模な研究に基づくものである。

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 Table 22:
 Summary of studies identified per outcome subgroup with more than 4 studies (NA: not available)

Outcome	N studies	Meta-analysis done	Previous meta- analysis result
Type 1 diabetes	13	Yes	NA
Type 2 diabetes	6	Yes	NA
Gestational diabetes	2	No	NA
Insulin/ Glucose tolerance	2	No	NA

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# 表 22:4 研究以上のサブグループごとに確認された研究の概要(NA:利用不可)

影響	研究数	メタアナリシス実施	前回のメタアナリシス結果
1型糖尿病	13	Yes	NA
2型糖尿病	6	Yes	NA
妊娠糖尿病	2	No	NA
インスリン・グルコース耐性	2	No	NA

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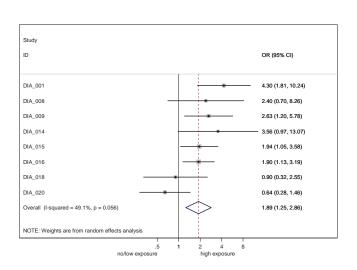


Figure 35: Summary odds ratio (OR) for the association between DDE exposure and type 1 diabetes

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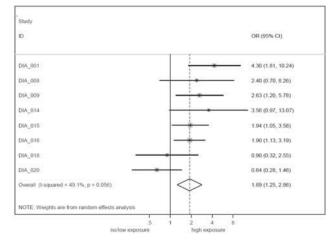


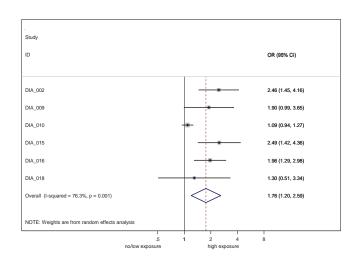
図35:DDEばく露と1型糖尿病との関連のサマリーオッズ比(OR)

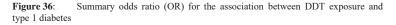
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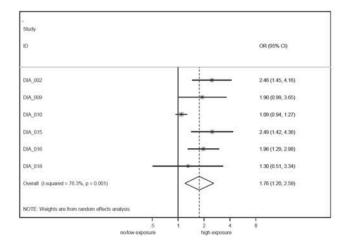


図36:DDTばく露と1型糖尿病との関連のサマリーオッズ比(OR)

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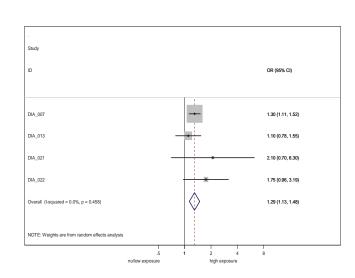


Figure 37: Summary odds ratio (OR) for the association between DDE exposure and type 2 diabetes

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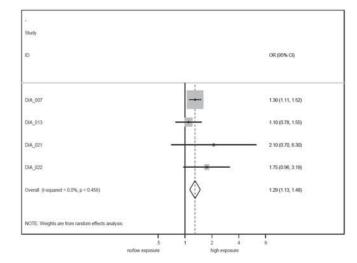


図37:DDEばく露と2型糖尿病との関連のサマリーオッズ比(OR)

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### 17. Cardiovascular diseases

This section includes hard cardiovascular outcomes (myocardial infraction, stroke etc.), cardiovascular risk factors (lipids, blood pressure) and other cardiometabolic outcomes (metabolic syndrome and obesity). No previous meta-analysis has been identified for any of these traits. The evidence collected in this systematic review provides weak suggestions of associations in particular regarding cardiometabolic risk factors and organochlorines; however, other classes of pesticides were not studied and even results on organochlorines were limited and require prospective replication.

#### 17.1. Hard cardiovascular outcomes

Five studies examined hard cardiovascular outcomes including myocardial infarction (ID CVD 005, ID CVD 006), peripheral arterial disease (PAD) (ID CVD 007), stroke (ID CVD 008), and composite cardiovascular disease (ID CVD 009). The Agricultural Health Study (AHS) contributed two prospective analyses (ID CVD 005, ID CVD 006) and National Health and Nutrition Examination Survey (NHANES) other two cross-sectional analyses (ID CVD 007, ID CVD 009). Studies on myocardial infarction (ID CVD 005, ID CVD 006) showed no evidence of an association between having used pesticides, individually or by class, and myocardial infarction mortality among men in the AHS. Similarly, among women of AHS, no overall association with pesticide use and myocardial infarction was seen. Six of 27 individual pesticides evaluated were significantly associated with nonfatal myocardial infarction among women (ID CVD 006), including chlorpyrifos, coumaphos, carbofuran, metalaxyl, pendimethalin, and trifluralin, which all had relatively high odds ratios (>1.7) but also high probability of false positive due to multiple testing.

Another prospective study (8) examined 21 persistent organic pollutants (POPs) in relation to stroke. After adjusting for known stroke risk factors, most polychlorinated biphenyls (PCBs) with 4, 5, or 6 chlorine atoms, p.p'-DDE, trans-nonachlor, and octachlorodibenzo-p-dioxin significantly predicted the risk of stroke. Nonetheless, results need replication from future studies. Peripheral arterial disease (PAD) and composite cardiovascular disease were studied in the cross-sectional NHANES cohort in relation to POPs. Compared with subjects without PAD, those with PAD had significantly higher concentrations of organochlorine pesticides but associations were not seen among non-obese participants. For composite cardiovascular disease, significant associations were observed for chlordane only. These findings need to be carefully interpreted because of the cross-sectional design and use of self-reported cardiovascular disease.

Overall, evidence for associations between pesticide exposure and cardiovascular outcomes is weak and mainly concentrated on organochlorine pesticides.

# 17.2. Cardiovascular risk factors

### 17.2.1. Blood pressure

Five studies examined associations between pesticides and blood pressure (ID CVD 002, ID CVD 003, ID CVD 004, ID CVD 010, ID CVD 011). All but one study (ID CVD 011) had cross-sectional

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### 17. 循環器疾患

重篤な心血管影響(心筋梗塞、脳卒中など)、心血管リスク因子(脂質、血圧)、その他の心血管影響(メタボリックシンドローム、肥満)を含む。これらのいずれについても、これまでのメタアナリシスは同定されていない。この系統的レビューで収集されたエビデンスは、特に心血管リスク因子と有機塩素に関する関連を弱く示唆しているが、他のクラスの農薬は研究されておらず、有機塩素に関する結果でさえ限られており、前向き研究の反復が必要である。

# 17.1. 重篤な心血管影響

心筋梗塞(ID CVD 005、ID CVD 006)、末梢動脈疾患(PAD)(ID CVD 007)、脳卒中(ID CVD 008)、 複合心血管疾患(ID CVD 009)を含む5つの研究が重篤な心血管影響を調査した。Agricultural Health Study (AHS)は2つの前向き分析(ID CVD 005、ID CVD 006)、National Health and Nutrition Examination Survey (NHANES)は他の2つの横断分析(ID CVD 007、ID CVD 009)を提供している。心筋梗塞に関す る研究(ID CVD 005、ID CVD 006)では、AHSの男性の心筋梗塞死亡率と農薬使用(個人またはクラス 別)との関連を示すエビデンスは示されなかった。同様に、AHSの女性では、農薬の使用と心筋梗塞と の全体的な関連は認められなかった。評価された27種類の農薬のうち、クロルビリホス、クマホス、カ ルボフラン、メタラキシル、ペンディメタリン、トリフラリンを含む6種類の農薬が女性の非致死的心 筋梗塞と有意に関連し(ID CVD 006)、いずれも比較的高いオッズ比(1.7以上)を示したが、複数回 の検査による偽陽性の確率も高かった。

別の前向き研究(8)では、脳卒中との関連で21種類の残留性有機汚染物質(POPs)を調査した。既 知の脳卒中リスク因子を調整した後、塩素原子が4、5、または6個のボリ塩化ビフェニル(PCB)、p、 p'-DDE、トランスノナクロル及びオクタクロロジベンゾーp-ジオキシンのほとんどが脳卒中のリスク を有意に予測した。にもかかわらず、結果は今後の研究で再現する必要がある。末梢動脈疾患(PAD) 及び複合心血管疾患が、横断的なNHANESコホートでPOPsとの関連で研究された。PADのない被験者と比 較して、PADのある被験者では有機塩素系殺虫剤の濃度が有意に高かったが、肥満でない被験者では関 連は認められなかった。複合心血管疾患については、クロルデンのみに有意な関連が観察された。横断 的なデザインと自己申告による心血管疾患のため、これらの所見は慎重に解釈する必要がある。

全体的に、農薬ばく露と心血管影響との間の関連エビデンスは弱く、主に有機塩素系農薬に集中している。

# 17.2. 心血管リスク因子

# 17.2.1. 血圧

5件の研究で農薬と血圧の関連が調査された(ID CVD 002、ID CVD 003、ID CVD 004、ID CVD 010、 ID CVD 011)。1件の研究(ID CVD 011)では、横断的な研究が行われていた。

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designs. All effect sizes were very small and not suggestive of an association between pesticide exposure and blood pressure.

### 17.2.2. Metabolic syndrome components

Nine studies examined components of metabolic syndrome in relation to pesticide exposure including lipids levels, glucose and insulin levels. All but one study examined exposure to organochlorine pesticides and significant associations for some classes and lipid levels or glucose levels were observed. Highest quality evidence comes from the prospective Coronary Artery Risk Development in Young Adults (CARDIA) Study (ID CVD 016). In CARDIA, p.p<sup>+</sup>-DDE most consistently predicted higher triglycerides, and homeostasis model assessment value for insulin resistance (HOMA–IR) and lower High Density Lipoprotein (HDL)-cholesterol at year 20 after adjusting for various confounders. Oxychlordane, trans-nonachlor, and hexachlorobenzene also significantly predicted higher triglycerides. Finally, a case-control study in China, examined differences in glucose regulation in participants highly exposed to pyrethroids (occupational exposure) (OR = 1.48, 95%CI = 1.24–1.77) (ID CVD 021). However, these results need external replication in other populations as the study is retrospective and residual confounding cannot be excluded.

### 17.2.3. Subclinical atherosclerosis

The population-based Prospective Investigation of the Vasculature in Uppsala Seniors examined in a cross-sectional study, whether POP levels were related to subclinical atherosclerosis. Circulating levels of PCBs were associated with atherosclerotic plaques and echogenicity of the intima-media complex independent of cardiovascular risk factors, but associations need to be confirmed in prospective studies.

# 17.3. Metabolic syndrome and obesity

Three studies (ID CVD 010, ID CVD 011) examined associations between organochlorine exposure and prevalence of metabolic syndrome. In National Health and Nutrition Examination Survey (NHANES) (ID CVD 010) significant association between organochlorine exposure and prevalence of Metabolic Syndrome was reported with ORs of 1.0, 1.5, 2.3 and 5.3 across organochlorine pesticide quartiles (p for trend <0.01). In the other case-control study (ID CVD 011) significant associations were noted for heptachlor only.

Overall, 12 cross-sectional studies examined associations between pesticide exposure and measures of body fatness or obesity. Also, 10 out of 12 studies examined associations between organochlorines and obesity or body fatness; evidence around other pesticide classes was scarce. Three studies (ID CVD 012, ID CVD 013, ID CVD 014) only presented correlation analysis with measures of body fatness. The remaining studies have shown some significant associations between waist circumference, Body Mass Index (BMI) and organochlorines (DDT and chlordane) but the evidence is limited to cross-sectional analysis and results are only suggestive of an association.

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すべての効果量は非常に小さく、農薬ばく露と血圧との関連を示唆するものではなかった。

# 17.2.2. メタボリックシンドロームの構成要素

9件の研究では、脂質レベル、グルコース、インスリンレベルを含む農薬ばく露に関連したメタボリ ックシンドロームの構成要素が調査された。1件を除くすべての研究で有機塩素系農薬へのばく露が調 査され、いくつかのクラスと脂質レベルまたはグルコースレベルとの有意な関連が観察された。最も 質の高いエビデンスは、前向きCARDIA(Coronary Artery Risk Development in Young Adults)研究 (ID CVD 016)から得られている。CARDIAでは、様々な交絡因子を調整した後、20年目にp,p'-DDEが 最も一貫してトリグリセリド、インスリン抵抗性のホメオスタシスモデル評価値(HOMA-IR)及び高密 度リポ蛋白(HDL)コレステロールの低下を予測していた。また、オキシクロルデン、トランスノナク ロル、ヘキサクロロベンゼンもトリグリセリドの上昇を有意に予測した。最後に、中国で行われた症例 対照研究では、ピレスロイド(職業ばく露)に高度にばく露された参加者のグルコース調節の違いが調 査された。その結果、ピレスロイドにばく露されると異常なグルコース調節のリスクが高まることが 示された(OR = 1.48、95%CI = 1.24-1.77)(ID CVD 021)。しかし、この研究は後ろ向きであり、 残留交絡因子を除外できないため、これらの結果は他の集団で外部で再現の必要がある。

# 17.2.3. 無症候性アテローム性動脈硬化症

集団ベースの Prospective Investigation of the Vasculature in Uppsala Seniors (ウプサラ高 齢者の血管系に関する前向き調査)では、POPのレベルが無症候性アテローム性動脈硬化症と関連して いるかどうかを横断研究で調査した。循環中のPCBレベルは、心血管リスク因子とは無関係に、アテロ ーム性動脈硬化性斑点と内膜複合体の超音波反射性と関連していたが、関連は前向き研究で確認する 必要がある。

# 17.3. メタボリックシンドロームと肥満

3件の研究(ID CVD 010、ID CVD 011)で有機塩素ばく露とメタボリックシンドローム罹患率との関 連を調査した。国民健康・栄養調査(NHANES)(ID CVD 010)では、有機塩素系農薬の四分位間の0Rが 1.0、1.5、2.3、5.3であり、有機塩素ばく露とメタボリックシンドロームの罹患率との間に有意な関連 が報告された(傾向<0.01の場合はp)。他の症例対照研究(ID CVD 011)では、ヘプタクロルのみで 有意な関連が認められた。

全体として、12件の横断研究で農薬ばく露と体脂肪率または肥満度の測定値との関連が調査された。 また、12件中10件の研究では有機塩素系農薬と肥満または体脂肪率との関連が調査されていたが、他 のクラスの農薬についてはエビデンスが乏しかった。3件の研究(ID CVD 012、ID CVD 013、ID CVD 014)では、体脂肪率の測定値との相関分析のみが示された。残りの研究では、ウエスト周囲長、体格 指数(BMI)、有機塩素系農薬(DDTとクロルデン)との間に何らかの有意な関連が示されているが、エ ビデンスは横断的な分析に限られており、結果は関連を示唆するものに過ぎない。

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# 18. Mortality

Overall, 11 publications examined the effect of pesticide exposure on mortality (median sample size: 1,986), contributing 318 separate analyses in the *data extraction database*. This section consists of a heterogeneous group of publications, which assessed associations between pesticides and all cause mortality of major mortality outcomes. Despite the fact that these studies were large, they were of modest quality and they are not very informative as they test a wide range of diseases simultaneously without corrections for multiple testing. The results do not show any apparent trend of pesticide exposure with overall mortality.

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# 18. 死亡率

全体では 11 の論文が農薬ばく露の死亡率への影響を調査し (サンプルサイズ中央値:1,986)、デ ータ抽出データベースでは 318 の個別分析が行われた。このセクションは、農薬と主要な死因との関 連を評価した異種の出版物群で構成されている。これらの研究は大規模なものであったにもかかわら ず、質は中等度であり、多重検定の補正を行わずに種々の疾患を同時に検定しているため、あまり有益 ではなかった。結果は、農薬ばく露と死亡との明らかな傾向を示していない。

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### 19. Immune/ Autoimmune diseases

Overall, 10 publications examined the effect of pesticide exposure on immune disorders (median sample size: 196, IQR 81-476), contributing 67 separate analyses in the *data extraction database*. Sixty studies were conducted in America, 3 in Europe and one study was Asian. Various health related outcomes including arthritis, osteoarthritis, rheumatoid arthritis and an extensive list of various antibodies, cytokines etc. as summarized in Table 23. Seven out of the 10 publications referred to prospective cohort studies whereas 2 studies were cross-sectional and only one was case-control. The sample size in the reported analyses was rather small; it ranged between 19 and 532 participants with the largest study being the Carolina Lupus Study. Half of the studies assess occupational exposures and information on biomarkers of exposure was available in 2 studies whereas 4 studies used both biomarkers and questionnaires. As seen with other outcomes, the diversity of the exposure definition and the outcomes assessed are extensive and poses special challenges to data synthesis. No single outcome was assessed in more than two studies therefore synthesis of the data was not feasible for the field of immune disorders.

# Table 23: Health outcomes assessed in the field of immune disorders

Health outcome	
Antinuclear antibodies	Interleukin-4 (IL-4)
Arthritis	Interleukin-13 (IL-13)
Complement components C3, C4	Immunologic effects
Eosinophils	Leucocyte counts
Erythrocyte counts	Lymphocyte levels
Glycoproteins	Neutrophils
Hematocrit/Hemoglobin	Natural Killers (NK) cells
Interferon-γ (IFN-γ)	Osteoarthritis
Immunoglobulin 1 (IgG1)	Rheumatoid arthritis
Immunoglobulin 4 (IgG4)	Systematic Lupus
Immunoglobulin M (IgM)	

### 19. 免疫疾患/自己免疫疾患

全体では、農薬ばく露が免疫障害に及ぼす影響を調査した論文は 10 (サンプルサイズ中央値 196、 IQR 81-476)で、データ抽出データベースでは 67 の個別の分析が行われた。60の研究はアメリカで 実施され、3つの研究はヨーロッパで実施され、1つの研究はアジアで実施された。関節炎、変形性関節 炎、関節リウマチ、様々な抗体、サイトカインなどの広範なリストを含む様々な健康関連影響が表 23 にまとめられている。10の出版物のうち7つは前向きコホート研究に言及していたが、2つの研究は横 断的であり、1つだけが症例対照であった。報告されている分析のサンプル数はかなり少なく、19~532 人で、最大の研究はCarolina Lupus Studyであった。研究の半分は職業ばく露を評価しており、ばく 露のバイオマーカーに関する情報は2つの研究で得られたが、4つの研究ではバイオマーカーと質問紙 の両方を使用していた。他の影響に見られるように、ばく露の定義と評価された影響はまちまちであ り、データ統合に特別な問題をもたらしている。2件以上の研究で単一の影響が評価されたものはなく、 免疫障害の分野ではデータの統合は不可能であった。

# 表23:免疫障害の分野で評価された健康影響

健康影響	
抗核抗体	インターロイキン-4 (IL-4
関節炎	インターロイキン13(IL-13
補体成分 C3、C4	免疫学的効果
好酸球	白血球数
赤血球数	リンパ球レベル
糖タンパク質	好中球
ヘマトクリット/ヘモグロビン	ナチュラルキラー (NK) 細胞
インターフェロンγ	変形性関節症
免疫グロブリン1 (IgG1)	関節リウマチ
免疫グロブリン4 (IgG4)	全身性エリテマトーデス
免疫グロブリン M (IgM)	

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### 20. Allergic diseases

Nine studies from eight different populations reported associations between pesticide exposure and allergic disorders. Seven studies examined occupational exposure whereas two studies examined environmental exposure. Eight studies were cross-sectional investigations and therefore conclusions are prone to reverse causality and other biases. In terms of outcomes examined, five studies examined self-reported allergic rhinitis, one examined self-reported asthma and the remaining 3 examined selfreported skin irritation, contact dermatitis, food allergy, hay fever and fragrance allergies. Statistically significant results were reported by four studies on allergic rhinitis (ID ALL 003, ID ALL 004, ID ALL 005, ID ALL 006). These studies reported significant association between various pesticide classes and allergic rhinitis. In particular, the Agricultural Health Study (AHS) reported significant association between allergic rhinitis and exposure to the herbicides 2,4-Dichlorophenoxyacetic acid (2,4-D) glyphosate and petroleum oil, the insecticide diazinon and the fungicide benomyl. However, the study has many limitations and results need cautious interpretation and require replication by future prospective studies. The study is limited by its ability to distinguish allergic from non-allergic symptoms of rhinitis and to establish temporality between exposure and symptoms due to its crosssectional design. One study with low overall quality reported high effect sizes (OR, 12.50; 95% CI, 2.00-78.05) for allergic rhinitis in greenhouse flower and ornamental plant growers with pesticide application by hand pump vs. without (ID ALL 006). Again, the study has low overall quality, concerns a heavily exposed population with definition of exposure related to the method of application rather than a chemical class. Overall, the evidence around allergic disorders and pesticide exposure is weak.

### 20. アレルギー疾患

8つの異なる集団における9つの研究が、農薬ばく露とアレルギー性障害との関連を報告した。7件の 研究では職業ばく露が調査され、2件の研究では環境ばく露が調査された。8件の研究は横断的な調査 であったため、結論は逆因果関係やその他のバイアスがかかりやすい。結果については、5件の研究が 自己申告によるアレルギー性鼻炎、1件の研究が自己申告による喘息、残りの3件の研究が自己申告に よる皮膚刺激、接触性皮膚炎、食物アレルギー、花粉症、香料アレルギーを調査した。アレルギー性鼻 炎に関する4件の研究 (ID ALL\_003、ID ALL\_004、ID ALL\_005、ID ALL\_006) で統計学的に有意な結果 が報告された。これらの研究では、様々な農薬クラスとアレルギー性鼻炎との間に有意な関連が報告 されている。特に、Agricultural Health Study (AHS) では、アレルギー性鼻炎と除草剤である2.4-ジ クロロフェノキシ酢酸(2.4-D)グリホサート及び石油油、殺虫剤であるジアジノン及び殺菌剤である ベノミルへのばく露との間に有意な関連が報告されている。しかし、この研究には多くの限界があり、 結果は慎重な解釈が必要であり、将来の前向き研究での再現が必要である。この研究は、アレルギー性 鼻炎と非アレルギー性鼻炎の症状を区別できているか、また、横断的なデザインのためにばく露と症 状の間の時系列を示せるかという点で制限されている。全体的に質の低い1件の研究では、温室内の花 卉及び観賞用植物の栽培者におけるアレルギー性鼻炎について、手押しポンプによる農薬散布と農薬 散布なしの比較で高い効果量(OR、12.50;95%CI、2.00-78.05)が報告されている(ID ALL\_006)。 ここでも、この研究は全体的に質が低く、化学物質の種類ではなく散布方法に関連して定義された高 濃度ばく露集団に関係している。全体的に、アレルギー性障害と農薬ばく露に関するエビデンスは弱 W.

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## 21. Haematological diseases

### 21.1. Aplastic anaemia

Three studies examined associations between pesticide exposure and aplastic anaemia; a rare hematologic condition. All studies were case-control designs and had small sample sizes (range 9-310). Two studies reported significant associations with large effect sizes but it is difficult to draw firm conclusions due to the small number of studies available and the limitations of these studies (Table 24). The other case control study (ID APL\_002) did not report effect sizes but only the p value of association, which was non-significant. Further evidence is required to throw light into these suggestive results.

 Table 24:
 Summary of results between pesticide exposure and aplastic anemia in 2 case-control studies that reported effect sizes

Study ID	Pesticide assessed	Comparison		Lower 95% Cl	Higher 95% CI	N cases	N controls
APL_001	Organophosphates	yes/no	2.1	1.1	4.2	21	32
APL_001	DDT	yes/no	6.7	1.5	30	5	4
APL_001	Carbamates	yes/no	7.4	1.7	31	8	3
APL_001	Paraquat	yes/no	2.3	1	5.1	12	24
APL_001	Other occupational pesticides	yes/no	1	0.4	2.2	11	32
APL_001	Any household pesticides	yes/no	1.3	0.9	1.9	64	238
APL_001	Organophosphates	yes/no	2.1	1	4.4	17	26
APL_001	Paraquat	yes/no	1.9	0.7	4.9	7	20
APL_001	Other occupational pesticides	yes/no	1.1	0.4	2.7	9	24
APL_003	Agricultural use of pesticides	yes/no	2.2	1.1	4.7	12	23
APL_003	Home use of pesticides	yes/no	1.3	0.9	1.9	70	240
APL_003	Organophosphorates	highest tertile of exposure/no exposure	3	0.9	10.1	5	7
APL_003	Pyrenthroids	highest tertile of exposure/no exposure	1.8	1	3.1	23	57
APL_003	Herbicides	yes/no	2.4	0.9	6	8	15

### 21.2. Haematological and biochemical alterations

Fourteen studies examined various haematological and biochemical alterations in relation to pesticide exposure. Main alterations studied were basic haematology and vitamin levels. The sample size ranged between 51 and 1,275. The quality of these studies was modest to low. Most studies reported unadjusted correlation statistics or means between haematological parameters and pesticide exposure and no effect sizes beyond the p values were reported. All studies provided cross-sectional evidence. Despite the fact than many of the reported analyses were statistically significant, results should not be interpreted at this stage due the limited evidence and modest quality associated with these data.

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# 21. 血液疾患

# 21.1. 再生不良性貧血

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3件の研究では、農薬ばく露と再生不良性貧血(まれな血液疾患)との関連が調査された。すべての 研究は症例対照デザインであり、サンプルサイズは小さい(9~310の範囲)。2件の研究では、大きな 効果量で有意な関連が報告されたが、利用可能な研究の数が少ないことと、これらの研究の限界があ るため、確実な結論を出すことは難しい(表24)。もう1件の症例対照研究(ID APL\_002)では、効果 量は報告されていないが、関連のp値のみが報告されており、有意ではなかった。これらの示唆に富む 結果を明らかにするためには、さらなるエビデンスが必要である。

# 表 24:効果量を報告した 2 つの症例対照研究における農薬ばく露と再生不良性貧 血の結果のまとめ

				下位	上位		コントロ
Study ID	調査した農薬	比較	OR	95% CI	95% CI	ケース数	ール数
APL_001	有機リン剤	yes/no	2.1	1.1	4.2	21	32
APL_001	DDT	yes/no	6.7	1.5	30	5	4
APL_001	カーバメート	yes/no	7.4	1.7	31	8	3
APL_001	パラコート	yes/no	2.3	1	5.1	12	24
APL_001	職業的ばく露の農薬	yes/no	1	0.4	2.2	11	32
APL_001	住居用農薬	yes/no	1.3	0.9	1.9	64	238
APL_001	有機リン剤	yes/no	2.1	1	4.4	17	26
APL_001	パラコート	yes/no	1.9	0.7	4.9	7	20
APL_001	職業的ばく露の農薬	yes/no	1.1	0.4	2.7	9	24
APL_003	農業用農薬	yes/no	2.2	1.1	4.7	12	23
APL_003	住居用農薬	yes/no	1.3	0.9	1.9	70	240
APL_003	有機リン剤	高濃度ばく露	3	0.9	10.1	5	7
		/ばく露なし					
APL_003	ピレスロイド	高濃度ばく露	1.8	1	3.1	23	57
		/ばく露なし					
APL_003	除草剤	yes/no	2.4	0.9	6	8	15

# 21.2. 血液学的及び生化学的変化

14の研究では、農薬ばく露に関連した様々な血液学的及び生化学的変化を調べた。主に一般血液検 査とビタミンレベルであった。サンプル数は51~1,275人であった。これらの研究の質は中等度から低 度であった。ほとんどの研究では、血液学的パラメータと農薬ばく露との間の無補正相関統計値また は平均値が報告されており、p値以外に効果量は報告されていない。すべての研究は横断的なエビデン スを提供している。報告された分析の多くが統計的に有意であったにもかかわらず、これらのデータ に関連するエビデンスが限られており、質も中等度であるため、現段階では結果を解釈すべきではな い。

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# 22. Other outcomes

Overall, 30 publications examined the effect of pesticide exposure on other outcomes. Based on our criteria for data synthesis no meta-analysis was performed for those outcomes.

# 22.1. Bone diseases

Three studies examined the effect of pesticide exposure on osteoporosis including 13 different analyses. We identified two European cross-sectional studies and one Asian cohort (median sample size: 176, IQR: 153-908). All studies assess environmental exposure with information on biomarkers of exposure and all studies examined exposure to organochlorines only. Osteoporosis was assessed via ultrasound measurements and bone mineral density. The largest study of 908 women showed that p,p'-DDE was positively associated with bone mineral density, the association remained after adjustment for confounders, but the effect was weak.

### 22.2. Skin diseases

Six studies examined the effect of pesticide exposure on skin lesion (median sample size: 356, IQR 262-2203) including 11 analyses. Four studies used cross-sectional design. Environmental exposure was assessed in 3 studies. The definition of outcome was often skin rash or eczema. The resulst were largely not statistical significant. One prospective study (ID SKD 004) on 5,042 men from the Health Effects of Arsenic Longitudinal Study in Araihazar reported highly significant effect sizes for skin lesions and pesticide use but study also evaluated arsenic exposure and it is difficult to differentiate between the effect of each exposure.

# 22.3. Dental diseases

One study cross-sectional study from America including 496 participants assessed two outcomes. The study assessed environmental exposure with information of biomarkers (ID PER 001). In this study, organochlorine (OC) pesticides were strongly associated with periodontal disease.

# 22.4. Metabolic diseases

One European cross-sectional study assessed the effect of pesticides on metabolic diseases and specifically on levels of various prorfyrins including 8 analyses but no significant results were reported. Environmental exposure was studied using biomarkers for the assessment of exposure.

# 22.5. Men health

One case-control study reported association between pesticide exposure and erectile dysfunction. The study focused on organochlorine pesticides and compared 101 cases with erectile dysfunction to 234 comparable control subjects. The results were no statistically significant and do not provide evidence of an association.

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# 22. その他の影響

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全体では 30 の論文がその他の影響に対する農薬ばく露の効果を調査した。データ統合のための 我々の基準に基づき、これらの影響に対するメタアナリシスは実施されなかった。

# 21.1. 骨疾患

農薬ばく露が骨粗鬆症に及ぼす影響を調査した研究は3件あり、13の異なる分析を行った。我々は2 つのヨーロッパの横断研究と1つのアジアのコホート研究を同定した(サンプルサイズ中央値:176、 IQR:153-908)。すべての研究は、ばく露のバイオマーカーに関する情報とともに環境ばく露を評価し ており、すべての研究は有機塩素へのばく露のみを調査している。骨粗鬆症は超音波測定と骨密度に よって評価された。908名の女性を対象とした最大の研究では、p,p'-DDEが骨密度と明確な関連を示 し、交絡因子を調整した後も関連は維持されたが、効果は弱いことが示された。

# 22.2. 皮膚疾患

11件の分析のうち農薬ばく露が皮膚病変に及ぼす影響を調査した研究は6件(サンプルサイズ中央値 356、IQR 26-2203)であった。4件の研究では横断的デザインが用いられていた。環境ばく露は3件の研 究で評価された。影響の定義は多くの場合、発疹や湿疹であった。結果はほとんど統計的に有意ではな かった。1つの前向き研究(ID SKD 004)では、男性5,042人を対象としたAraihazarのヒ素の健康影響 縦断研究から、皮膚病変と農薬使用について非常に有意な効果量が報告されているが、この研究では ヒ素ばく露も評価されており、それぞれのばく露の効果を区別することは困難であった。

# 22.3. 歯科疾患

アメリカで行われた1つの横断研究では、496人の参加者が2つの影響で評価された。この研究では、 バイオマーカー (ID PER 001)の情報を用いて環境ばく露を評価した。この研究では、有機塩素系(0C) 農薬は歯周病と強く関連していた。

# 22.4. 代謝性疾患

ヨーロッパで行われた横断研究では、農薬が代謝性疾患、特に様々なprorfyrins (誤植?)のレベル に及ぼす影響を評価し、8つの分析を行ったが、有意な結果は報告されていない。環境ばく露は、ばく 露評価のためのバイオマーカーを用いて研究された。

# 22.5. 男性機能疾患

1件の症例対照研究では、農薬ばく露と勃起不全との関連が報告されている。この研究では有機塩素 系農薬に焦点を当て、勃起不全の症例101例を234例の同等の対照群と比較した。結果は統計的に有意 ではなく、関連を示すエビデンスとはならなかった。

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### 22.6. Gynaecological diseases

In this group we included gynaecological outcomes not included in the previous outcome categories. Four studies are included in this group, three examined endometriosis and one the timing of menopause. The three studies on endometriosis (ID GYN 001, ID GYN 002, ID GYN 003) were all cross-sectional and all examined organochlorines. One out of 12 separate analyses on endometriosis and organochlorines was statistically significant; the highest tertile of aromatic fungicide was associated with a fivefold risk of endometriosis (OR = 5.3; 95% CI, 1.2-23.6) compared to the lowest tertile. This effect size is large and requires independent replication in other prospective studies.

Data from the Agricultural Health Study (AHS) was used to study associations between exposure to pesticides and age at menopause in a prospective investigation of pre-menopausal women. After control for age, smoking status, and past use of oral contraceptives, the median time to menopause increased by approximately 3 months for women who used pesticides (HR 0.87, 95% CI: 0.78, 0.97) and by approximately 5 months for women who used hormonally active pesticides (HR 0.77, 95% CI: 0.65, 0.92). Pesticide use may be associated with a later age at menopause based on these results; however results are prone to false positive bias and independent replication is needed.

## 22.7. Symptoms and general health

Five studies examined general health symptoms such as nausea, fatigue, dizziness, and shortness of breath. The definition of these outcomes is very hard and associated with large measurement errors. Studies were of modest to low quality and all concerned occupational exposures. Some statistically significant results were observed but are far form conclusive at this stage due to heterogeneity of data reported and the limitations associated with these studies.

## 22.8. Kidney diseases

Three studies examined kidney diseases including chronic kidney disease and gallstone disease. One study reported statistically significant results between DDE and DDT residues and gallstone disease.

### 22.9. Benign tumours

One a population-based case-control study on acoustic neuroma found no link between pesticide exposure and acoustic neuroma.

# 22.10. Gastrointestinal diseases

Seven studies examining associations between pesticide exposure and liver enzymes were identified. All studies were cross-sectional or case-control. One study, the National Health and Nutrition Examination Survey (NHANES), examined organochlorines, another one examined exposure to 2,4dichlorophenoxyacetic acid (2,4-D) and paraquat and the remaining studies examined broadly defined pesticide categories. The studies were of modest and low quality and presented only the means of enzymes in exposed and unexposed participants often without adjustments. Almost all studies reported statistically significant results with higher level of liver enzymes (e.g. Gamma-glutamyltransferase (GGT), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)) in participants exposed to pesticides. However, due to the low quality of the data and the limited number of studies firm conclusions cannot be drawn and data is only suggestive of associations at this stage.

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# 22.6. 婦人科疾患

このグループには、上述の影響カテゴリーには含まれていない婦人科学的影響が含まれている。こ のグループには4件の研究が含まれており、3件は子宮内膜症、1件は閉経のタイミングを調査した。子 宮内膜症に関する3件の研究(ID GYN 001、ID GYN 002、ID GYN 003)はすべて横断的であり、すべて 有機塩素を調査していた。子宮内膜症と有機塩素に関する12の個別分析のうち1つは統計学的に有意で あった;芳香族系殺菌剤の上位3位までは、下位3位までと比較して子宮内膜症のリスクが5倍(OR = 5.3;95%CI、1.2-23.6)であった。この効果量は大きく、他の前向き研究で独立した再現が必要であ る。

農業健康調査(Agricultural Health Study: AHS)のデータを用いて、閉経前の女性を対象とした前 向き調査において、農薬へのばく露と閉経時年齢との関連を調査した。年齢、喫煙状況、経口避妊薬の 過去の使用状況をコントロールした後、閉経までの期間の中央値は、農薬を使用した女性では約3ヵ月 (HR 0.87、95%CI:0.78、0.97)、ホルモン活性農薬を使用した女性では約5ヵ月(HR 0.77、95%CI: 0.65、0.92)増加した。農薬の使用は、これらの結果に基づいて、閉経年齢の遅延と関連している可能 性がある。しかしながら、結果は偽陽性バイアスがかかりやすく、独立した再現が必要である。

# 22.7. 症状及び一般的な疾患

吐き気、倦怠感、めまい、息切れなどの一般的な疾患を5つの研究で調査した。これらの結果の定義 は非常に難しく、大きな測定誤差と関連している。研究の質は中等度から低度で、すべての研究が職業 ばく露に関係していた。いくつかの統計的に有意な結果が観察されたが、報告されたデータの不均一 性とこれらの研究に関連する限界のため、現段階では決定的な結論には程遠い。

# 22.8. 腎臟疾患

3つの研究では、慢性腎臓病や結石疾患などの腎臓病を調査した。1件の研究では、DDE及びDDT残留農 薬と結石疾患との間に統計的に有意な結果が報告された。

# 22.9. 良性腫瘍

聴神経腫瘍に関する集団ベースの症例対照研究では、農薬ばく露と聴神経腫瘍との関連は認められ なかった。

# 22.10. 消化器疾患

農薬ばく露と肝臓酵素との関連を調査した7件の研究が同定された。すべての研究は横断的または 症例対照であった。1件の研究、国民健康・栄養調査(NHANES)では有機塩素について、もう1件の研究 では2,4-ジクロロフェノキシ酢酸(2,4-D)とパラコートへのばく露について、残りの研究では広く定 義された農薬のカテゴリーについて調査が行われた。これらの研究は限られた規模で質が低く、多く の場合調整なしで、被ばく露者と非ばく露者の酵素の平均値のみが提示されていた。ほとんどすべて の研究で、農薬にばく露された参加者の肝臓酵素(例:y-グルタミルトランスフェラーゼ(GGT)、ア ラニンアミノトランスフェラーゼ(ALT)、アスパラギン酸アミノトランスフェラーゼ(AST))のレベ ルが高いほど、統計的に有意な結果が報告されている。しかし、データの質の低さと研究の数が限られ ているため、しっかりとした結論を出すことはできず、データは現段階では関連をほのめかすだけで ある。

### CONCLUSIONS

After an exhaustive and comprehensive search of almost 46,000 scientific publications we identified 602 publications, which examine epidemiologic associations between pesticide exposure and diverse health outcomes. The entire spectrum of health outcomes related to pesticide exposure has not been studied before. Our results show a very wide spectrum including 24 major disease categories. Few environmental exposures have been associated with such a wide range of outcomes. The most prevalent outcomes are cancers and mother and child health outcomes. But other disease categories have received considerable attention such as neurological conditions and reproductive diseases. Despite the large volume of available data and the large number (>6,000) of analyses available, firm conclusions cannot be made for the majority of the outcomes studied. This observation is disappointing especially when one accounts for the large volume of research in the area. However, this observation is in line with previous studies on environmental epidemiology and in particular on pesticides which all acknowledge that such epidemiological studies suffer from many limitations and that the heterogeneity of data is such that does not allows firm conclusions to de made.

The range of categories of pesticide studied is wide but studies very often concentrate on a broadly defined pesticide category, and it is hard to understand which pesticide the population is exposed to. Studies often examine pesticides that have already been banned in western populations and the European Union. The use of biomarkers as means of exposure assessment is infrequent but still available in almost half of the studies. In addition, cohort studies represent a minority of this literature with case control and cross-sectional studies representing an approximately equal proportion of eligible articles. Case-control and cross-sectional evidence does not allow the study of temporal relations and thus are unable to provide support regarding the causality of associations. The assessment of exposure is perhaps the most important methodological limitation of the studies. Studies used different methods for exposure assessment and assignment. Most studies were based on selfreported exposure to pesticides, defined as ever versus never use or as regular versus non-regular use. Such methods suffer from high misclassification rates and especially in the case of retrospective studies where misclassification would be differential with higher exposures reported in participants with disease (recall bias). Above all, such questionnaires might be capable of differentiating subjects with very high and very low exposure levels but are not capable of valid exposure classification across an exposure gradient thus not allowing the study of dose-response relationships. Also, the accuracy of exposure might be high for broad categories of pesticides and commonly used pesticides, but not for specific pesticides. It is important that questionnaires used for exposure assessment are validated. However, studies largely used "home- made" versions of questionnaires, sometimes not giving the information on the actual questions used to assess exposure. In addition, exposure simultaneously in multiple agents is common which may introduce further bias in the results. For example, occupational exposure to pesticides is likely to coexist with exposure to benzene, heavy metals, solvents, suspended particulate matter etc. all of which have adverse health outcomes. It is essential to account for confounding from exposure to multiple agents in order to delineate true associations but this has not been possible in the overwhelming majority of evidence assessed herein.

In addition, the evidence collected and appraised herein is likely to suffer from selective reporting and multiple testing. The studies reported a very wide range of analyses; 602 publications resulted in 6000 analyses. The amount of multiple hypothesis testing is enormous. These analyses need to be adjusted for multiple hypothesis testing else the results suffer from high false positive rate. Even when studies present only one analysis, selective reporting is always a possibility as has been shown in other epidemiological fields as well. In addition, when interpreting results one should also take into account that, especially for certain outcomes (e.g. cancers), the majority of evidence comes from single study populations and the AHS in particular.

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# 結論

約46,000の科学出版物を網羅的かつ包括的に検索した結果、602の出版物を確認した。農薬ばく露に 関連した健康影響の全領域については、これまでにも研究されたことがなかった。我々の結果は、24の 主要な疾患カテゴリーを含む非常に幅広いスペクトルを示している。これほど広範囲の影響と関連し た環境ばく露はこれまでほとんどなかった。最も一般的な影響は、がんと母子の健康影響である。しか し、神経疾患や生殖器疾患など、他の疾患カテゴリーにも注目が集まっている。利用可能なデータが大 量にあり、利用可能な分析の数が多い(6,000件以上)にもかかわらず、研究された影響の大部分につ いて確固たる結論を出すことはできない。この調査は、この分野の研究量の多さを考慮すると、特に残 念な結果となった。しかし、この調査は環境疫学、特に農薬に関するこれまでの研究と一致しており、 疫学研究には多くの限界があり、データの不均一性から確固たる結論を出すことができないことを認 めている。

研究されている農薬のカテゴリーの範囲は広いが、研究は多くの場合、広く定義された農薬のカテ ゴリーに集中しており、集団がどの農薬にばく露されているかを理解するのは難しい。研究では、欧米 の集団や欧州連合ですでに禁止されている農薬を調査することが多い。ばく露評価の手段としてバイ オマーカーを使用することはほとんどないが、ほぼ半数の研究ではまだ使用可能である。さらに、コホ ート研究はこの文献の中では少数派であり、症例対照研究と横断研究が対象となる論文のほぼ同じ割 合を占めている。症例対照研究や横断研究では、時間的関係を研究することができないため、関連の因 果関係に関する裏付けを提供することができない。ばく露の評価は、おそらくこれらの研究の最も重 要な方法論的限界である。研究では、ばく露の評価と割り付けに異なる方法が用いられていた。ほとん どの研究では、農薬へのばく露を、使用したことがあるかないか、あるいは定期的に使用したことがあ るかないかという自己申告に基づいていた。このような方法は、高い誤分類率に悩まされ、特に後ろ向 き研究の場合には、病気のある参加者で報告されたばく露量が多いほど誤分類に差が出てしまう(リ コールバイアス)。とりわけ、このような質問紙は、非常に高いばく露量の被験者と非常に低いばく露 量の被験者を区別することができるかもしれないが、ばく露の段階にわたって有効なばく露分類がで きないため、用量反応関係の研究を行うことができない。また、幅広いカテゴリーの農薬や一般的に使 用されている農薬ではばく露の精度が高いかもしれないが、特定の農薬ではそうではない。ばく露評 価に使用される質問紙の妥当性が確認されていることが重要である。しかし、研究では、ほとんどの場 合、質問紙の「自己流」バージョンが使用されており、ばく露評価に使用された実際の質問紙に関する 情報が得られないことがある。さらに、複数の物質への同時ばく露は一般的であり、結果にさらなる偏 りが生じる可能性がある。例えば、農薬への職業ばく露は、ベンゼン、重金属、溶剤、浮遊粒子状物質 などへのばく露と共存している可能性が高い。真の関連を明らかにするためには、複数の物質へのば く露による交絡を考慮することが不可欠であるが、ここで評価されたエビデンスの圧倒的多数では、 これは不可能であった。

さらに、ここで収集されて評価されたエビデンスは、選択的な報告と多重検定に悩まされている可 能性が高い。研究は非常に広範囲の分析を報告しており、602件の論文で6000件の分析が行われていた。 多重仮説検定の量は膨大である。これらの分析は多重仮説検定のために調整する必要があり、そうし ないと結果は高い偽陽性率に悩まされることになる。研究が1つの分析しか行われていない場合でも、 他の疫学分野でも示されているように、選択的な報告が行われる可能性は常にある。さらに、結果を解 釈する際には、特に特定の影響(がんなど)については、エビデンスの大部分が単一の研究集団と特に

Beyond definition of exposure, the definition of clinical outcomes displayed large variability in eligible epidemiological studies, which can further cause the variability in results. Perhaps most important in this setting is the use of surrogate outcomes examined. Here we observed a great number of surrogate outcomes. Surrogate outcomes are biomarkers or physical measures that are generally accepted as substitutes for or predictors of specific clinical outcomes. However, many times these surrogate outcomes can be defined as possible predictors of clinical outcomes but do not fulfil the criteria for a surgate outcome. It is essential that the evidence around unvalidated surrogate outcomes are appraised taking into account the implicit assumptions of unvalidated surrogate outcomes.

Acknowledging these limitations we attempted to summarise the evidence retrieved in this report. An added important limitation here is the fact that this review is limited to publications after 2006. This allows us only to review recent evidence and any meta-analysis needs very cautious interpretation, as it does not include all available evidence. Results might be biased if data published after 2006 are different from earlier evidence. To this end, we also provided updated meta-analysis for major outcomes and for those that a relevant meta-analysis published after 2006 was identified. This has only been possible for childhood leukaemia and for Parkinson's disease. For both these outcomes we found significant associations between pesticide exposure and disease in line with previous evidence. Significant summary estimates have also been reported for other outcomes as summarised in Table 25 below. However, as they represent studies form 2006 onwards results should be regarded as suggestive of associations only and limitations especially regarding the heterogeneity of exposure should always been take into consideration.

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AHSから得られていることも考慮に入れるべきである。

ばく露の定義以外に、臨床所見の定義は妥当な疫学研究においても大きなばらつきを示しており、 これが結果のばらつきの原因となっている。おそらく、このような状況で最も重要なのは、調査された 代替健康影響の使用であろう。ここでは非常に多くの代替健康影響が観察された。代替健康影響とは、 特定の臨床所見の代用または予測因子として一般的に受け入れられているバイオマーカーまたは身体 測定値である。しかし、多くの場合、これらの代替健康影響は検証されておらず、代替健康影響の厳密 な定義を満たしていない。このような影響は、臨床所見の予測因子として定義される可能性はあるが、 代替健康影響の基準を満たしていない。検証されていない代替健康影響に関するエビデンスは、検証 されていない代替健康影響の暗黙の想定を考慮に入れて評価されることが不可欠である。

これらの限界を認識した上で、我々は本報告書で検索されたエビデンスを要約することを試みた。 ここで追加された重要な制限は、このレビューが2006年以降の出版物に限定されているという事実で ある。これにより、最近のエビデンスのみをレビューすることが可能となり、メタアナリシスは利用可 能なすべてのエビデンスを含んでいるわけではないため、非常に慎重な解釈が必要となる。2006年以 降に発表されたデータがそれ以前のエビデンスと異なる場合、結果に偏りが生じる可能性がある。こ の目的のために、主要な影響及び2006年以降に発表された関連するメタアナリシスが確認されたもの については、更新されたメタアナリシスも提供した。これは小児白血病とパーキンソン病についての み可能である。これらの影響については、以前のエビデンスに沿って、農薬ばく露と疾患との間に有意 な関連があることがわかった。有意な要約推定値は他の影響についても報告されており、以下の表25 にまとめられている。しかし、これらは2006年以降の研究であるため、結果はあくまでも関連を示唆す るものであり、特にばく露の不均一性に関する限界を常に考慮に入れるべきである。

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# Table 25: Summary of meta-analyses performed in this report

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Health outcome	N	Meta-analysis	$I^2$
	studies	result	
Leukemia	6	1.26 (0.93,1.71)	59.4%
Hodgkin's Lymphoma	7	1.29 (0.81, 2.06)	81.6%
Childhood Leukemia (exposure to pesticides	6	1.67 (1.25, 2.23)	81.2%
during pregnancy)			
Childhood Leukemia (exposure to insecticides	5	1.55 (1.14, 2.11)	65%
during pregnancy)			
Childhood Leukemia (exposure to insecticides	9	1.69 (1.35, 2.11)	49.8%
during pregnancy-update Turner 2010)			
Childhood Leukemia (exposure to unspecified	5	2.00 (1.73, 2.30)	39.6%
pesticides during pregnancy)			
Childhood Leukemia (exposure to unspecified	11	1.30 (1.09, 1.56)	26.5%
pesticides during pregnancy-update Turner			
2010)			
Childhood Leukemia (exposure to pesticides	7	1.27 (0.96, 1.69)	61.1%
during childhood)			
Childhood Leukemia (exposure to insecticides	8	1.51 (1.28, 1.78)	0%
during childhood-update Turner 2010)			
Childhood Leukemia (exposure to unspecified	11	1.36 (1.19, 1.55)	0%
pesticides during childhood-update Turner			
2010)		4 40 (0 04 4 57)	00/
Breast Cancer (DDE exposure)	5	1.13 (0.81, 1.57)	0%
Breast Cancer	11	1.24 (1.08, 1.43)	0%
Testicular Cancer (DDE exposure)	5	1.40 (0.82, 2.39)	59.5%
Stomach Cancer	6	1.79 (1.30, 2.47)	0%
Liver Cancer	5	2.50 (1.57, 3.98)	25.4%
Cryptorchidism	8	1.19 (0.96, 1.49)	23.9%
Cryptorchidism (DDT exposure)	4	1.47 (0.98, 2.20)	51%
Hypospadias (general pesticide exposure)	6	1.01 (0.74, 1.39)	71.5%
Hypospadias (exposure to specific pesticides)	9	1 (0.84, 1.18)	65.9%
Abortion	6	1.52 (1.09, 2.13)	63.1%
Parkinson's disease	26	1.49 (1.28, 1.73)	54.6%
Parkinson's disease (DDT exposure)	5	1.01 (0.78, 1.30)	0%
Parkinson's disease (paraquat exposure)	9	1.32 (1.09, 1.60)	34.1%
Amyotrophic Lateral Sclerosis	6	1.58 (1.31, 1.90)	10%
Asthma (DDT exposure)	5	1.29 (1.14, 1.45)	0%
Asthma (paraquat exposure)	6	1.40 (0.95, 2.06)	53.3%
Asthma (chlorpyrifos exposure)	5	1.03 (0.82, 1.28)	0%
Type 1 Diabetes (DDE exposure)	8	1.89 (1.25, 2.86)	49%
Type 1 Diabetes (DDT exposure)	6	1.76 (1.20, 2.59)	76.3%
Type 2 Diabetes (DDE exposure)	4	1.29 (1.13, 1.48)	0%
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# 表25:本報告書で実施されたメタアナリシスの要約

健康影響	研究数	メタアナリシス結果	$I^2$
白血病	6	1.26 (0.93, 1.71)	59.40%
ホジキンリンパ腫	7	1.29 (0.81, 2.06)	81.60%
小児白血病(妊娠期の農薬ばく露)	6	1.67 (1.25, 2.23)	81.20%
小児白血病(妊娠期の殺虫剤ばく露)	5	1.55 (1.14, 2.11)	65%
小児白血病(妊娠期の殺虫剤ばく露-ターナー2010 年に更 新)	9	1.69 (1.35, 2.11)	49.80%
小児白血病(妊娠期の不特定農薬ばく露)	5	2.00 (1.73, 2.30)	39.60%
小児白血病(妊娠期の不特定殺虫剤ばく露-ターナー2010 年に更新)	11	1.30 (1.09, 1.56)	26.50%
小児白血病(小児期の農薬ばく露)	7	1.27 (0.96, 1.69)	61.10%
小児白血病(小児期の殺虫剤ばく露-ターナー2010 年に更 新)	8	1.51 (1.28, 1.78)	0%
小児白血病(小児期の不特定殺虫剤ばく露-ターナー2010 年に更新)	11	1.36 (1.19, 1.55)	0%
乳がん(DDE ばく露)	5	1.13 (0.81, 1.57)	0%
乳がん	11	1.24 (1.08, 1.43)	0%
精巣がん(DDE ばく露)	5	1.40 (0.82, 2.39)	59.50%
胃がん	6	1.79 (1.30, 2.47)	0%
肝臓がん	5	2.50 (1.57, 3.98)	25.40%
停留精巣	8	1.19 (0.96, 1.49)	23.90%
停留精巣(DDT ばく露)	4	1.47 (0.98, 2.20)	51%
尿道下裂(一般的な農薬ばく露)	6	1.01 (0.74, 1.39)	71.50%
尿道下裂(特定の農薬ばく露)	9	1 (0.84, 1.18)	65.90%
流産	6	1.52 (1.09, 2.13)	63.10%
パーキンソン病	26	1.49 (1.28, 1.73)	54.60%
パーキンソン病(DDT ばく露)	5	1.01 (0.78, 1.30)	0%
パーキンソン病(パラコートばく露)	9	1.32 (1.09, 1.60)	34.10%
筋萎縮性側索硬化症	6	1.58 (1.31, 1.90)	10%
喘息(DDT ばく露)	5	1.29 (1.14, 1.45)	0%
喘息(パラコートばく露)	6	1.40 (0.95, 2.06)	53.30%
喘息(クロルピリホスばく露)	5	1.03 (0.82, 1.28)	0%
1 型糖尿病(DDE ばく露)	8	1.89 (1.25, 2.86)	49%
1 型糖尿病(DDT ばく露)	6	1.76 (1.20, 2.59)	76.30%
2 型糖尿病(DDE ばく露)	4	1.29 (1.13, 1.48)	0%

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#### RECOMMENDATIONS

As discussed above, the extensive evidence gathered for this report highlights that there is immense amount of information available on pesticide exposure and health outcomes from epidemiological studies. Nonetheless, the quality of this evidence is usually low and many biases are likely to affect the results to an extent that firm conclusions cannot be made. Childhood cancers and Parkinson's disease are the two outcomes for which a corresponding meta-analysis after 2006 was found and for which data are consistent to show an increased risk associated with pesticide exposure. Nonetheless, the exposure needs to be studies further in order to disentangle the effect of specific pesticide classes or even individual pesticides. Effects on other outcomes, such as endocrine disorders, asthma and allergies, diabetes and obesity, are showing increased risk and should be explored further. This report concentrated on examining separately health outcomes. An alternative approach would be to look for pesticide classes, subclasses or even individual pesticides across a range of outcomes. These approaches could highlight whether a pesticide class has a particular detrimental effect across a variety of disease endpoints. Finally, exposure epidemiology has long suffered from exposure measurement and definition and in particular for pesticides this has always been exceptionally difficult to assess and define. Technological advances now enable us to measure in a large scale and agnostic way biomarkers of exposure using high throughput technologies of omics. For example, metabolomic analysis offers a way to capture a whole range of environmental exposures with minimal measurement error and ability to specify the exposure. These approaches are now being developed and are likely to offer much clearer view on the associations between environmental exposures, including dietary exposures, and health outcomes.

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### 推奨事項

上述したように、本報告書のために収集された広範なエビデンスは、疫学研究から得られる農薬ば く露と健康影響に関する膨大な量の情報があることを浮き彫りにしている。しかし、これらのエビデ ンスの質は通常低く、多くのバイアスが結果に影響を与え、確固とした結論を出すことができない可 能性が高い。小児がんとパーキンソン病は、2006年以降に対応するメタアナリシスが行われた2つの 影響であり、農薬ばく露に関連したリスクの増加を示すデータが一貫している。しかし、特定の農薬ク ラスや個々の農薬の影響を切り離すためには、ばく露の研究をさらに進める必要がある。内分泌疾患、 喘息、アレルギー、糖尿病、肥満などの他の影響への影響はリスクの増加を示しており、さらに調査が 必要である。本報告書では、健康影響を個別に調査することに集中した。別のアプローチとしては、農 薬のクラス、サブクラス、あるいは個々の農薬でさえも、さまざまな影響にわたって調べることであろ う。これらのアプローチにより、ある農薬クラスが様々な疾患エンドポイントにおいて特定の有害な 影響を及ぼすかどうかを明らかにすることができる。最後に、ばく露疫学は長い間、ばく露の測定と定 義に悩まされてきたが、特に農薬については、これは常に評価と定義が非常に困難であった。技術的な 進歩により、オミクスのハイスループット技術を用いて、大規模かつ断定的でない方法でばく露のバ イオマーカーを測定することが可能になった。例えば、メタボローム分析は、最小限の測定誤差とばく 露を特定する能力で、環境ばく露の全範囲を捕捉する方法を提供している。これらのアプローチは現 在開発が進められており、食事ばく露を含む環境ばく露と健康影響との関連について、より明確な見 解を提供してくれる可能性が高い。

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### REFERENCES

- ALLEN, M. T. & LEVY, L. S. 2013. Parkinson's disease and pesticide exposure--a new assessment. Crit Rev Toxicol, 43, 515-34.
- BAILEY, H. D., ARMSTRONG, B. K., DE KLERK, N. H., FRITSCHI, L., ATTIA, J., SCOTT, R. J., SMIBERT, E. & MILNE, E. 2011. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer, 129, 1678-88.
- BUDNIK, L. T., KLOTH, S., VELASCO-GARRIDO, M. & BAUR, X. 2012. Prostate cancer and toxicity from critical use exemptions of methyl bromide: environmental protection helps protect against human health risks. Environ Health, 11, 5.
- COOPER, G. S. & JONES, S. 2008. Pentachlorophenol and cancer risk: focusing the lens on specific chlorophenols and contaminants. Environ Health Perspect, 116, 1001-8.
- DICK, F. D. 2006. Parkinson's disease and pesticide exposures. Br Med Bull, 79-80, 219-31.
- GOVARTS, E., NIEUWENHUIJSEN, M., SCHOETERS, G., BALLESTER, F., BLOEMEN, K., DE BOER, M., CHEVRIER, C., EGGESBO, M., GUXENS, M., KRAMER, U., LEGLER, J., MARTINEZ, D., PALKOVICOVA, L., PATELAROU, E., RANFT, U., RAUTIO, A., PETERSEN, M. S., SLAMA, R., STIGUM, H., TOFT, G., TRNOVEC, T., VANDENTORREN, S., WEIHE, P., KUPERUS, N. W., WILHELM, M., WITTSIEPE, J. & BONDE, J. P. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect, 120, 162-70.
- IOANNIDIS, J. P., PATSOPOULOS, N. A. & EVANGELOU, E. 2007. Uncertainty in heterogeneity estimates in meta-analyses. BMJ, 335, 914-6.
- ISMAIL, A. A., BODNER, T. E. & ROHLMAN, D. S. 2012. Neurobehavioral performance among agricultural workers and pesticide applicators: a meta-analytic study. Occup Environ Med, 69, 457-64.
- KAMEL, F., UMBACH, D. M., BEDLACK, R. S., RICHARDS, M., WATSON, M., ALAVANJA, M. C., BLAIR, A., HOPPIN, J. A., SCHMIDT, S. & SANDLER, D. P. 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology, 33, 457-62.
- KHANJANI, N., HOVING, J. L., FORBES, A. B. & SIM, M. R. 2007. Systematic review and metaanalysis of cyclodiene insecticides and breast cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 25, 23-52.
- KOUREAS, M., TSAKALOF, A., TSATSAKIS, A. & HADJICHRISTODOULOU, C. 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett, 210, 155-68.
- LAU, J., IOANNIDIS, J. P. & SCHMID, C. H. 1997. Quantitative synthesis in systematic reviews. Ann Intern Med, 127, 820-6.
- LI, A. A., LOWE, K. A., MCINTOSH, L. J. & MINK, P. J. 2012. Evaluation of epidemiology and animal data for risk assessment: chlorpyrifos developmental neurobehavioral outcomes. J Toxicol Environ Health B Crit Rev, 15, 109-84.
- LOPEZ-CERVANTES, M., TORRES-SANCHEZ, L., TOBIAS, A. & LOPEZ-CARRILLO, L. 2004. Dichlorodiphenyldichloroethane burden and breast cancer risk: a meta-analysis of the epidemiologic evidence. Environ Health Perspect, 112, 207-14.

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### 参考文献

- ALLEN, M. T. & LEVY, L. S. 2013. Parkinson's disease and pesticide exposure--a new assessment. Crit Rev Toxicol, 43, 515-34.
- BAILEY, H. D., ARMSTRONG, B. K., DE KLERK, N. H., FRITSCHI, L., ATTIA, J., SCOTT, R. J., SMIBERT, E. & MILNE, E. 2011. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer, 129, 1678-88.
- BUDNIK, L. T., KLOTH, S., VELASCO-GARRIDO, M. & BAUR, X. 2012. Prostate cancer and toxicity from critical use exemptions of methyl bromide: environmental protection helps protect against human health risks. Environ Health, 11, 5.
- COOPER, G. S. & JONES, S. 2008. Pentachlorophenol and cancer risk: focusing the lens on specific chlorophenols and contaminants. Environ Health Perspect, 116, 1001-8.
- DICK, F. D. 2006. Parkinson's disease and pesticide exposures. Br Med Bull, 79-80, 219-31.
- GOVARTS, E., NIEUWENHUIJSEN, M., SCHOETERS, G., BALLESTER, F., BLOEMEN, K., DE BOER, M., CHEVRIER, C., EGGESBO, M., GUXENS, M., KRAMER, U., LEGLER, J., MARTINEZ, D., PALKOVICOVA, L., PATELAROU, E., RANFT, U., RAUTIO, A., PETERSEN, M. S., SLAMA, R., STIGUM, H., TOFT, G., TRNOVEC, T., VANDENTORREN, S., WEIHE, P., KUPERUS, N. W., WILHELM, M., WITTSIEPE, J. & BONDE, J. P. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect, 120, 162-70.
- IOANNIDIS, J. P., PATSOPOULOS, N. A. & EVANGELOU, E. 2007. Uncertainty in heterogeneity estimates in meta-analyses. BMJ, 335, 914-6.
- ISMAIL, A. A., BODNER, T. E. & ROHLMAN, D. S. 2012. Neurobehavioral performance among agricultural workers and pesticide applicators: a meta-analytic study. Occup Environ Med, 69, 457- 64.
- KAMEL, F., UMBACH, D. M., BEDLACK, R. S., RICHARDS, M., WATSON, M., ALAVANJA, M. C., BLAIR, A., HOPPIN, J. A., SCHMIDT, S. & SANDLER, D. P. 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology, 33, 457-62.
- KHANJANI, N., HOVING, J. L., FORBES, A. B. & SIM, M. R. 2007. Systematic review and metaanalysis of cyclodiene insecticides and breast cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 25, 23-52.
- KOUREAS, M., TSAKALOF, A., TSATSAKIS, A. & HADJICHRISTODOULOU, C. 2012. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicol Lett, 210, 155-68.
- LAU, J., IOANNIDIS, J. P. & SCHMID, C. H. 1997. Quantitative synthesis in systematic reviews. Ann Intern Med, 127, 820-6.
- LI, A. A., LOWE, K. A., MCINTOSH, L. J. & MINK, P. J. 2012. Evaluation of epidemiology and animal data for risk assessment: chlorpyrifos developmental neurobehavioral outcomes. J Toxicol Environ Health B Crit Rev, 15, 109-84.
- LOPEZ-CERVANTES, M., TORRES-SANCHEZ, L., TOBIAS, A. & LOPEZ-CARRILLO, L. 2004. Dichlorodiphenyldichloroethane burden and breast cancer risk: a meta-analysis of the epidemiologic evidence. Environ Health Perspect, 112, 207-14.

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- MALEK, A. M., BARCHOWSKY, A., BOWSER, R., YOUK, A. & TALBOTT, E. O. 2012. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS. Environ Res, 117, 112-9.
- MERHI, M., RAYNAL, H., CAHUZAC, E., VINSON, F., CRAVEDI, J. P. & GAMET-PAYRASTRE, L. 2007. Occupational exposure to pesticides and risk of hematopoietic cancers: meta-analysis of case-control studies. Cancer Causes Control, 18, 1209-26.
- PEZZOLI, G. & CEREDA, E. 2013. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology, 80, 2035-41.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & PRIYADARSHI, S. S. 2001. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res, 86, 122-7.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & SHRIVASTAVA, S. 2000. A metaanalysis of Parkinson's disease and exposure to pesticides. Neurotoxicology, 21, 435-40.
- SAPHIR, A. 1998. Farmers and cancer: old crop of data gets new scrutiny. J Natl Cancer Inst, 90, 651-3.
- SHIRANGI, A., NIEUWENHUIJSEN, M., VIENNEAU, D. & HOLMAN, C. D. 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. Paediatr Perinat Epidemiol, 25, 172-91.
- SNIJDER, C. A., TE VELDE, E., ROELEVELD, N. & BURDORF, A. 2012. Occupational exposure to chemical substances and time to pregnancy: a systematic review. Hum Reprod Update, 18, 284-300.
- SUTEDJA, N. A., VELDINK, J. H., FISCHER, K., KROMHOUT, H., HEEDERIK, D., HUISMAN, M. H., WOKKE, J. H. & VAN DEN BERG, L. H. 2009. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph Lateral Scler, 10, 302-9.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Environ Health Perspect, 118, 33-41.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Cien Saude Colet, 16, 1915-31.
- VAN DER MARK, M., BROUWER, M., KROMHOUT, H., NIJSSEN, P., HUSS, A. & VERMEULEN, R. 2012. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect, 120, 340-7.
- VAN MAELE-FABRY, G., DUHAYON, S., MERTENS, C. & LISON, D. 2008. Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. Environ Res, 106, 121-37.
- VAN MAELE-FABRY, G., HOET, P., VILAIN, F. & LISON, D. 2012. Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. Environ Int, 46, 30-43.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2010. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control, 21, 787-809.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2011. Residential exposure to pesticides and childhood leukaemia: a systematic review and meta-analysis. Environ Int, 37, 280-91.

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- Pesticide epidemiology
- MALEK, A. M., BARCHOWSKY, A., BOWSER, R., YOUK, A. & TALBOTT, E. O. 2012. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS. Environ Res, 117, 112-9.
- MERHI, M., RAYNAL, H., CAHUZAC, E., VINSON, F., CRAVEDI, J. P. & GAMET-PAYRASTRE, L. 2007. Occupational exposure to pesticides and risk of hematopoietic cancers: meta-analysis of case-control studies. Cancer Causes Control, 18, 1209-26.
- PEZZOLI, G. & CEREDA, E. 2013. Exposure to pesticides or solvents and risk of Parkinson disease. Neurology, 80, 2035-41.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & PRIYADARSHI, S. S. 2001. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res, 86, 122-7.
- PRIYADARSHI, A., KHUDER, S. A., SCHAUB, E. A. & SHRIVASTAVA, S. 2000. A meta-analysis of Parkinson's disease and exposure to pesticides. Neurotoxicology, 21, 435-40.
- SAPHIR, A. 1998. Farmers and cancer: old crop of data gets new scrutiny. J Natl Cancer Inst, 90, 651-3.
- SHIRANGI, A., NIEUWENHUIJSEN, M., VIENNEAU, D. & HOLMAN, C. D. 2011. Living near agricultural pesticide applications and the risk of adverse reproductive outcomes: a review of the literature. Paediatr Perinat Epidemiol, 25, 172-91.
- SNIJDER, C. A., TE VELDE, E., ROELEVELD, N. & BURDORF, A. 2012. Occupational exposure to chemical substances and time to pregnancy: a systematic review. Hum Reprod Update, 18, 284-300.
- SUTEDJA, N. A., VELDINK, J. H., FISCHER, K., KROMHOUT, H., HEEDERIK, D., HUISMAN, M. H., WOKKE, J. H. & VAN DEN BERG, L. H. 2009. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph Lateral Scler, 10, 302-9.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Environ Health Perspect, 118, 33-41.
- TURNER, M. C., WIGLE, D. T. & KREWSKI, D. 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Cien Saude Colet, 16, 1915-31.
- VAN DER MARK, M., BROUWER, M., KROMHOUT, H., NIJSSEN, P., HUSS, A. & VERMEULEN, R. 2012. Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ Health Perspect, 120, 340-7.
- VAN MAELE-FABRY, G., DUHAYON, S., MERTENS, C. & LISON, D. 2008. Risk of leukaemia among pesticide manufacturing workers: a review and meta-analysis of cohort studies. Environ Res, 106, 121-37.
- VAN MAELE-FABRY, G., HOET, P., VILAIN, F. & LISON, D. 2012. Occupational exposure to pesticides and Parkinson's disease: a systematic review and meta-analysis of cohort studies. Environ Int, 46, 30-43.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2010. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control, 21, 787-809.
- VAN MAELE-FABRY, G., LANTIN, A. C., HOET, P. & LISON, D. 2011. Residential exposure to pesticides and childhood leukaemia: a systematic review and meta-analysis. Environ Int, 37, 280- 91.

VINSON, F., MERHI, M., BALDI, I., RAYNAL, H. & GAMET-PAYRASTRE, L. 2011. Exposure to

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#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I Pestic

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- VINSON, F., MERHI, M., BALDI, I., RAYNAL, H. & GAMET-PAYRASTRE, L. 2011. Exposure to pesticides and risk of childhood cancer: a meta-analysis of recent epidemiological studies. Occup Environ Med, 68, 694-702.
- WIGLE, D. T., TURNER, M. C. & KREWSKI, D. 2009. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ Health Perspect, 117, 1505-13.

pesticides and risk of childhood cancer: a meta-analysis of recent epidemiological studies. Occup Environ Med, 68, 694-702.

WIGLE, D. T., TURNER, M. C. & KREWSKI, D. 2009. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ Health Perspect, 117, 1505-13.

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### APPENDICES

## **APPENDIX I. EXTENDED SEARCH ALGORITHM IN MEDLINE**

Pesticid\* OR Pesticide OR pest control OR "pest control" OR (Chemosteril\* OR Chemosterilant OR Fungicide OR fungicide OR Fungicide, Industrial OR Herbicide OR Herbicide OR Defoliant\* OR Defoliant, Chemical OR Insect Repellent\*OR Insect Repellent OR Insecticid\* OR Insecticide OR Molluscacide OR Molluscacide OR Pesticide Synergist\* OR Pesticide Synergist OR Rodenticid\* OR Rodenticide OR organochlor\* OR organochloride OR organochlorine OR chlorocarbon OR chlorinated hydrocarbon OR chlorinated solvent OR organophosphat\* OR organophosphate OR carbamat\* OR carbamate OR pyrethroid\* OR pyrethroid) OR (1,2-dibromo-3-chloropropane OR 1,3dichloro-1-propene OR 1-(4-ethynylphenyl)-4-propyl-2,6,7-trioxabicyclo(2.2.2)octane OR 1-Methyl-4-phenylpyridiniumOR 2,4,5-Trichlorophenoxyacetic Acid OR 2,4-Dichlorophenoxyacetic AcidOR 2dichlorobenzeneOR 2-Methyl-4-chlorophenoxyacetic Acid OR 2-methyl-4-chlorophenoxyacetic acid dicamba herbicide solution OR 2-phenylphenol OR 3,5,6-trichloro-2-pyridinolOR 4"-epiacetylamino-4"-deoxyavermectin B1 OR 4-dichlorobenzeneOR abamectin OR acephate OR acetochlor OR acifluorfen ORAgent OrangeOR alachlor OR Aldicarb OR Aldrin OR Allethrin OR allosamidin OR alpha-Chlorohydrin OR alpha-naphthyl thiourea OR alpha-naphthylphthalamic acid OR aluminum phosphide OR aminocarb OR amitrazOR AnabasineOR arsenic acidOR Atrazine OR avermectinOR azadirachtin OR AzinphosmethylOR Bacillus thuringiensis protoxinOR bendiocarbOR BenomylOR bentazoneOR benthiocarbOR benzyl benzoate OR bialaphos OR binB protein Bacillus sphaericus OR bioallethrinOR bioresmethrin OR bis(tri-n-butyltin)oxideOR boric acid OR bromacil OR bromadiolone OR bromfenacoumOR bullatacinOR butachlorOR butyl phosphorotrithioate OR Cacodylic Acid OR captafol OR CaptanOR Carbaryl OR Carbofuran OR CarboxinOR Chloranil OR ChlordanOR ChlordeconeOR Chlorfenvinphos OR chlorocresol OR chlorophacinoneOR ChlorphenamidineOR Chlorpropham OR Chlorpyrifos OR chlorsulfuronOR chlortoluronOR cismethrinOR closantel OR CoumaphosOR crotamiton OR cyanazine OR cyclonite OR cyfluthrinOR cyhalothrinOR cyhexatinOR cypermethrinOR cyromazineOR cythioateOR daminozideOR decamethrinOR DEETOR dexon (fungicide)OR diallyl trisulfideOR Diazinon OR Dicamba OR dichlobanilOR Dichlorodiphenyl DichloroethyleneOR DichlorodiphenyldichloroethaneOR dichlorodiphenyltrichloroethane OR DDT OR Dichlorvos OR Dicofol OR dieldrin OR difenacoumOR DimethoateOR dimethyl 4,4'-o-phenylene bis (3-thioallophanate) with carbamic acid ethylene bis (dithio)-mangenese zinc complexOR dimethyl 4-phthalateOR dimethyl phthalateOR Dinitrophenols OR dinosebOR diphenylOR DiquatOR DisulfotonOR DiuronOR doramectin OR EndosulfanOR EndrinOR ethionOR Ethylmercuric Chloride OR Ethylmercury Compounds OR famophos OR fenarimol OR FenitrothionOR fenoxycarb OR fenpropimorphOR Fenthion OR fenyalerate OR fipronil OR fluazifop OR fluazifop-butyl OR fluoroacetic acid OR fluphenacur OR fluridoneOR fluvalinate OR folpet OR FonofosOR glyphosateOR hedolit OR Hempa OR HeptachlorOR Heptachlor Epoxide OR heptenophosOR HexachlorobenzeneOR hexachlorobutadiene OR hexazinoneOR hydramethylnonOR imazalilOR imidaclopridOR insecticidal crystal protein Bacillus ThuringiensisOR iprodioneOR isofenphosOR isoproturonOR IvermectinOR jasplakinolideOR LeptophosOR linaloolOR LindaneOR Linuron ORmalachite greenOR malaoxonOR MalathionOR Maleic HydrazideOR mancozebOR ManebOR mecarzoleOR mecopropOR metalaxylOR metaldehydeOR methamidophosOR methidathionOR MethiocarbOR MethomyIOR MethoxychlorOR methyl demetonOR methyl isothiocyanateOR Methyl ParathionOR methylbromfenvinphosOR methyldithiocarbamateOR methyllycaconitineOR metolachlorOR metribuzinOR MevinphosOR milbemycinOR molinateOR MonocrotophosOR monomethylarsonic acidOR N.Ndiethylphenylacetamide OR N-(3,5-dichlorophenyl)succinimideOR N-bromoacetamideOR nhexanalOR Naled OR neem oilOR neosaxitoxinOR Niclosamide OR nitrofenOR nonachlor OR norbormideOR norflurazoneOR nornicotine OR octamethyl pyrophosphoramideOR oryzalinOR

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## 付録

# 付録 I. MEDLINE における拡張検索アルゴリズム

Pesticid\* OR Pesticide OR pest control OR "pest control" OR (Chemosteril\* OR Chemosterilant OR Fungicid\* OR fungicide OR Fungicide, Industrial OR Herbicid\* OR Herbicide OR Defoliant\* OR Defoliant, Chemical OR Insect Repellent\*OR Insect Repellent OR Insecticid\* OR Insecticide OR Molluscacid\* OR Molluscacide OR Pesticide Synergist\* OR Pesticide Synergist OR Rodenticid\* OR Rodenticide OR organochlor\* OR organochloride OR organochlorine OR chlorocarbon OR chlorinated hydrocarbon OR chlorinated solvent OR organophosphat\* OR organophosphate OR carbamat\* OR carbamate OR pyrethroid\* OR pyrethroid) OR (1, 2-dibromo-3-chloropropane OR 1, 3- dichloro-1-propene OR 1-(4-ethynylphenyl)-4propyl-2, 6, 7-trioxabicyclo(2.2.2) octane OR 1-Methyl- 4-phenylpyridiniumOR 2, 4, 5-Trichlorophenoxyacetic Acid OR 2, 4-Dichlorophenoxyacetic AcidOR 2- dichlorobenzeneOR 2-Methyl-4-chlorophenoxyacetic Acid OR 2-methyl-4-chlorophenoxyacetic acid dicamba herbicide solution OR 2-phenylphenol OR 3, 5, 6-trichloro-2-pyridinolOR 4''-epiacetylamino- 4''deoxyavermectin B1 OR 4-dichlorobenzeneOR abamectin OR acephate OR acetochlor OR acifluorfen ORAgent OrangeOR alachlor OR Aldicarb OR Aldrin OR Allethrin OR allosamidin OR alpha-Chlorohydrin OR alpha-naphthyl thiourea OR alpha-naphthylphthalamic acid OR aluminum phosphide OR aminocarb OR amitrazOR AnabasineOR arsenic acidOR Atrazine OR avermectinOR azadirachtin OR AzinphosmethylOR Bacillus thuringiensis protoxinOR bendiocarbOR BenomylOR bentazoneOR benthiocarbOR benzyl benzoate OR bialaphos OR binB protein Bacillus sphaericus OR bioallethrinOR bioresmethrin OR bis(tri-n-butyltin)oxideOR boric acid OR bromacil OR bromadiolone OR bromfenacoumOR bullatacinOR butachlorOR butyl phosphorotrithioate OR Cacodylic Acid OR captafol OR CaptanOR Carbaryl OR Carbofuran OR CarboxinOR Chloranil OR ChlordanOR ChlordeconeOR Chlorfenvinphos OR chlorocresol OR chlorophacinoneOR ChlorphenamidineOR Chlorpropham OR Chlorpyrifos OR chlorsulfuronOR chlortoluronOR cismethrinOR closantel OR CoumaphosOR crotamiton OR cvanazine OR cvclonite OR cvfluthrinOR cyhalothrinOR cyhexatinOR cypermethrinOR cyromazineOR cythioateOR daminozideOR decamethrinOR DEETOR dexon (fungicide)OR diallyl trisulfideOR Diazinon OR Dicamba OR dichlobanilOR Dichlorodiphenyl DichloroethyleneOR DichlorodiphenyldichloroethaneOR dichlorodiphenvltrichloroethane OR DDT OR Dichlorvos OR Dicofol OR dieldrin OR difenacoumOR DimethoateOR dimethyl 4,4'-o-phenylene bis (3-thioallophanate) with carbamic acid ethylene bis (dithio)-mangenese zinc complexOR dimethyl 4-phthalateOR dimethyl phthalateOR Dinitrophenols OR dinosebOR diphenylOR DiquatOR DisulfotonOR DiuronOR doramectin OR EndosulfanOR EndrinOR ethionOR Ethylmercuric Chloride OR Ethylmercury Compounds OR famophos OR fenarimol OR FenitrothionOR fenoxycarb OR fenpropimorphOR Fenthion OR fenvalerate OR fipronil OR fluazifop OR fluazifop-butyl OR fluoroacetic acid OR fluphenacur OR fluridoneOR fluvalinate OR folpet OR FonofosOR glyphosateOR hedolit OR Hempa OR HeptachlorOR Heptachlor Epoxide OR heptenophosOR HexachlorobenzeneOR hexachlorobutadiene OR hexazinoneOR hydramethylnonOR imazalilOR imidaclopridOR insecticidal crystal protein Bacillus ThuringiensisOR iprodioneOR isofenphosOR isoproturonOR IvermectinOR jasplakinolideOR LeptophosOR linaloolOR LindaneOR Linuron ORmalachite greenOR malaoxonOR MalathionOR Maleic HydrazideOR mancozebOR ManebOR mecarzoleOR mecopropOR metalaxylOR metaldehydeOR methamidophosOR methidathionOR MethiocarbOR MethomylOR MethoxychlorOR methyl demetonOR methyl isothiocyanateOR Methyl ParathionOR methylbromfenvinphosOR methyldithiocarbamateOR methyllycaconitineOR metolachlorOR metribuzinOR MevinphosOR milbemycinOR molinateOR MonocrotophosOR monomethylarsonic acidOR N, N- diethylphenylacetamide OR N-(3, 5dichlorophenvl)succinimideOR N-bromoacetamideOR n- hexanalOR Naled OR neem oilOR neosaxitoxinOR Niclosamide OR nitrofenOR nonachlor OR norbormideOR norflurazoneOR nornicotine OR octamethyl pyrophosphoramideOR oryzalinOR ParaoxonOR ParaouatOR ParathionOR

ParaoxonOR ParaquatOR ParathionOR pendimethalin OR pentachlorobenzeneOR PentachlorophenoIOR PermethrinOR phenothrinOR phenthoateOR phentin acetate OR Phenylmercuric Acetate OR phenylmercuric nitrate, basicOR Phenylmercury CompoundsOR Phenylphosphonothioic Acid 2-Ethyl 2-(4-Nitrophenyl) EsterOR Phorate OR phosaloneOR PhosmetOR PhosphamidonOR phosphineOR phosphinothricinOR phoxim OR Picloram OR Piperonyl ButoxideOR pirimicarbOR pirimiphos methylOR precocene IIOR prochlorazOR procymidoneOR profenofosOR PrometryneOR propachlorOR PropanilOR PropoxurOR PyrethrinsOR pyriminil OR quinalphos OR quintozene OR RotenoneOR S,S'-(2-(dimethylamino)-1,3-propanediyl)thiosulfuric acid ester OR SimazineOR sodium chlorateOR spinosadOR sulfamic acidOR sulfometuron methyl OR tebufenozideOR TemefosOR terbutryneOR terbutylazineOR terthienyl OR tetrachloroisophthalonitrileOR TetrachlorvinphosOR tetramethrinOR thallium sulfate OR ThiophanateOR ThiramOR ToxapheneOR triadimefon OR Triallate OR TrichlorfonOR triclopyrOR triflumuron OR Trifluralin OR vinclozolin OR Warfarin OR zinc phosphide OR Zineb OR Zinam)

(LIMITS: HUMAN, 1/1/2006 - 1/10/2012)

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pendimethalin OR pentachlorobenzeneOR PentachlorophenolOR PermethrinOR phenothrinOR phenthoateOR phentin acetate OR Phenylmercuric Acetate OR phenylmercuric nitrate, basicOR Phenylmercury CompoundsOR Phenylphosphonothioic Acid 2-Ethyl 2-(4-Nitrophenyl) EsterOR Phorate OR phosaloneOR PhosmetOR PhosphamidonOR phosphineOR phosphinothricinOR phoxim OR Picloram OR Piperonyl ButoxideOR pirimicarbOR pirimiphos methylOR preocene IIOR prochlorazOR procymidoneOR profenofosOR PrometryneOR propachlorOR PropanilOR PropoxurOR PyrethrinsOR pyriminil OR quinalphos OR quintozene OR RotenoneOR S, S'-(2-(dimethylamino)-1, 3-propanediyl)thiosulfuric acid ester OR SimazineOR sodium chlorateOR spinosadOR sulfamic acidOR sulfometuron methyl OR tebufenozideOR TemefosOR terbutryneOR terbutylazineOR terthienyl OR tetrachloroisophthalonitrileOR TetrachlorvinphosOR tetramethrinOR thallium sulfate OR ThiophanateOR ThiramOR ToxapheneOR triadimefon OR Triallate OR TrichlorfonOR triclopyrOR triflumuron OR Trifluralin OR vinclozolin OR Warfarin OR zinc phosphide OR Zineb OR Ziram)

(LIMITS: HUMAN, 1/1/2006 - 1/10/2012)

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# APPENDIX II. EXPLANATIONS TO THE DATA EXTRACTION DATABASE

Study ID	This is the unique ID of the study given sequentially for each
	study major outcome
PUBMED_ID	This is the PUBMED ID of the study (if not available ID in
	EMBASE was provided and when this was not available the
	title of the study was provided)
First author	First author's last name
Journal	Journal in which the study was published
Year	Year of publication
Country	Country where the study was conducted
Location (continent)	Continent where the study was conducted
Recruitment period	Period during which the study participants were recruited
Exposure Period (preconception,	Growth period in which the pesticide exposure occurred
infancy, childhood, adulthood,	(preconception, pregnancy, infancy, childhood, adolescence,
pregnancy)	adulthood)
Follow-up period	Follow-up calendar period for prospective/ retrospective studies
Follow-up duration (maximum)	Maximum follow-up period in years for prospective/
	retrospective studies
Follow-up duration (years)	Mean or median follow-up period in years for prospective/
(median/mean)	retrospective studies
Study type (cohort, nested case-	The epidemiological study design: cohort, nested case-control,
control, case-control, cross- sectional)	case-control, cross-sectional
Cohort name	The name of the epidemiological study
Age (years) (range/mean/median)	The age of the population studied (preference is to provide
	the mean or meadian age, when not available the range is
	given). Data is presented in years unless otherwise stated.
Gender (% male)	Percentage of males in study population
Active substance assessed	Pesticide assessed in the study as defined/named in the study
Active substance category	Chemical or functional pesticide category in which the
	pesticide is classified
Authorisation status	Pesticide active substances authorized within EU
	(06/09/2013). Yes/No/NA (NA=not applicable)
Biomarker name	The name of the biomarker of exposure to pesticide (if
	measured)
Control definition	Definition of the control group in case-control studies
Pesticide co-exposure (measured)	Did the study provided information on other co-exposed pesticides? (yes, no)
Population characteristics	Description of the population examined (gender, location,
	disease status)
Type of exposure (occupational,	What is the source of exposure to pesticides: occupational (if
environmental, both)	the exposure is related to a specific occupational activity);
	environmental (if the exposure is not related to any

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# 付録 II. データ抽出データベースの説明

Study ID	これは、研究の主要な影響ごとに順次与えられる研究の固有 ID である。
PUBMED _ID	これがその研究の PUBMED ID である(EMBASE の ID が提供されてい
	ない場合に PUBMED ID が提供され、PUBMED ID が提供されていない
	場合は研究のタイトルが提供された)。
筆頭著者	筆頭著者の姓
ジャーナル	研究が掲載された雑誌
年	出版年
围	研究実施国
場所(大陸)	研究実施大陸
募集期間	研究参加者を募集した期間
ばく露期間(妊娠前、乳児期、小児期、成	農薬ばく露が発生した発育期(妊娠前、妊娠期、乳児期、小児
人期、妊娠期)	期、思春期、成人期)
追跡期間	前向き/後ろ向き研究の追跡予定期間
追跡期間(最大)	前向き/後ろ向き研究の最大追跡期間は年単位である。
追跡期間(年)(中央値/平均値)	前向き/後ろ向き研究の平均または中央値の追跡期間 (年)。
研究の種類(コホート、コホート内症例対	疫学研究のデザイン:コホート、コホート内症例対照、症例対
照、症例対照、横断的)	照、横断的
コホート名	疫学研究の名称
年齢(年)(範囲/平均/中央値)	調査対象となった集団の年齢(平均年齢または中央値年齢を提示
	することを好む。データは、別段の記載がない限り、年単位で表
	示される。
性別(男性の割合)	調査対象集団における男性の割合
評価された有効成分	試験で定義・命名された試験で評価された農薬
有効成分のカテゴリー	農薬が分類されている化学的または機能的な農薬の分類
認可状況	EU 城内で認可された農薬有効成分(2013/09/06) Yes/No/NA (NA=該 当なし)
バイオマーカー名	農薬ばく露のバイオマーカー名(測定した場合)
対照定義	症例対照研究における対照群の定義
農薬の共ばく露(測定値)	その研究では、他の共ばく露農薬に関する情報を提供したか?(は
	い、いいえ)
集団の特徴	調査した集団の説明(性別、場所、病状)
ばく露の種類(職業的、環境的、両方)	農薬へのばく露源は何か:職業的(ばく露が特定の職業活動に関
	連している場合);環境的(ばく露がいかなる職業活動にも関連し
	ていない場合(例えば、住居内での農薬使用、ガーデニングでの
	農薬使用、ガーデニングに関連したばく露など);両方(職業ばく
	露と環境ばく露の両方が存在する場合)。
ばく露評価の種類(直接ばく露についての	農薬ばく露の測定方法:直接ばく露についての質問紙(面接また
質問紙/バイオマーカー/居住履歴/職歴	は自己記入);体液中のバイオマーカーの測定;居住歴;職業歴;
/JEM/専門家評価/環境 odeling 誤植と	職業ばく露マトリックス (JEM)。
思われる	

	occupational activity (e.g. domestic use of pesticides, use of pesticides in gardening, exposure related to gardening etc.); both (when both occupation and environmental exposure is present).
Type of exposure assessment (direct exposure questionnaire/ biomarker/residential history/occupational history/ JEM/ expert evaluation/ environmental odeling)	Means of measuring pesticide exposure: direct exposure questionnaire (interview or self-administered); measurement of biomarker in biological fluids; residential history; occupational history; Job Exposure Matrix (JEM)
Exposure definition	Definition of exposure as described in the study
Questionnaire type	Questionnaire type (interview or self administrated) (for studies which assessed exposure through questionnaires, else state n/a)
Measurement of biomarker (whole blood, plasma, urine, breast milk, placenta, nails, hair, saliva, adipose tissue)	Body fluid or tissue in which the biomarker was measured (whole blood, plasma, urine, breast milk, placenta, nails, hair, saliva, adipose tissue etc.)
Assay type	Type of biochemical assay used for biomarker measurement
Exposure duration	Duration of exposure to pesticides in years (when available)
Pediatric exposure type (mother, father, child, combinations)	For studies on child outcomes, describe means of exposure through self-exposure or parental exposure (mother, father, child, combinations)
Pediatric exposure time (preconception, pregnancy, combination)	For studies on child outcomes, was parental exposure during preconception, pregnancy or combinations?
Health outcome	Health outcome as described in the study
Outcome definition	Health outcome definition used in the study
Disease category	Disease category
Effect estimate type (RR, OR, HR, beta, MD, SMD)	Type of effect estimate for the assessment of pesticide and health outcome relationship (RR, OR, HR, beta, MD, SMD)
Effect (binary, continuous)	Effect estimated on a binary or continuous manner (binary, continuous)
Comparison unit (yes/no, unit increase,)	The definition of comparison for the calculation of the effect size (yes/no, unit increase etc.)
Effect estimate	Value of effect estimate
SE/SD effect stimate	Standard error/Standard deviation of effect estimate
Lower 95% CI	Lower 95% confidence interval of the effect estimate
Higher 95% CI	Higher 95% confidence interval of the effect estimate
Adjustment for	Confounders/ variables for which the effect estimate was adjusted for
Controls matched for	Variables for which controls were matched to cases (case control studies only)
Sample size	Total number of participants
N cases	Number of cases
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バノボージャ	研究に記掛きしていてばく雪のウギ
ばく 露の 定義	研究に記載されているばく露の定義 質問紙の種類(面接または自己管理)(質問紙を用いてばく露を評
資間紙の種類	負向紙の種類(面接または自己管理)(負向紙を用いてはく露を許 価した研究の場合、そうでない場合は該当なし)
バイオマーカーの測定(全血、血漿、尿、	バイオマーカーが測定された体液または組織(全血、血漿、尿、
母乳、胎盤、爪、毛髪、唾液、脂肪組織)	母乳、胎盤、爪、毛髪、唾液、脂肪組織など)
アッセイタイプ	バイオマーカー測定に使用した生化学的アッセイの種類
ばく露期間	年単位での農薬ばく露期間(入手可能な場合)
小児ばく露タイプ(母、父、小児、組合わ	小児の影響に関する研究については、自己ばく露または親のばく
せ)	露(母親、父親、小児、組合わせ)を通じたばく露手段を記述
小児ばく露時期(妊娠前、妊娠期、両方)	小児の影響に関する研究では、親のばく露は妊娠前、妊娠期、または両方の期間に行われたか?
健康影響	研究に記載されている通りの健康影響
影響の定義	研究で使用された健康影響の定義
疾患カテゴリ	疾患カテゴリ
効果推定タイプ (RR、OR、HR、β、MD、	農薬と健康影響の関係を評価するための効果推定値の種類(RR,
SMD)	OR, HR, $\beta$ , MD, SMD)
効果(2変数、連続変数)	2変数法または連続変数法で推定された効果(2変数、連続変数)
比較単位(はい/いいえ、単位増加、	効果量を算出するための比較の定義(はい/いいえ、単位増加な ど)
効果推定	効果推定値
SE/SD 効果推定	効果推定値の標準誤差・標準偏差
95% CI の下限	効果推定値の 95%信頼区間の下限
95% CI の上限	効果推定値の 95%信頼区間の上限
調整	効果推定値の交絡因子/変数の調整
マッチした対照	対照の変数を症例にマッチさせた(症例対照研究のみ)
サンプルサイズ	参加者総数
N症例	症例数
N 対照	対照数
統計的手法	効果量の計算に用いられる統計的手法
試験デザイン(前向き、後ろ向き、混合、	研究デザインの前向き型または後ろ向き型(前向き、後ろ向き、
横断)	混合、横断)
包含/除外の基準の明記	研究参加者(母集団)の説明は詳細に行われていましたか?(はい
(はい、一部、いいえ)	/一部/いいえ)
著者による検出力の言及	著者らは、統計分析の前または後に論文で検出力について言及し
(はい、いいえ)	ているか?(はい/いいえ)
ばく露レベル(高、中、低)の詳細記述	農薬ばく露の定義でばく露レベル(高、中、低)の詳細記述
ばく露量の妥当な測定 :	ばく露量の測定は妥当であったか:バイオマーカー(はい);小規
(バイオマーカー(有);小規模区域の生態	模区域の生態学的測定、職種、質問紙(一部);大規模区域の生態
学的測定、職種、質問紙(一部); 大規模区	学的測定に基づいた(いいえ)
域の生態学的測定に基づいた(無))	
ばく露量の測定は特定のものであったか?	ばく露量の測定は特定のものであったか?(はい);より広範で化
はい;より広範で化学的に関連したグルー	学的に関連したグループに基づく(一部);多様な化学的及び毒性
プに基づく (一部);多様な化学的及び毒	学的特性の広範なグループ化に基づく(いいえ)

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N controls	Number of controls
Statistical method	Statistical method used to calculate the effect size
Study design (prospective, retrospective, mixed, cross- sectional)	Prospective or retrospective type of study design (prospective, retrospective, mixed, cross-sectional)
Inclusion/exclusion criteria clearly stated (yes, partially, no) Authors mention power calculations (yes, no) Level of detail in describing exposure (high, medium, low) Robust measurement of exposure. (biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)	Was the description of study participants (population) inclusion and exclusion criteria detailed? (yes/partially/no) Do the authors mention power calculations in the manuscript preceding or proceeding their statistical analysis (yes/no) Level of detail in which the definition of exposure to pesticides is provided (high/medium/low) Was the measurement of exposure robust: biomarker (yes); small area ecological measures, job titles, questionnaire (partial); was based on large area ecological measures (no)
Were measures of exposure specific? Yes; based on broader, chemically-related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)	Were measures of exposure specific? (yes); based on broader, chemically-related groups (partial); based on broad groupings of diverse chemical and toxicological properties (no)
Attempt to balance the allocation between the groups (e.g., through stratification, matching)	Was an attempt to balance the allocation between the groups in case-control studies either through stratification or matching (yes/no)?
Adjustment performed for potential confounders (yes, some, no)	Was the effect size adjusted for potential confounders (yes, some, no)?
Assessors blinded to exposure status (for cohort studies)	Were the assessors blinded to exposure status in cohort studies (yes/no/;n/a:not available or not applicable when studies are not cohorts)?
Outcomes assessed using valid and reliable measures, implemented consistently across all study participants?	Were the outcomes assessed using valid and reliable measures implemented consistently across all study participants (yes/no)
Sample size (top [991], middle, bottom quartiles[104])	The size of the sample
Was source of funding acknowledged	Do the authors acknowledge any possible source of funding (yes/no)
Rough quality assessment	Rough quality assessment taking into account the data in all other columns of the quality assessment of data extraction form
COMMENTS	Any comments related to the study that help interpretation of the data extracted

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性学的特性の広範なグループに基づく(い いえ)	
グループ間の配分のバランスを図る(層別 化、マッチングなど)	症例対照研究では、層別化またはマッチングによってグループ間 の配分のバランスをとる試みが行われたか?(はい/いいえ)
潜在的な交絡因子の調整を行った(はい、 いくつか、いいえ)	潜在的な交絡因子について効果量を調整したか?(はい、いくつ か、いいえ)
ばく露状況を盲検化された評価者(コホー ト研究の場合)	コホート研究では、評価者はばく露状況を盲検化されていたか? (はい/いいえ/:n/a:コホート研究でない場合は、成果なし、また は該当なし)
影響は、すべての研究参加者に一貫して実 施された有効かつ信頼性のある測定を用い て評価されたか?	影響は、すべての研究参加者に一貫して実施された有効で信頼性 のある測定を用いて評価されたか? (はい/いいえ)
サンプルサイズ(四分位数の上部[991]、 中間部、下部[104])	サンプルの大きさ
資金源の承認	著者は、資金調達の可能性があることを認めているか(はい/いい え)
大まかな品質評価	データ抽出フォームの品質評価の他のすべての列のデータを考慮 した大まかな品質評価
コメント	抽出されたデータの解釈に役立つ研究に関するコメント

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#### **APPENDIX III. REFERENCES TO THE DATA EXTRACTION DATABASE**

- A.S Al-Sarar, Y. Abo Bakr, G.S Al-Erimah, H.I Hussein, A.E Bayoumi. Hematological and biochemical alterations in occupationally pesticide-exposed workers of Riyadh municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology 3 (4) : 179-185, 2009 ISSN 1819-3420
- Abadi-Korek I, Stark B, Zaizov R, Shaham J. Parental occupational exposure and the risk of acute lymphoblastic leukemia in offspring in israel. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:165-174
- Abdel Rasoul GM, Abou Salem ME, Mechael AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. Neurotoxicology. 2008;29:833-838
- Abdelouahab N, Ainmelk Y, Takser L. Polybrominated diphenyl ethers and sperm quality. Reprod Toxicol. 2011;31:546-550
- Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, Spear PA, Chan HM. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of quebec (canada). Environmental research. 2008;107:380-392
- Abu Sham'a F, Skogstad M, Nijem K, Bjertness E, Kristensen P. Lung function and respiratory symptoms in male palestinian farmers. Arch Environ Occup Health. 2010;65:191-200
- Ahamed M, Anand M, Kumar A, Siddiqui MK. Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. Clinical biochemistry. 2006;39:762-766
- Ahrens W, Mambetova C, Bourdon-Raverdy N, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Zambon P. Occupational exposure to endocrine-disrupting compounds and biliary tract cancer among men. Scand J Work Environ Health. 2007;33:387-396
- Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. Diabetes care. 2011;34:1972-1979
- Al-Saleh I, Al-Doush I, Alsabbaheen A, Mohamed Gel D, Rabbah A. Levels of ddt and its metabolites in placenta, maternal and cord blood and their potential influence on neonatal anthropometric measures. The Science of the total environment. 2012;416:62-74
- Alavanja MCR, Sandler DP, Hoppin JA, Schroeder P, Lynch CF, Blair A, Mahajan R. Fonofos exposure and cancer incidence in the agricultural health study. Environmental Health Perspectives. 2006
- Albers JW, Garabrant DH, Mattsson JL, Burns CJ, Cohen SS, Sima C, Garrison RP, Richardson RJ, Berent S. Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. Toxicological sciences: an official journal of the Society of Toxicology. 2007;97:196-204

Alderton LE, Spector LG, Blair CK, Roesler M, Olshan AF, Robison LL, Ross JA. Child and

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environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:165-174 Abdel Rasoul GM, Abou Salem ME, Mechael AA, Hendy OM, Rohlman DS, Ismail AA. Effects of

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付録 III. データ抽出データベースの参考文献

3 (4) : 179-185, 2009 ISSN 1819-3420

occupational pesticide exposure on children applying pesticides. Neurotoxicology. 2008;29:833-838

A.S Al-Sarar, Y. Abo Bakr, G.S Al-Erimah, H.I Hussein, A.E Bayoumi. Hematological and biochemical alterations in occupationally pesticide-exposed workers of Rivadh

Abadi-Korek I, Stark B, Zaizov R, Shaham J. Parental occupational exposure and the risk

municipality, Kingdom of Saudi Arabia. Research Journal of Environmental Toxicology

- Abdelouahab N, Ainmelk Y, Takser L. Polybrominated diphenyl ethers and sperm quality. Reprod Toxicol. 2011;31:546-550
- Abdelouahab N, Mergler D, Takser L, Vanier C, St-Jean M, Baldwin M, Spear PA, Chan HM. Gender differences in the effects of organochlorines, mercury, and lead on thyroid hormone levels in lakeside communities of quebec (canada). Environmental research. 2008;107:380-392
- Abu Sham'a F, Skogstad M, Nijem K, Bjertness E, Kristensen P. Lung function and respiratory symptoms in male palestinian farmers. Arch Environ Occup Health. 2010;65:191-200
- Ahamed M, Anand M, Kumar A, Siddiqui MK. Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. Clinical biochemistry. 2006;39:762-766
- Ahrens W, Mambetova C, Bourdon-Raverdy N, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Zambon P. Occupational exposure to endocrine-disrupting compounds and biliary tract cancer among men. Scand J Work Environ Health. 2007;33:387-396
- Airaksinen R, Rantakokko P, Eriksson JG, Blomstedt P, Kajantie E, Kiviranta H. Association between type 2 diabetes and exposure to persistent organic pollutants. Diabetes care. 2011;34:1972-1979
- Al-Saleh I, Al-Doush I, Alsabbaheen A, Mohamed Gel D, Rabbah A. Levels of ddt and its metabolites in placenta, maternal and cord blood and their potential influence on neonatal anthropometric measures. The Science of the total environment. 2012;416:62-74
- Alavanja MCR, Sandler DP, Hoppin JA, Schroeder P, Lynch CF, Blair A, Mahajan R. Fonofos exposure and cancer incidence in the agricultural health study. Environmental Health Perspectives. 2006
- Albers JW, Garabrant DH, Mattsson JL, Burns CJ, Cohen SS, Sima C, Garrison RP, Richardson RJ, Berent S. Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. Toxicological sciences: an official journal of the Society of Toxicology. 2007;97:196-204

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of acute lymphoblastic leukemia in offspring in israel. Journal of occupational and

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maternal household chemical exposure and the risk of acute leukemia in children with Down's syndrome: A report from the children's oncology group. American journal of epidemiology. 2006;164:212-221

- Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009;43:7909-7915
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, Sunyer J. Thyroid disruption at birth due to prenatal exposure to betahexachlorocyclohexane. Environment international. 2008;34:737-740
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Grimalt JO, Sunyer J. Effects of pcbs, p,p'-ddt, p,p'-dde, hcb and beta-hch on thyroid function in preschool children. Occupational and environmental medicine. 2008;65:452-457
- Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jorgensen E, Kjaerstad MB, Baelum J, Nielsen JB, Skakkebaek NE, Main KM. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 2008;116:566-572
- Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, Silverman DT, Alavanja MC. Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort. International journal of cancer. Journal international du cancer. 2009;124:2495-2500
- Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental ddt exposure in young men living in a malaria area in the limpopo province, south africa. Journal of andrology. 2007;28:423-434
- Araoud M, Neffeti F, Douki W, Najjar MF, Kenani A. Paraoxonase 1 correlates with butyrylcholinesterase and gamma glutamyl transferase in workers chronically exposed to pesticides. Journal of occupational health. 2010;52:383-388
- Arcury TA, Feldman SR, Schulz MR, Vallejos Q, Verma A, Fleischer AB, Jr., Rapp SR, Davis SF, Preisser JS, Quandt SA. Diagnosed skin diseases among migrant farmworkers in North Carolina: Prevalence and risk factors. Journal of agricultural safety and health. 2007;13:407-418
- Arguelles LM, Liu X, Venners SA, Ronnenberg AG, Li Z, Yang F, Yang J, Xu X, Wang X. Serum folate and DDT isomers and metabolites are inversely associated in Chinese women: A cross-sectional analysis. Journal of the American College of Nutrition. 2009;28:380-387
- Aronson KJ, Wilson JW, Hamel M, Diarsvitri W, Fan W, Woolcott C, Heaton JP, Nickel JC, Macneily A, Morales A. Plasma organochlorine levels and prostate cancer risk. Journal of exposure science & environmental epidemiology. 2010;20:434-445
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of ddt and their association with reproductive hormones in adult men from northern thailand. The Science of the total environment. 2006;355:98-105
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand. Environment international. 2006;32:554-559
- Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, Schwarzschild MA, Thun MJ. Pesticide exposure and risk for Parkinson's disease. Annals of neurology.

- Alderton LE, Spector LG, Blair CK, Roesler M, Olshan AF, Robison LL, Ross JA. Child and maternal household chemical exposure and the risk of acute leukemia in children with Down"s syndrome: A report from the children's oncology group. American journal of epidemiology. 2006;164:212-221
- Alvarez-Pedrerol M, Guxens M, Ibarluzea J, Rebagliato M, Rodriguez A, Espada M, Goni F, Basterrechea M, Sunyer J. Organochlorine compounds, iodine intake, and thyroid hormone levels during pregnancy. Environmental science & technology. 2009;43:7909-7915
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, Sunyer J. Thyroid disruption at birth due to prenatal exposure to betahexachlorocyclohexane. Environment international. 2008;34:737-740
- Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Grimalt JO, Sunyer J. Effects of pcbs, p,p'-ddt, p,p'-dde, hcb and beta-hch on thyroid function in preschool children. Occupational and environmental medicine. 2008;65:452-457
- Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jorgensen E, Kjaerstad MB, Baelum J, Nielsen JB, Skakkebaek NE, Main KM. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ Health Perspect. 2008;116:566-572
- Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, Silverman DT, Alavanja MC. Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort. International journal of cancer. Journal international du cancer. 2009;124:2495-2500
- Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental ddt exposure in young men living in a malaria area in the limpopo province, south africa. Journal of andrology. 2007;28:423-434
- Araoud M, Neffeti F, Douki W, Najjar MF, Kenani A. Paraoxonase 1 correlates with butyrylcholinesterase and gamma glutamyl transferase in workers chronically exposed to pesticides. Journal of occupational health. 2010;52:383-388
- Arcury TA, Feldman SR, Schulz MR, Vallejos Q, Verma A, Fleischer AB, Jr., Rapp SR, Davis SF, Preisser JS, Quandt SA. Diagnosed skin diseases among migrant farmworkers in North Carolina: Prevalence and risk factors. Journal of agricultural safety and health. 2007;13:407-418
- Arguelles LM, Liu X, Venners SA, Ronnenberg AG, Li Z, Yang F, Yang J, Xu X, Wang X. Serum folate and DDT isomers and metabolites are inversely associated in Chinese women: A cross-sectional analysis. Journal of the American College of Nutrition. 2009;28:380-387
- Aronson KJ, Wilson JW, Hamel M, Diarsvitri W, Fan W, Woolcott C, Heaton JP, Nickel JC, Macneily A, Morales A. Plasma organochlorine levels and prostate cancer risk. Journal of exposure science & environmental epidemiology. 2010;20:434-445
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of ddt and their association with reproductive hormones in adult men from northern thailand. The Science of the total environment. 2006;355:98-105
- Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand. Environment international. 2006;32:554-559

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

2006;60:197-203

- Avivar Oyonarte C., Duran Salas I., Molina Arrebola M.A., Castilla Alcala J.A., Olea Serrano N., Fernandez Cabrera M. Pesticide exposure and decreased sperm count. Revista del Laboratorio Clinico (2010) 3:1 (4-11).
- Axmon A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Zvyezday V, Ludwicki JK, Jonsson BA, Toft G, Bonde JP, Hagmar L. Time to pregnancy as a function of male and female serum concentrations of 2.24,4'5,5'-hexachlorobiphenyl (cb-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-dde). Hum Reprod. 2006;21:657-665
- Azmi MA, Naqvi SN, Akhtar K, Moinuddin, Parveen S, Parveen R, Aslam M. Effect of pesticide residues on health and blood parameters of farm workers from rural gadap. karachi, pakistan. Journal of environmental biology / Academy of Environmental Biology, India. 2009;30:747-756
- Baharuddin MR, Sahid IB, Noor MA, Sulaiman N, Othman F. Pesticide risk assessment: A study on inhalation and dermal exposure to 2.4-d and paraguat among malaysian paddy farmers. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:600-607
- Bahena-Medina LA, Torres-Sanchez L, Schnaas L, Cebrian ME, Chavez CH, Osorio-Valencia E, Hernandez RM, Lopez-Carrillo L. Neonatal neurodevelopment and prenatal exposure to dichlorodiphenyldichloroethylene (dde): A cohort study in Mexico. Journal of exposure science & environmental epidemiology. 2011;21:609-614
- Bailey HD, Armstrong BK, de Klerk NH, Fritschi L, Attia J, Scott RJ, Smibert E, Milne E, Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. International journal of cancer. Journal international du cancer. 2011;129:1678-1688
- Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, Le ND. Prostate cancer risk and exposure to pesticides in British Columbia farmers. The Prostate. 2011;71:168-183
- Baranska M, Van Amelsvoort L, Birindelli S, Fustinoni S, Corsini E, Liesivuori J, Van Loveren H. Association of pesticide exposure, vaccination response, and interleukin-1 gene polymorphisms. Human & experimental toxicology. 2008;27:709-713
- Barczyk A, Sozanska E, Pierzchala W. [the influence of occupational exposure to pesticides on the frequency of chronic obstructive pulmonary diseases]. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2006;59:596-600
- Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in new jersey. The Science of the total environment. 2010;408:790-795
- Barry KH, Koutros S, Berndt SI, Andreotti G, Hoppin JA, Sandler DP, Burdette LA, Yeager M, Freeman LE, Lubin JH, Ma X, Zheng T, Alavanja MC. Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk. Environ Health Perspect. 2011:119:1726-1732
- Barry KH, Koutros S, Lubin JH, Coble JB, Barone-Adesi F, Beane Freeman LE, Sandler DP, Hoppin JA, Ma X, Zheng T, Alavanja MC. Methyl bromide exposure and cancer risk in the agricultural health study. Cancer causes & control: CCC. 2012;23:807-818

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Ascherio A, Chen H, Weisskopf MG, O'Reillv E, McCullough ML, Calle EE, Schwarzschild MA, Thun MJ. Pesticide exposure and risk for Parkinson"s disease. Annals of neurology. 2006;60:197-203
- Avivar Oyonarte C., Duran Salas I., Molina Arrebola M.A., Castilla Alcala J.A., Olea Serrano N., Fernandez Cabrera M. Pesticide exposure and decreased sperm count. Revista del Laboratorio Clinico (2010) 3:1 (4-11).
- Axmon A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Zvyezday V, Ludwicki JK, Jonsson BA, Toft G, Bonde JP, Hagmar L. Time to pregnancy as a function of male and female serum concentrations of 2, 2'4, 4'5, 5'-hexachlorobiphenyl (cb-153) and 1, 1-dichloro-2, 2-bis (p-chlorophenyl)-ethylene (p, p'-dde). Hum Reprod. 2006;21:657-665
- Azmi MA, Naqvi SN, Akhtar K, Moinuddin, Parveen S, Parveen R, Aslam M. Effect of pesticide residues on health and blood parameters of farm workers from rural gadap, karachi, pakistan. Journal of environmental biology / Academy of Environmental Biology, India. 2009;30:747-756
- Baharuddin MR, Sahid IB, Noor MA, Sulaiman N, Othman F. Pesticide risk assessment: A study on inhalation and dermal exposure to 2,4-d and paraguat among malaysian paddy farmers. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:600-607
- Bahena-Medina LA, Torres-Sanchez L, Schnaas L, Cebrian ME, Chavez CH, Osorio-Valencia E, Hernandez RM, Lopez-Carrillo L. Neonatal neurodevelopment and prenatal exposure to dichlorodiphenyldichloroethylene (dde): A cohort study in Mexico. Journal of exposure science & environmental epidemiology. 2011;21:609-614
- Bailey HD, Armstrong BK, de Klerk NH, Fritschi L, Attia J, Scott RJ, Smibert E, Milne E. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. International journal of cancer. Journal international du cancer. 2011;129:1678-1688
- Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP, Le ND. Prostate cancer risk and exposure to pesticides in British Columbia farmers. The Prostate. 2011;71:168-183
- Baranska M, Van Amelsvoort L, Birindelli S, Fustinoni S, Corsini E, Liesivuori J, Van Loveren H. Association of pesticide exposure, vaccination response, and interleukin-1 gene polymorphisms. Human & experimental toxicology. 2008;27:709-713
- Barczyk A, Sozanska E, Pierzchala W. [the influence of occupational exposure to pesticides on the frequency of chronic obstructive pulmonary diseases]. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2006;59:596-600
- Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in new jersey. The Science of the total environment. 2010;408:790-795
- Barry KH, Koutros S, Berndt SI, Andreotti G, Hoppin IA, Sandler DP, Burdette LA, Yeager M, Freeman LE, Lubin JH, Ma X, Zheng T, Alavanja MC. Genetic variation in base excision repair pathway genes, pesticide exposure, and prostate cancer risk. Environ Health Perspect. 2011;119:1726-1732
- Barry KH, Koutros S, Lubin JH, Coble JB, Barone-Adesi F, Beane Freeman LE, Sandler DP, Hoppin IA, Ma X, Zheng T, Alavanja MC, Methyl bromide exposure and cancer risk in the agricultural health study. Cancer causes & control: CCC. 2012;23:807-818

<sup>113</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

- Bayrami M, Hashemi T, Malekirad AA, Ashayeri H, Faraji F, Abdollahi M. Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. Toxicology and industrial health. 2012;28:90-96
- Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, Sandler DP, Kamel F. Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. Environ Health Perspect. 2011;119:1610-1615
- Behrens T, Lynge E, Cree I, Lutz JM, Eriksson M, Guenel P, Merletti F, Morales-Suarez-Varela M, Afonso N, Stengrevics A, Fevotte J, Sabroe S, Llopis-Gonzalez A, Gorini G, Hardell L, Stang A, Ahrens W. Pesticide exposure in farming and forestry and the risk of uveal melanoma. Cancer causes & control: CCC. 2012;23:141-151
- Beltrame D, Lo Cascio N, Miotto D, Mapp CE, De Rosa E, Boschetto P. [occupational exposure and chronic heart failure severity]. Giornale italiano di medicina del lavoro ed ergonomia. 2007;29:438-439
- Bergonzi R, De Palma G, Specchia C, Dinolfo M, Tomasi C, Frusca T, Apostoli P. Persistent organochlorine compounds in fetal and maternal tissues: Evaluation of their potential influence on several indicators of fetal growth and health. The Science of the total environment. 2011;409:2888-2893
- Bertrand KA, Spiegelman D, Aster JC, Altshul LM, Korrick SA, Rodig SJ, Zhang SM, Kurth T, Laden F. Plasma organochlorine levels and risk of non-hodgkin lymphoma in a cohort of men. Epidemiology. 2010;21:172-180
- Beseler C, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:1005-1013
- Beseler CL, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures among private pesticide applicators enrolled in the agricultural health study. Environ Health Perspect. 2008;116:1713-1719
- Bhalli JA, Khan QM, Haq MA, Khalid AM, Nasim A. Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis. 2006;21:143-148
- Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, Doody DR, Fish S, Needham LL, Chen C, Schwartz SM. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: A population-based case-control study. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:2012-2018
- Blanco-Munoz J, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Bassol S, Cebrian ME, Lopez-Flores I, Ruiz-Perez I. Effect of exposure to p.p'-dde on male hormone profile in mexican flower growers. Occupational and environmental medicine. 2012;69:5-11
- Blanco-Munoz J, Morales MM, Lacasana M, Aguilar-Garduno C, Bassol S, Cebrian ME. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of morelos, Mexico. Hum Reprod. 2010;25:1787-1795

Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Cause-specific

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Bayrami M, Hashemi T, Malekirad AA, Ashayeri H, Faraji F, Abdollahi M. Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. Toxicology and industrial health. 2012;28:90-96
- Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, Sandler DP, Kamel F. Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. Environ Health Perspect. 2011;119:1610-1615
- Behrens T, Lynge E, Cree I, Lutz JM, Eriksson M, Guenel P, Merletti F, Morales-Suarez-Varela M, Afonso N, Stengrevics A, Fevotte J, Sabroe S, Llopis-Gonzalez A, Gorini G, Hardell L, Stang A, Ahrens W. Pesticide exposure in farming and forestry and the risk of uveal melanoma. Cancer causes & control: CCC. 2012;23:141-151
- Beltrame D, Lo Cascio N, Miotto D, Mapp CE, De Rosa E, Boschetto P. [occupational exposure and chronic heart failure severity]. Giornale italiano di medicina del lavoro ed ergonomia. 2007;29:438-439
- Bergonzi R, De Palma G, Specchia C, Dinolfo M, Tomasi C, Frusca T, Apostoli P. Persistent organochlorine compounds in fetal and maternal tissues: Evaluation of their potential influence on several indicators of fetal growth and health. The Science of the total environment. 2011;409:2888-2893
- Bertrand KA, Spiegelman D, Aster JC, Altshul LM, Korrick SA, Rodig SJ, Zhang SM, Kurth T, Laden F. Plasma organochlorine levels and risk of non-hodgkin lymphoma in a cohort of men. Epidemiology. 2010;21:172-180
- Beseler C, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:1005-1013
- Beseler CL, Stallones L, Hoppin JA, Alavanja MC, Blair A, Keefe T, Kamel F. Depression and pesticide exposures among private pesticide applicators enrolled in the agricultural health study. Environ Health Perspect. 2008;116:1713-1719
- Bhalli JA, Khan QM, Haq MA, Khalid AM, Nasim A. Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis. 2006;21:143-148
- Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, Doody DR, Fish S, Needham LL, Chen C, Schwartz SM. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: A population-based case-control study. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:2012-2018
- Blanco-Munoz J, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Bassol S, Cebrian ME, Lopez-Flores I, Ruiz-Perez I. Effect of exposure to p,p'-dde on male hormone profile in mexican flower growers. Occupational and environmental medicine. 2012;69:5-11
- Blanco-Munoz J, Morales MM, Lacasana M, Aguilar-Garduno C, Bassol S, Cebrian ME. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of morelos, Mexico. Hum Reprod. 2010;25:1787-1795
- Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Cause-specific mortality of dutch chlorophenoxy herbicide manufacturing workers. Occupational and environmental medicine. 2010;67:24-31

<sup>114</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

mortality of dutch chlorophenoxy herbicide manufacturing workers. Occupational and environmental medicine. 2010;67:24-31

- Boers D, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Campo L, Bosetti C, La Vecchia C, Vergieva T, Tarkowski M, Liesivuori J, Steerenberg P, van Loveren H. Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the europit field studies. Human & experimental toxicology. 2008;27:721-727
- Bonde JP, Toft G, Rylander L, Rignell-Hydbom A, Giwercman A, Spano M, Manicardi GC, Bizzaro D, Ludwicki JK, Zvyezday V, Bonefeld-Jorgensen EC, Pedersen HS, Jonsson BA, Thulstrup AM. Fertility and markers of male reproductive function in Inuit and European populations spanning large contrasts in blood levels of persistent organochlorines. Environ Health Perspect. 2008;116:269-277
- Bonner MR, Coble J, Blair A, Beane Freeman LE, Hoppin JA, Sandler DP, Alavanja MC. Malathion exposure and the incidence of cancer in the agricultural health study. American journal of epidemiology. 2007;166:1023-1034
- Bonner MR, Williams BA, Rusiecki JA, Blair A, Beane Freeman LE, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC. Occupational exposure to terbufos and the incidence of cancer in the agricultural health study. Cancer causes & control: CCC. 2010;21:871-877
- Bonvicini F, Marcello N, Mandrioli J, Pietrini V, Vinceti M. Exposure to pesticides and risk of amyotrophic lateral sclerosis: A population-based case-control study. Annali dell'Istituto superiore di sanita. 2010;46:284-287
- Borkowski WJ, Riederer A, Prapamontol T. Neurological evaluation of newborn infants of mothers working in citrus groves in northern Thailand. International journal of occupational and environmental health. 2011;17:135-143
- Bornman R, de Jager C, Worku Z, Farias P, Reif S. Ddt and urogenital malformations in newborn boys in a malarial area. BJU international. 2010;106:405-411
- Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics. 2010;125:e1270-1277
- Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr DB, Eskenazi B. Prenatal exposure to organophosphate pesticides and iq in 7-year-old children. Environ Health Perspect. 2011;119:1189-1195
- Bräuner EV, Sørensen M, Gaudreau E, LeBlanc A, Eriksen KT, Tjønneland A, Overvad K, Raaschou-Nielsen O. A prospective study of organochlorines in adipose tissue and risk of non-hodgkin lymphoma. Environmental Health Perspectives. 2011;120:105-111
- Brender JD, Felkner M, Suarez L, Canfield MA, Henry JP. Maternal pesticide exposure and neural tube defects in Mexican Americans. Annals of epidemiology. 2010;20:16-22
- Bretveld R, Zielhuis GA, Roeleveld N. Time to pregnancy among female greenhouse workers. Scandinavian Journal of Work, Environment & Health. 2006;32:359-367
- Bretveld RW, Hooiveld M, Zielhuis GA, Pellegrino A, van Rooij IA, Roeleveld N. Reproductive disorders among male and female greenhouse workers. Reprod Toxicol. 2008;25:107-114
- Brighina L, Frigerio R, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Farrer

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EFSA supporting publication 2013:EN-497

### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Pesticide epidemiology
- Boers D, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Campo L, Bosetti C, La Vecchia C, Vergieva T, Tarkowski M, Liesivuori J, Steerenberg P, van Loveren H. Asthmatic symptoms after exposure to ethylenebisdithiocarbamates and other pesticides in the europit field studies. Human & experimental toxicology. 2008;27:721-727
- Bonde JP, Toft G, Rylander L, Rignell-Hydbom A, Giwercman A, Spano M, Manicardi GC, Bizzaro D, Ludwicki JK, Zvyezday V, Bonefeld-Jorgensen EC, Pedersen HS, Jonsson BA, Thulstrup AM. Fertility and markers of male reproductive function in Inuit and European populations spanning large contrasts in blood levels of persistent organochlorines. Environ Health Perspect. 2008;116:269-277
- Bonner MR, Coble J, Blair A, Beane Freeman LE, Hoppin JA, Sandler DP, Alavanja MC. Malathion exposure and the incidence of cancer in the agricultural health study. American journal of epidemiology. 2007;166:1023-1034
- Bonner MR, Williams BA, Rusiecki JA, Blair A, Beane Freeman LE, Hoppin JA, Dosemeci M, Lubin J, Sandler DP, Alavanja MC. Occupational exposure to terbufos and the incidence of cancer in the agricultural health study. Cancer causes & control: CCC. 2010;21:871-877
- Bonvicini F, Marcello N, Mandrioli J, Pietrini V, Vinceti M. Exposure to pesticides and risk of amyotrophic lateral sclerosis: A population-based case-control study. Annali dell'Istituto superiore di sanita. 2010;46:284-287
- Borkowski WJ, Riederer A, Prapamontol T. Neurological evaluation of newborn infants of mothers working in citrus groves in northern Thailand. International journal of occupational and environmental health. 2011;17:135-143
- Bornman R, de Jager C, Worku Z, Farias P, Reif S. Ddt and urogenital malformations in newborn boys in a malarial area. BJU international. 2010;106:405-411
- Bouchard MF, Bellinger DC, Wright RO, Weisskopf MG. Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. Pediatrics. 2010;125:e1270- 1277
- Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr DB, Eskenazi B. Prenatal exposure to organophosphate pesticides and iq in 7-year-old children. Environ Health Perspect. 2011;119:1189-1195
- Bräuner EV, Sørensen M, Gaudreau E, LeBlanc A, Eriksen KT, Tjønneland A, Overvad K, Raaschou-Nielsen O. A prospective study of organochlorines in adipose tissue and risk of non-hodgkin lymphoma. Environmental Health Perspectives. 2011;120:105-111
- Brender JD, Felkner M, Suarez L, Canfield MA, Henry JP. Maternal pesticide exposure and neural tube defects in Mexican Americans. Annals of epidemiology. 2010;20:16-22
- Bretveld R, Zielhuis GA, Roeleveld N. Time to pregnancy among female greenhouse workers. Scandinavian Journal of Work, Environment & Health. 2006;32:359-367
- Bretveld RW, Hooiveld M, Zielhuis GA, Pellegrino A, van Rooij IA, Roeleveld N. Reproductive disorders among male and female greenhouse workers. Reprod Toxicol. 2008;25:107-114
- Brighina L, Frigerio R, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Farrer MJ, Lincoln SJ, Checkoway H, Rocca WA, Maraganore DM. Alpha-synuclein, pesticides, and Parkinson disease: A case-control study. Neurology. 2008;70:1461-1469

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

MJ, Lincoln SJ, Checkoway H, Rocca WA, Maraganore DM. Alpha-synuclein, pesticides, and Parkinson disease: A case-control study. Neurology. 2008;70:1461-1469

- Brooks K, Hasan H, Samineni S, Gangur V, Karmaus W. Placental p,p'dichlorodiphenyldichloroethylene and cord blood immune markers. Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2007;18:621-624
- Brouwers MM, Feitz WF, Roelofs LA, Kiemeney LA, de Gier RP, Roeleveld N. Risk factors for hypospadias. European journal of pediatrics. 2007;166:671-678
- Browne RO, Moyal-Segal LB, Zumsteg D, David Y, Kofman O, Berger A, Soreq H, Friedman A. Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2006;20:1733-1735
- Brucker-Davis F, Ducot B, Wagner-Mahler K, Tommasi C, Ferrari P, Pacini P, Boda-Buccino M, Bongain A, Azuar P, Fenichel P. [environmental pollutants in maternal milk and cryptorchidism]. Gynecologie, obstetrique & fertilite. 2008;36:840-847
- Brucker-Davis F, Ferrari P, Boda-Buccino M, Wagner-Mahler K, Pacini P, Gal J, Azuar P, Fenichel P. Cord blood thyroid tests in boys born with and without cryptorchidism: Correlations with birth parameters and in utero xenobiotics exposure. Thyroid: official journal of the American Thyroid Association. 2011;21:1133-1141
- Brucker-Davis F, Wagner-Mahler K, Bornebusch L, Delattre I, Ferrari P, Gal J, Boda-Buccino M, Pacini P, Tommasi C, Azuar P, Bongain A, Fenichel P. Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from nice area (france). Chemosphere. 2010;81:169-176
- Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A, Kurzenne JY, Mas JC, Fenichel P. Cryptorchidism at birth in nice area (France) is associated with higher prenatal exposure to pcbs and dde, as assessed by colostrum concentrations. Hum Reprod. 2008;23:1708-1718
- Brulls C., Niggemann H., Weissbach W., Dott W., Fischer M., Merk H.F., Blomeke B., Isselstein J., Ilgner, Westhofen M., Wiesmuller G.A.. Pilot study on living conditions and living factors investigated in patients suffering from self-reported multiple chemical sensitivity, fragrance allergies or polyposis nasi. Atemwegs- und Lungenkrankheiten (2008) 34:5 (187-198)
- Buck Louis GM, Rios LI, McLain A, Cooney MA, Kostyniak PJ, Sundaram R. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere. 2011;85:1742-1748
- Burdorf A, Brand T, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA. The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: The generation r study. Occupational and environmental medicine. 2011;68:197-204
- Burns JS, Williams PL, Sergeyev O, Korrick SA, Lee MM, Revich B, Altshul L, Del Prato JT, Humblet O, Patterson DG, Turner WE, Starovoytov M, Hauser R. Serum concentrations of organochlorine pesticides and growth among russian boys. Environ Health Perspect. 2012;120:303-308

Bustamante Montes LP, Waliszewski S, Hernandez-Valero M, Sanin-Aguirre L, Infanzon-Ruiz

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# EFSA supporting publication 2013:EN-497

### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Brooks K, Hasan H, Samineni S, Gangur V, Karmaus W. Placental p, p'dichlorodiphenyldichloroethylene and cord blood immune markers. Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2007;18:621-624
- Brouwers MM, Feitz WF, Roelofs LA, Kiemeney LA, de Gier RP, Roeleveld N. Risk factors for hypospadias. European journal of pediatrics. 2007;166:671-678
- Browne RO, Moyal-Segal LB, Zumsteg D, David Y, Kofman O, Berger A, Soreq H, Friedman A. Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2006;20:1733-1735
- Brucker-Davis F, Ducot B, Wagner-Mahler K, Tommasi C, Ferrari P, Pacini P, Boda-Buccino M, Bongain A, Azuar P, Fenichel P. [environmental pollutants in maternal milk and cryptorchidism]. Gynecologie, obstetrique & fertilite. 2008;36:840-847
- Brucker-Davis F, Ferrari P, Boda-Buccino M, Wagner-Mahler K, Pacini P, Gal J, Azuar P, Fenichel P. Cord blood thyroid tests in boys born with and without cryptorchidism: Correlations with birth parameters and in utero xenobiotics exposure. Thyroid: official journal of the American Thyroid Association. 2011;21:1133-1141
- Brucker-Davis F, Wagner-Mahler K, Bornebusch L, Delattre I, Ferrari P, Gal J, Boda-Buccino M, Pacini P, Tommasi C, Azuar P, Bongain A, Fenichel P. Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from nice area (france). Chemosphere. 2010;81:169-176
- Brucker-Davis F, Wagner-Mahler K, Delattre I, Ducot B, Ferrari P, Bongain A, Kurzenne JY, Mas JC, Fenichel P. Cryptorchidism at birth in nice area (France) is associated with higher prenatal exposure to pcbs and dde, as assessed by colostrum concentrations. Hum Reprod. 2008;23:1708-1718
- Brulls C., Niggemann H., Weissbach W., Dott W., Fischer M., Merk H.F., Blomeke B., Isselstein J., Ilgner, Westhofen M., Wiesmuller G.A.. Pilot study on living conditions and living factors investigated in patients suffering from self-reported multiple chemical sensitivity, fragrance allergies or polyposis nasi. Atemwegs- und Lungenkrankheiten (2008) 34:5 (187-198)
- Buck Louis GM, Rios LI, McLain A, Cooney MA, Kostyniak PJ, Sundaram R. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere. 2011;85:1742-1748
- Burdorf A, Brand T, Jaddoe VW, Hofman A, Mackenbach JP, Steegers EA. The effects of workrelated maternal risk factors on time to pregnancy, preterm birth and birth weight: The generation r study. Occupational and environmental medicine. 2011;68:197-204
- Burns JS, Williams PL, Sergeyev O, Korrick SA, Lee MM, Revich B, Altshul L, Del Prato JT, Humblet O, Patterson DG, Turner WE, Starovoytov M, Hauser R. Serum concentrations of organochlorine pesticides and growth among russian boys. Environ Health Perspect. 2012;120:303-308
- Bustamante Montes LP, Waliszewski S, Hernandez-Valero M, Sanin-Aguirre L, Infanzon-Ruiz RM, Janas AG. [prenatal exposure to organochlorine pesticides and cryptorchidism]. Ciencia & saude coletiva. 2010;15 Suppl 1:1169-1174

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

RM, Janas AG. [prenatal exposure to organochlorine pesticides and cryptorchidism]. Ciencia & saude coletiva. 2010;15 Suppl 1:1169-1174

- Carbone P, Giordano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamanca I. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural Sicily. International journal of andrology. 2007;30:3-13
- Carmichael SL, Herring AH, Sjodin A, Jones R, Needham L, Ma C, Ding K, Shaw GM. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80:641-646
- Carozza SE, Li B, Wang Q, Horel S, Cooper S. Agricultural pesticides and risk of childhood cancers. International journal of hygiene and environmental health. 2009;212:186-195
- Cha ES, Lee YK, Moon EK, Kim YB, Lee YJ, Jeong WC, Cho EY, Lee IJ, Hur J, Ha M, Lee WJ. Paraquat application and respiratory health effects among South Korean farmers. Occupational and environmental medicine. 2012;69:398-403
- Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in india. Journal of occupational health. 2009;51:488-497
- Chang CK, Astrakianakis G, Thomas DB, Seixas NS, Ray RM, Gao DL, Wernli KJ, Fitzgibbons ED, Vaughan TL, Checkoway H. Occupational exposures and risks of liver cancer among shanghai female textile workers--a case-cohort study. International journal of epidemiology. 2006;35:361-369
- Chang YL, Li J, Yao SQ, Hu WN, Jiang SF, Guo Z, Yang L, Li DD, Li YM, Liu Y. [a casecontrol study on serum organochlorines residues, genetic polymorphisms of glutathione stransferase t1 and the risks of breast cancer]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2008;29:763-766
- Charles LE, Burchfiel CM, Fekedulegn D, Gu JK, Petrovitch H, Sanderson WT, Masaki K, Rodriguez BL, Andrew ME, Ross GW. Occupational exposure to pesticides, metals, and solvents: The impact on mortality rates in the Honolulu heart program. Work. 2010;37:205-215
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational exposures and movement abnormalities among japanese-american men: The honolulu-asia aging study. Neuroepidemiology. 2006;26:130-139
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Sanderson WT, Petrovitch H. Occupational and other risk factors for hand-grip strength: The honolulu-asia aging study. Occupational and environmental medicine. 2006;63:820-827
- Chatzi L, Alegakis A, Kruger-Krasagakis S, Lionis C. Skin symptoms and work-related skin symptoms among grape farmers in crete, greece. American journal of industrial medicine. 2006;49:77-84
- Chatzi L, Alegakis A, Tzanakis N, Siafakas N, Kogevinas M, Lionis C. Association of allergic rhinitis with pesticide use among grape farmers in crete, greece. Occupational and environmental medicine. 2007;64:417-421
- Chen SC, Wong RH, Shiu LJ, Chiou MC, Lee H. Exposure to mosquito coil smoke may be a risk factor for lung cancer in Taiwan. Journal of epidemiology / Japan Epidemiological

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Carbone P, Giordano F, Nori F, Mantovani A, Taruscio D, Lauria L, Figa-Talamanca I. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: A population-based case-control study in rural Sicily. International journal of andrology. 2007;30:3-13
- Carmichael SL, Herring AH, Sjodin A, Jones R, Needham L, Ma C, Ding K, Shaw GM. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80:641-646
- Carozza SE, Li B, Wang Q, Horel S, Cooper S. Agricultural pesticides and risk of childhood cancers. International journal of hygiene and environmental health. 2009;212:186-195
- Cha ES, Lee YK, Moon EK, Kim YB, Lee YJ, Jeong WC, Cho EY, Lee IJ, Hur J, Ha M, Lee WJ. Paraquat application and respiratory health effects among South Korean farmers. Occupational and environmental medicine. 2012;69:398-403
- Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in india. Journal of occupational health. 2009;51:488-497
- Chang CK, Astrakianakis G, Thomas DB, Seixas NS, Ray RM, Gao DL, Wernli KJ, Fitzgibbons ED, Vaughan TL, Checkoway H. Occupational exposures and risks of liver cancer among shanghai female textile workers--a case-cohort study. International journal of epidemiology. 2006;35:361-369
- Chang YL, Li J, Yao SQ, Hu WN, Jiang SF, Guo Z, Yang L, Li DD, Li YM, Liu Y. [a casecontrol study on serum organochlorines residues, genetic polymorphisms of glutathione s- transferase t1 and the risks of breast cancer]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2008;29:763-766
- Charles LE, Burchfiel CM, Fekedulegn D, Gu JK, Petrovitch H, Sanderson WT, Masaki K, Rodriguez BL, Andrew ME, Ross GW. Occupational exposure to pesticides, metals, and solvents: The impact on mortality rates in the Honolulu heart program. Work. 2010;37:205-215
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Petrovitch H, Sanderson WT. Occupational exposures and movement abnormalities among japanese-american men: The honolulu-asia aging study. Neuroepidemiology. 2006;26:130-139
- Charles LE, Burchfiel CM, Fekedulegn D, Kashon ML, Ross GW, Sanderson WT, Petrovitch H. Occupational and other risk factors for hand-grip strength: The honolulu-asia aging study. Occupational and environmental medicine. 2006;63:820-827
- Chatzi L, Alegakis A, Kruger-Krasagakis S, Lionis C. Skin symptoms and work-related skin symptoms among grape farmers in crete, greece. American journal of industrial medicine. 2006;49:77-84
- Chatzi L, Alegakis A, Tzanakis N, Siafakas N, Kogevinas M, Lionis C. Association of allergic rhinitis with pesticide use among grape farmers in crete, greece. Occupational and environmental medicine. 2007;64:417-421
- Chen SC, Wong RH, Shiu LJ, Chiou MC, Lee H. Exposure to mosquito coil smoke may be a risk factor for lung cancer in Taiwan. Journal of epidemiology / Japan Epidemiological Association. 2008;18:19-25

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Association. 2008:18:19-25

- Chen Z, Robison L, Giller R, Krailo M, Davis M, Davies S, Shu XO. Environmental exposure to residential pesticides, chemicals, dusts, fumes, and metals, and risk of childhood germ cell tumors. International journal of hygiene and environmental health. 2006;209:31-40
- Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, Durand G, Cordier S. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the pelagie birth cohort. Environ Health Perspect. 2011;119:1034-1041
- Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008;168:298-310
- Chitra GA, Muraleedharan VR, Swaminathan T, Veeraraghavan D. Use of pesticides and its impact on health of farmers in south india. International journal of occupational and environmental health. 2006;12:228-233
- Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-hodgkin lymphoma. Blood. 2006;108:1363-1369
- Christensen CH, Platz EA, Andreotti G, Blair A, Hoppin JA, Koutros S, Lynch CF, Sandler DP, Alavanja MC. Coumaphos exposure and incident cancer among male participants in the agricultural health study (ahs). Environ Health Perspect. 2010;118:92-96
- Cocco P, Brennan P, Ibba A, de Sanjose Llongueras S, Maynadie M, Nieters A, Becker N, Ennas MG, Tocco MG, Boffetta P. Plasma polychlorobiphenyl and organochlorine pesticide level and risk of major lymphoma subtypes. Occupational and environmental medicine. 2008;65:132-140
- Cockburn M, Mills P, Zhang X, Zadnick J, Goldberg D, Ritz B. Prostate cancer and ambient pesticide exposure in agriculturally intensive areas in california. American journal of epidemiology. 2011;173:1280-1288
- Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environ Health Perspect. 2007;115:1442-1447
- Cohn BA, Cirillo PM, Christianson RE. Prenatal ddt exposure and testicular cancer: A nested case-control study. Arch Environ Occup Health. 2010;65:127-134
- Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. Ddt and breast cancer in young women: New data on the significance of age at exposure. Environ Health Perspect. 2007;115:1406-1414
- Cole DC, Wainman B, Sanin LH, Weber JP, Muggah H, Ibrahim S. Environmental contaminant levels and fecundability among non-smoking couples. Reprod Toxicol. 2006;22:13-19
- Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2009;51:1212-1219
- Colt JS, Davis S, Severson RK, Lynch CF, Cozen W, Camann D, Engels EA, Blair A, Hartge P. Residential insecticide use and risk of non-hodgkin's lymphoma. Cancer epidemiology,

EFSA supporting publication 2013:EN-497

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Chen Z, Robison L, Giller R, Krailo M, Davis M, Davies S, Shu XO. Environmental exposure to residential pesticides, chemicals, dusts, fumes, and metals, and risk of childhood germ cell tumors. International journal of hygiene and environmental health. 2006;209:31-40
- Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, Durand G, Cordier S. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the pelagie birth cohort. Environ Health Perspect. 2011;119:1034-1041
- Chevrier J, Eskenazi B, Holland N, Bradman A, Barr DB. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. American journal of epidemiology. 2008;168:298-310
- Chitra GA, Muraleedharan VR, Swaminathan T, Veeraraghavan D. Use of pesticides and its impact on health of farmers in south india. International journal of occupational and environmental health. 2006;12:228-233
- Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-hodgkin lymphoma. Blood. 2006;108:1363-1369
- Christensen CH, Platz EA, Andreotti G, Blair A, Hoppin JA, Koutros S, Lynch CF, Sandler DP, Alavanja MC. Coumaphos exposure and incident cancer among male participants in the agricultural health study (ahs). Environ Health Perspect. 2010;118:92-96
- Cocco P, Brennan P, Ibba A, de Sanjose Llongueras S, Maynadie M, Nieters A, Becker N, Ennas MG, Tocco MG, Boffetta P. Plasma polychlorobiphenyl and organochlorine pesticide level and risk of major lymphoma subtypes. Occupational and environmental medicine. 2008;65:132-140
- Cockburn M, Mills P, Zhang X, Zadnick J, Goldberg D, Ritz B. Prostate cancer and ambient pesticide exposure in agriculturally intensive areas in california. American journal of epidemiology. 2011;173:1280-1288
- Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environ Health Perspect. 2007;115:1442-1447
- Cohn BA, Cirillo PM, Christianson RE. Prenatal ddt exposure and testicular cancer: A nested case-control study. Arch Environ Occup Health. 2010;65:127-134
- Cohn BA, Wolff MS, Cirillo PM, Sholtz RI. Ddt and breast cancer in young women: New data on the significance of age at exposure. Environ Health Perspect. 2007;115:1406-1414
- Cole DC, Wainman B, Sanin LH, Weber JP, Muggah H, Ibrahim S. Environmental contaminant levels and fecundability among non-smoking couples. Reprod Toxicol. 2006;22:13-19
- Collins JJ, Bodner K, Aylward LL, Wilken M, Swaen G, Budinsky R, Rowlands C, Bodnar CM. Mortality rates among workers exposed to dioxins in the manufacture of pentachlorophenol. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2009;51:1212-1219
- Colt JS, Davis S, Severson RK, Lynch CF, Cozen W, Camann D, Engels EA, Blair A, Hartge P. Residential insecticide use and risk of non-hodgkin's lymphoma. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:251-257

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:251-257

- Colt JS, Rothman N, Severson RK, Hartge P, Cerhan JR, Chatterjee N, Cozen W, Morton LM, De Roos AJ, Davis S, Chanock S, Wang SS. Organochlorine exposure, immune gene variation, and risk of non-hodgkin lymphoma. Blood. 2009;113:1899-1905
- Cooney MA, Buck Louis GM, Hediger ML, Vexler A, Kostyniak PJ. Organochlorine pesticides and endometriosis. Reprod Toxicol. 2010;30:365-369
- Cooney MA, Daniels JL, Ross JA, Breslow NE, Pollock BH, Olshan AF. Household pesticides and the risk of wilms tumor. Environmental Health Perspectives. 2006;115:134-137
- Cooper GS, Parks CG, Schur PS, Fraser PA. Occupational and environmental associations with antinuclear antibodies in a general population sample. Journal of toxicology and environmental health. Part A. 2006;69:2063-2069
- Cornelis C, Schoeters G, Kellen E, Buntinx F, Zeegers M. Development of a gis-based indicator for environmental pesticide exposure and its application to a belgian case-control study on bladder cancer. International journal of hygiene and environmental health. 2009;212:172-185
- Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of california. American journal of epidemiology. 2009;169:919-926
- Cote S, Ayotte P, Dodin S, Blanchet C, Mulvad G, Petersen HS, Gingras S, Dewailly E. Plasma organochlorine concentrations and bone ultrasound measurements: A cross-sectional study in peri-and postmenopausal inuit women from greenland. Environmental health: a global access science source. 2006;5:33
- Cox S, Niskar AS, Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. Environ Health Perspect. 2007;115:1747-1752
- Crawford JM, Hoppin JA, Alavanja MC, Blair A, Sandler DP, Kamel F. Hearing loss among licensed pesticide applicators in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:817-826
- Cupul-Uicab LA, Gladen BC, Hernandez-Avila M, Weber JP, Longnecker MP. Dde, a degradation product of ddt, and duration of lactation in a highly exposed area of mexico. Environ Health Perspect. 2008;116:179-183
- Cupul-Uicab LA, Hernandez-Avila M, Terrazas-Medina EA, Pennell ML, Longnecker MP. Prenatal exposure to the major ddt metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) and growth in boys from mexico. Environmental research. 2010;110:595-603
- Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberte C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in quebec, canada. Environmental research. 2008;108:387-392
- Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in inuit adults. Environ Health Perspect. 2009;117:1380-1386

EFSA supporting publication 2013:EN-497

### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Colt JS, Rothman N, Severson RK, Hartge P, Cerhan JR, Chatterjee N, Cozen W, Morton LM, De Roos AJ, Davis S, Chanock S, Wang SS. Organochlorine exposure, immune gene variation, and risk of non-hodgkin lymphoma. Blood. 2009;113:1899-1905
- Cooney MA, Buck Louis GM, Hediger ML, Vexler A, Kostyniak PJ. Organochlorine pesticides and endometriosis. Reprod Toxicol. 2010;30:365-369
- Cooney MA, Daniels JL, Ross JA, Breslow NE, Pollock BH, Olshan AF. Household pesticides and the risk of wilms tumor. Environmental Health Perspectives. 2006;115:134-137
- Cooper GS, Parks CG, Schur PS, Fraser PA. Occupational and environmental associations with antinuclear antibodies in a general population sample. Journal of toxicology and environmental health. Part A. 2006;69:2063-2069
- Cornelis C, Schoeters G, Kellen E, Buntinx F, Zeegers M. Development of a gis-based indicator for environmental pesticide exposure and its application to a belgian case-control study on bladder cancer. International journal of hygiene and environmental health. 2009;212:172-185
- Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of california. American journal of epidemiology. 2009;169:919-926
- Cote S, Ayotte P, Dodin S, Blanchet C, Mulvad G, Petersen HS, Gingras S, Dewailly E. Plasma organochlorine concentrations and bone ultrasound measurements: A crosssectional study in peri-and postmenopausal inuit women from greenland. Environmental health: a global access science source. 2006;5:33
- Cox S, Niskar AS, Narayan KM, Marcus M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. Environ Health Perspect. 2007;115:1747-1752
- Crawford JM, Hoppin JA, Alavanja MC, Blair A, Sandler DP, Kamel F. Hearing loss among licensed pesticide applicators in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:817-826
- Cupul-Uicab LA, Gladen BC, Hernandez-Avila M, Weber JP, Longnecker MP. Dde, a degradation product of ddt, and duration of lactation in a highly exposed area of mexico. Environ Health Perspect. 2008;116:179-183
- Cupul-Uicab LA, Hernandez-Avila M, Terrazas-Medina EA, Pennell ML, Longnecker MP. Prenatal exposure to the major ddt metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (dde) and growth in boys from mexico. Environmental research. 2010;110:595-603
- Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberte C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in quebec, canada. Environmental research. 2008;108:387-392
- Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in inuit adults. Environ Health Perspect. 2009;117:1380-1386

<sup>119</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect. 2009;117:1014-1020

- Dallaire R, Muckle G, Rouget F, Kadhel P, Bataille H, Guldner L, Seurin S, Chajes V, Monfort C, Boucher O, Thome JP, Jacobson SW, Multigner L, Cordier S. Cognitive, visual, and motor development of 7-month-old guadeloupean infants exposed to chlordecone. Environmental research. 2012;118:79-85
- Damgaard IN, Skakkebæk NE, Toppari J, Virtanen HE, Shen H, Schramm K-W, Petersen JH, Jensen TK, Main KM. Persistent pesticides in human breast milk and cryptorchidism. Environmental Health Perspectives. 2006;114:1133-1138
- Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. Pop levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from uppsala, sweden. Environment international. 2010;36:180-187
- Dassanayake T, Gawarammana IB, Weerasinghe V, Dissanayake PS, Pragaash S, Dawson A, Senanayake N. Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2009;120:1693-1698
- Dayton SB, Sandler DP, Blair A, Alavanja M, Beane Freeman LE, Hoppin JA. Pesticide use and myocardial infarction incidence among farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2010;52:693-697
- De Fleurian G, Perrin J, Ecochard R, Dantony E, Lanteaume A, Achard V, Grillo JM, Guichaoua MR, Botta A, Sari-Minodier I. Occupational exposures obtained by questionnaire in clinical practice and their association with semen quality. Journal of andrology. 2009;30:566-579
- de Jager C, Aneck-Hahn NH, Bornman MS, Farias P, Leter G, Eleuteri P, Rescia M, Spano M. Sperm chromatin integrity in ddt-exposed young men living in a malaria area in the limpopo province, south africa. Hum Reprod. 2009;24:2429-2438
- De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, Dombrowski C, Rousseau F, Sanchez VD, Bailey JL. Reduced seminal parameters associated with environmental ddt exposure and p,p'-dde concentrations in men in chiapas, mexico: A crosssectional study. Journal of andrology. 2006;27:16-27
- de Souza A, Medeiros Ados R, de Souza AC, Wink M, Siqueira IR, Ferreira MB, Fernandes L, Loayza Hidalgo MP, Torres IL. [evaluation of the impact of exposure to pesticides on the health of the rural population: Vale do taquari, state of rio grande do sul (brazil)]. Ciencia & saude coletiva. 2011;16:3519-3528
- Delancey JO, Alavanja MC, Coble J, Blair A, Hoppin JA, Austin HD, Beane Freeman LE. Occupational exposure to metribuzin and the incidence of cancer in the agricultural health study. Annals of epidemiology. 2009;19:388-395
- Delport R, Bornman R, MacIntyre UE, Oosthuizen NM, Becker PJ, Aneck-Hahn NH, de Jager C. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to ddt. Environ Health Perspect. 2011;119:647-651

Demers PA, Davies HW, Friesen MC, Hertzman C, Ostry A, Hershler R, Teschke K. Cancer

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# EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, Sandau CD, Ayotte P. Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect. 2009;117:1014-1020
- Dallaire R, Muckle G, Rouget F, Kadhel P, Bataille H, Guldner L, Seurin S, Chajes V, Monfort C, Boucher O, Thome JP, Jacobson SW, Multigner L, Cordier S. Cognitive, visual, and motor development of 7-month-old guadeloupean infants exposed to chlordecone. Environmental research. 2012;118:79-85
- Damgaard IN, Skakkebæk NE, Toppari J, Virtanen HE, Shen H, Schramm K-W, Petersen JH, Jensen TK, Main KM. Persistent pesticides in human breast milk and cryptorchidism. Environmental Health Perspectives. 2006;114:1133-1138
- Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. Pop levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from uppsala, sweden. Environment international. 2010;36:180-187
- Dassanayake T, Gawarammana IB, Weerasinghe V, Dissanayake PS, Pragaash S, Dawson A, Senanayake N. Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2009;120:1693-1698
- Dayton SB, Sandler DP, Blair A, Alavanja M, Beane Freeman LE, Hoppin JA. Pesticide use and myocardial infarction incidence among farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2010;52:693-697
- De Fleurian G, Perrin J, Ecochard R, Dantony E, Lanteaume A, Achard V, Grillo JM, Guichaoua MR, Botta A, Sari-Minodier I. Occupational exposures obtained by questionnaire in clinical practice and their association with semen quality. Journal of andrology. 2009;30:566-579
- de Jager C, Aneck-Hahn NH, Bornman MS, Farias P, Leter G, Eleuteri P, Rescia M, Spano M. Sperm chromatin integrity in ddt-exposed young men living in a malaria area in the limpopo province, south africa. Hum Reprod. 2009;24:2429-2438
- De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, Dombrowski C, Rousseau F, Sanchez VD, Bailey JL. Reduced seminal parameters associated with environmental ddt exposure and p,p'-dde concentrations in men in chiapas, mexico: A cross- sectional study. Journal of andrology. 2006;27:16-27
- de Souza A, Medeiros Ados R, de Souza AC, Wink M, Siqueira IR, Ferreira MB, Fernandes L, Loayza Hidalgo MP, Torres IL. [evaluation of the impact of exposure to pesticides on the health of the rural population: Vale do taquari, state of rio grande do sul (brazil)]. Ciencia & saude coletiva. 2011;16:3519-3528
- Delancey JO, Alavanja MC, Coble J, Blair A, Hoppin JA, Austin HD, Beane Freeman LE. Occupational exposure to metribuzin and the incidence of cancer in the agricultural health study. Annals of epidemiology. 2009;19:388-395
- Delport R, Bornman R, MacIntyre UE, Oosthuizen NM, Becker PJ, Aneck-Hahn NH, de Jager C. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to ddt. Environ Health Perspect. 2011;119:647-651
- Demers PA, Davies HW, Friesen MC, Hertzman C, Ostry A, Hershler R, Teschke K. Cancer and occupational exposure to pentachlorophenol and tetrachlorophenol (canada). Cancer causes & control: CCC. 2006;17:749-758

EFSA 支援出版 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

and occupational exposure to pentachlorophenol and tetrachlorophenol (canada). Cancer causes & control: CCC. 2006;17:749-758

- Den Hond E, Dhooge W, Bruckers L, Schoeters G, Nelen V, van de Mieroop E, Koppen G, Bilau M, Schroijen C, Keune H, Baeyens W, van Larebeke N. Internal exposure to pollutants and sexual maturation in flemish adolescents. Journal of exposure science & environmental epidemiology. 2011;21:224-233
- Deng F, Tao FB, Liu DY, Xu YY, Hao JH, Sun Y, Su PY. Effects of growth environments and two environmental endocrine disruptors on children with idiopathic precocious puberty. European journal of endocrinology / European Federation of Endocrine Societies. 2012;166:803-809
- Dennis LK, Lowe JB, Lynch CF, Alavanja MC. Cutaneous melanoma and obesity in the agricultural health study. Annals of epidemiology. 2008;18:214-221
- Dennis LK, Lynch CF, Sandler DP, Alavanja MC. Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. Environ Health Perspect. 2010;118:812-817
- Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, Shepherd S. Pesticide/environmental exposures and parkinson's disease in east texas. Journal of agromedicine. 2008;13:37-48
- Dhooge W, Den Hond E, Koppen G, Bruckers L, Nelen V, Van De Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, Van Larebeke N. Internal exposure to pollutants and body size in flemish adolescents and adults: Associations and dose-response relationships. Environment international. 2010;36:330-337
- Dhooge W, den Hond E, Koppen G, Bruckers L, Nelen V, van de Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, van Larebeke N. Internal exposure to pollutants and sex hormone levels in flemish male adolescents in a cross-sectional study: Associations and dose-response relationships. Journal of exposure science & environmental epidemiology. 2011;21:106-113
- Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, Semple S, Dick S, Counsell C, Mozzoni P, Haites N, Wettinger SB, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A. Environmental risk factors for Parkinson's disease and Parkinsonism: The geoparkinson study. Occupational and environmental medicine. 2007;64:666-672
- Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, Mertens I, Van Gaal L. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring). 2011;19:709-714
- Djordjevic M, Sazdanovic P, Djordjevic G, Jovanovic B. Morbidity in newborns exposed to organophosphorus pesticides. Medicinski pregled. 2010;63:414-417
- Dugas J, Nieuwenhuijsen MJ, Martinez D, Iszatt N, Nelson P, Elliott P. Use of biocides and insect repellents and risk of hypospadias. Occupational and environmental medicine. 2010;67:196-200
- Duk-Hee Lee, Michael W. Steffes, Andreas Sjodin, Richard S. Jones, Larry L. Needham, David R., Jacobs Jr. Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. PLoS ONE 2011; 6(1): e15977

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Den Hond E, Dhooge W, Bruckers L, Schoeters G, Nelen V, van de Mieroop E, Koppen G, Bilau M, Schroijen C, Keune H, Baeyens W, van Larebeke N. Internal exposure to pollutants and sexual maturation in flemish adolescents. Journal of exposure science & environmental epidemiology. 2011;21:224-233
- Deng F, Tao FB, Liu DY, Xu YY, Hao JH, Sun Y, Su PY. Effects of growth environments and two environmental endocrine disruptors on children with idiopathic precocious puberty. European journal of endocrinology / European Federation of Endocrine Societies. 2012;166:803-809
- Dennis LK, Lowe JB, Lynch CF, Alavanja MC. Cutaneous melanoma and obesity in the agricultural health study. Annals of epidemiology. 2008;18:214-221
- Dennis LK, Lynch CF, Sandler DP, Alavanja MC. Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. Environ Health Perspect. 2010;118:812- 817
- Dhillon AS, Tarbutton GL, Levin JL, Plotkin GM, Lowry LK, Nalbone JT, Shepherd S. Pesticide/environmental exposures and parkinson's disease in east texas. Journal of agromedicine. 2008;13:37-48
- Dhooge W, Den Hond E, Koppen G, Bruckers L, Nelen V, Van De Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, Van Larebeke N. Internal exposure to pollutants and body size in flemish adolescents and adults: Associations and dose-response relationships. Environment international. 2010;36:330-337
- Dhooge W, den Hond E, Koppen G, Bruckers L, Nelen V, van de Mieroop E, Bilau M, Croes K, Baeyens W, Schoeters G, van Larebeke N. Internal exposure to pollutants and sex hormone levels in flemish male adolescents in a cross-sectional study: Associations and dose-response relationships. Journal of exposure science & environmental epidemiology. 2011;21:106-113
- Dick FD, De Palma G, Ahmadi A, Scott NW, Prescott GJ, Bennett J, Semple S, Dick S, Counsell C, Mozzoni P, Haites N, Wettinger SB, Mutti A, Otelea M, Seaton A, Soderkvist P, Felice A. Environmental risk factors for Parkinson's disease and Parkinsonism: The geoparkinson study. Occupational and environmental medicine. 2007;64:666-672
- Dirinck E, Jorens PG, Covaci A, Geens T, Roosens L, Neels H, Mertens I, Van Gaal L. Obesity and persistent organic pollutants: Possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity (Silver Spring). 2011;19:709-714
- Djordjevic M, Sazdanovic P, Djordjevic G, Jovanovic B. Morbidity in newborns exposed to organophosphorus pesticides. Medicinski pregled. 2010;63:414-417
- Dugas J, Nieuwenhuijsen MJ, Martinez D, Iszatt N, Nelson P, Elliott P. Use of biocides and insect repellents and risk of hypospadias. Occupational and environmental medicine. 2010;67:196-200
- Duk-Hee Lee, Michael W. Steffes, Andreas Sjodin, Richard S. Jones, Larry L. Needham, David R., Jacobs Jr. Low Dose Organochlorine Pesticides and Polychlorinated Biphenyls Predict Obesity, Dyslipidemia, and Insulin Resistance among People Free of Diabetes. PLoS ONE 2011 ; 6(1): e15977

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The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Eckerman DA, Gimenes LS, de Souza RC, Galvao PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in brazil. Neurotoxicology and teratology. 2007;29:164-175
- Eggesbo M, Stigum H, Longnecker MP, Polder A, Aldrin M, Basso O, Thomsen C, Skaare JU, Becher G, Magnus P. Levels of hexachlorobenzene (hcb) in breast milk in relation to birth weight in a norwegian cohort. Environmental research. 2009;109:559-566
- El-Helaly M, Abdel-Elah K, Haussein A, Shalaby H. Paternal occupational exposures and the risk of congenital malformations--a case-control study. International journal of occupational medicine and environmental health. 2011;24:218-227
- Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, Alperovitch A, Tzourio C. Professional exposure to pesticides and parkinson disease. Annals of neurology. 2009;66:494-504
- Elobeid MA, Padilla MA, Brock DW, Ruden DM, Allison DB. Endocrine disruptors and obesity: An examination of selected persistent organic pollutants in the nhanes 1999-2002 data. International journal of environmental research and public health. 2010;7:2988-3005
- Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal organophosphate metabolite and organochlorine levels and performance on the brazelton neonatal behavioral assessment scale in a multiethnic pregnancy cohort. American journal of epidemiology. 2007;165:1397-1404
- Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 2011;119:1182-1188
- English RG, Perry M, Lee MM, Hoffman E, Delport S, Dalvie MA. Farm residence and reproductive health among boys in rural South Africa. Environment international. 2012;47:73-79
- Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for nonhodgkin lymphoma including histopathological subgroup analysis. International journal of cancer. Journal international du cancer. 2008;123:1657-1663
- Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, Jewell NP. In utero exposure to dichlorodiphenyltrichloroethane (ddt) and dichlorodiphenyldichloroethylene (dde) and neurodevelopment among young mexican american children. Pediatrics. 2006;118:233-241
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate pesticide exposure and neurodevelopment in young mexican-american children. Environ Health Perspect. 2007;115:792-798
- Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Jr., Mainous AG, 3rd. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and ddt with diabetes in the 1999-2002 national health and nutrition examination survey. Environmental research. 2007;103:413-418
- Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination survey. Environment international. 2010;36:398-401

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Eckerman DA, Gimenes LS, de Souza RC, Galvao PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in brazil. Neurotoxicology and teratology. 2007;29:164-175
- Eggesbo M, Stigum H, Longnecker MP, Polder A, Aldrin M, Basso O, Thomsen C, Skaare JU, Becher G, Magnus P. Levels of hexachlorobenzene (hcb) in breast milk in relation to birth weight in a norwegian cohort. Environmental research. 2009;109:559-566
- El-Helaly M, Abdel-Elah K, Haussein A, Shalaby H. Paternal occupational exposures and the risk of congenital malformations--a case-control study. International journal of occupational medicine and environmental health. 2011;24:218-227
- Elbaz A, Clavel J, Rathouz PJ, Moisan F, Galanaud JP, Delemotte B, Alperovitch A, Tzourio C. Professional exposure to pesticides and parkinson disease. Annals of neurology. 2009;66:494-504
- Elobeid MA, Padilla MA, Brock DW, Ruden DM, Allison DB. Endocrine disruptors and obesity: An examination of selected persistent organic pollutants in the nhanes 1999-2002 data. International journal of environmental research and public health. 2010;7:2988-3005
- Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal organophosphate metabolite and organochlorine levels and performance on the brazelton neonatal behavioral assessment scale in a multiethnic pregnancy cohort. American journal of epidemiology. 2007;165:1397-1404
- Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 2011;119:1182-1188
- English RG, Perry M, Lee MM, Hoffman E, Delport S, Dalvie MA. Farm residence and reproductive health among boys in rural South Africa. Environment international. 2012;47:73-79
- Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for nonhodgkin lymphoma including histopathological subgroup analysis. International journal of cancer. Journal international du cancer. 2008;123:1657-1663
- Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, Jewell NP. In utero exposure to dichlorodiphenyltrichloroethane (ddt) and dichlorodiphenyldichloroethylene (dde) and neurodevelopment among young mexican american children. Pediatrics. 2006;118:233-241
- Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate pesticide exposure and neurodevelopment in young mexican-american children. Environ Health Perspect. 2007;115:792-798
- Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Jr., Mainous AG, 3rd. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and ddt with diabetes in the 1999-2002 national health and nutrition examination survey. Environmental research. 2007;103:413-418
- Everett CJ, Matheson EM. Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination survey. Environment international. 2010;36:398-401

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

F. Giordano, V. Dell'Orco, G. Galante, F. Giannandrea,

- Fang F, Quinlan P, Ye W, Barber MK, Umbach DM, Sandler DP, Kamel F. Workplace exposures and the risk of amyotrophic lateral sclerosis. Environ Health Perspect. 2009;117:1387-1392
- Farooq U, Joshi M, Nookala V, Cheriyath P, Fischman D, Graber NJ, Stellman SD, Muscat J. Self-reported exposure to pesticides in residential settings and risk of breast cancer: A casecontrol study. Environmental health : a global access science source. 2010;9:30
- Farr SL, Cai J, Savitz DA, Sandler DP, Hoppin JA, Cooper GS. Pesticide exposure and timing of menopause: The agricultural health study. American journal of epidemiology. 2006;163:731-742
- Fatemeh Tohidia, Farzaneh Farrokhib, Ali Taravatic. Effects of pesticide on the thyroid hormones of pesticide sprayers living in Mazandaran. Abstracts. doi:10.1016/j.clinbiochem.2011.08.1063
- Fear NT, Hey K, Vincent T, Murphy M. Paternal occupation and neural tube defects: A casecontrol study based on the oxford record linkage study register. Paediatric and perinatal epidemiology. 2007;21:163-168
- Fear NT, Vincent TJ, King JC, MacCarthy A, Bunch KJ, Murphy MF. Wilms tumour and paternal occupation: An analysis of data from the national registry of childhood tumours. Pediatric blood & cancer. 2009;53:28-32
- Feldman AL, Johansson AL, Nise G, Gatz M, Pedersen NL, Wirdefeldt K. Occupational exposure in parkinsonian disorders: A 43-year prospective cohort study in men. Parkinsonism & related disorders. 2011;17:677-682
- Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: Results of a casecontrol study. Birth defects research. Part A, Clinical and molecular teratology. 2008;82:98-105
- Feng Hong qi, Yang Lin, Guo Ling, Huang Wen li, Xu Bo nan, LI Zhao Xiang, Wang Tong. A case control study on the risk factors of Yunnan endemic sudden cardiac death. Chin J Endemiol Jul 20 2005 Vol 24 No.4; 1000-4955 2005 04-0414-03
- Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, Hubbard A, Barr DB. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environmental Health Perspectives. 2005;114:597-602
- Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. In utero exposure to ddt and performance on the brazelton neonatal behavioral assessment scale. Neurotoxicology. 2007;28:471-477
- Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p'-dde, hcb, pcbs and reproductive hormones among men of reproductive age. Reprod Toxicol. 2012;34:429-435
- Fernandez MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez JM, Cruz M, Olea-Serrano F, Olea N. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: A nested case-control study.

EFSA supporting publication 2013:EN-497

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F. Giordano, V. Dell'Orco, G. Galante, F. Giannandrea,

- Fang F, Quinlan P, Ye W, Barber MK, Umbach DM, Sandler DP, Kamel F. Workplace exposures and the risk of amyotrophic lateral sclerosis. Environ Health Perspect. 2009;117:1387-1392
- Farooq U, Joshi M, Nookala V, Cheriyath P, Fischman D, Graber NJ, Stellman SD, Muscat J. Self-reported exposure to pesticides in residential settings and risk of breast cancer: A case- control study. Environmental health : a global access science source. 2010;9:30
- Farr SL, Cai J, Savitz DA, Sandler DP, Hoppin JA, Cooper GS. Pesticide exposure and timing of menopause: The agricultural health study. American journal of epidemiology. 2006;163:731-742
- Fatemeh Tohidia, Farzaneh Farrokhib, Ali Taravatic. Effects of pesticide on the thyroid hormones of pesticide sprayers living in Mazandaran. Abstracts. doi:10.1016/j.clinbiochem.2011.08.1063
- Fear NT, Hey K, Vincent T, Murphy M. Paternal occupation and neural tube defects: A casecontrol study based on the oxford record linkage study register. Paediatric and perinatal epidemiology. 2007;21:163-168
- Fear NT, Vincent TJ, King JC, MacCarthy A, Bunch KJ, Murphy MF. Wilms tumour and paternal occupation: An analysis of data from the national registry of childhood tumours. Pediatric blood & cancer. 2009;53:28-32
- Feldman AL, Johansson AL, Nise G, Gatz M, Pedersen NL, Wirdefeldt K. Occupational exposure in parkinsonian disorders: A 43-year prospective cohort study in men. Parkinsonism & related disorders. 2011;17:677-682
- Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: Results of a case- control study. Birth defects research. Part A, Clinical and molecular teratology. 2008;82:98-105
- Feng Hong qi, Yang Lin, Guo Ling, Huang Wen li, Xu Bo nan, LI Zhao Xiang, Wang Tong. A case control study on the risk factors of Yunnan endemic sudden cardiac death. Chin J Endemiol Jul 20 2005 Vol 24 No.4 ; 1000-4955 2005 04-0414-03
- Fenster L, Eskenazi B, Anderson M, Bradman A, Harley K, Hernandez H, Hubbard A, Barr DB. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environmental Health Perspectives. 2005;114:597-602
- Fenster L, Eskenazi B, Anderson M, Bradman A, Hubbard A, Barr DB. In utero exposure to ddt and performance on the brazelton neonatal behavioral assessment scale. Neurotoxicology. 2007;28:471-477
- Ferguson KK, Hauser R, Altshul L, Meeker JD. Serum concentrations of p, p'-dde, hcb, pcbs and reproductive hormones among men of reproductive age. Reprod Toxicol. 2012;34:429-435
- Fernandez MF, Olmos B, Granada A, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez JM, Cruz M, Olea-Serrano F, Olea N. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: A nested casecontrol study. Environ Health Perspect. 2007;115 Suppl 1:8-14

<sup>123</sup> 

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Pesticide epidemiology

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Environ Health Perspect. 2007;115 Suppl 1:8-14

- Fieten KB, Kromhout H, Heederik D, van Wendel de Joode B. Pesticide exposure and respiratory health of indigenous women in costa rica. American journal of epidemiology. 2009:169:1500-1506
- Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Jr., Checkoway H. Occupational factors and risk of Parkinson's disease: A populationbased case-control study. American journal of industrial medicine. 2010;53:217-223
- Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, Cheng CW. Pesticide exposure on southwestern Taiwanese with mnsod and ngo1 polymorphisms is associated with increased risk of parkinson's disease. Clinica chimica acta; international journal of clinical chemistry. 2007:378:136-141
- Fortes C, Mastroeni S, Melchi F, Pilla MA, Alotto M, Antonelli G, Camaione D, Bolli S, Luchetti E, Pasquini P. The association between residential pesticide use and cutaneous melanoma, Eur J Cancer, 2007:43:1066-1075
- Freeman LE, Rusiecki JA, Hoppin JA, Lubin JH, Koutros S, Andreotti G, Zahm SH, Hines CJ, Coble JB, Barone-Adesi F, Sloan J, Sandler DP, Blair A, Alavanja MC. Atrazine and cancer incidence among pesticide applicators in the agricultural health study (1994-2007). Environ Health Perspect. 2011;119:1253-1259
- Freire C, Lopez-Espinosa MJ, Fernandez M, Molina-Molina JM, Prada R, Olea N. Prenatal exposure to organochlorine pesticides and tsh status in newborns from southern spain. The Science of the total environment, 2011:409:3281-3287
- Freire C, Ramos R, Amaya E, Fernandez MF, Santiago-Fernandez P, Lopez-Espinosa MJ, Arrebola JP, Olea N. Newborn tsh concentration and its association with cognitive development in healthy boys. European journal of endocrinology / European Federation of Endocrine Societies. 2010;163:901-909
- Friesen MC, Davies HW, Teschke K, Ostry AS, Hertzman C, Demers PA. Impact of the specificity of the exposure metric on exposure-response relationships. Epidemiology. 2007;18:88-94
- Frigerio R, Sanft KR, Grossardt BR, Peterson BJ, Elbaz A, Bower JH, Ahlskog JE, de Andrade M, Maraganore DM, Rocca WA. Chemical exposures and Parkinson's disease: A population-based case-control study. Movement disorders: official journal of the Movement Disorder Society. 2006;21:1688-1692
- Fritschi L, Glass DC, Tabrizi JS, Leavy JE, Ambrosini GL. Occupational risk factors for prostate cancer and benign prostatic hyperplasia: A case-control study in Western Australia. Occupational and environmental medicine. 2007;64:60-65
- Gabel P. Jensen MS. Andersen HR. Baelum J. Thulstrup AM, Bonde JP. Toft G. The risk of cryptorchidism among sons of women working in horticulture in Denmark: A cohort study. Environmental health: a global access science source. 2011;10:100
- Gallagher RP, Macarthur AC, Lee TK, Weber JP, Leblanc A, Mark Elwood J, Borugian M, Abanto Z, Spinelli JJ. Plasma levels of polychlorinated biphenyls and risk of cutaneous malignant melanoma: A preliminary study. International journal of cancer. Journal international du cancer. 2011;128:1872-1880

Ganesh B, Sushama S, Monika S, Suvarna P. A case-control study of risk factors for lung

EFSA supporting publication 2013:EN-497

# Fieten KB, Kromhout H, Heederik D, van Wendel de Joode B. Pesticide exposure and respiratory health of indigenous women in costa rica. American journal of epidemiology. 2009;169:1500-1506

- Firestone JA, Lundin JI, Powers KM, Smith-Weller T, Franklin GM, Swanson PD, Longstreth WT, Jr., Checkoway H. Occupational factors and risk of Parkinson"s disease: A population- based case-control study. American journal of industrial medicine. 2010;53:217-223
- Fong CS, Wu RM, Shieh JC, Chao YT, Fu YP, Kuao CL, Cheng CW. Pesticide exposure on southwestern Taiwanese with mnsod and ngol polymorphisms is associated with increased risk of parkinson's disease. Clinica chimica acta; international journal of clinical chemistry. 2007;378:136-141
- Fortes C, Mastroeni S, Melchi F, Pilla MA, Alotto M, Antonelli G, Camaione D, Bolli S, Luchetti E. Pasquini P. The association between residential pesticide use and cutaneous melanoma. Eur J Cancer. 2007;43:1066-1075
- Freeman LE, Rusiecki IA, Hoppin IA, Lubin IH, Koutros S, Andreotti G, Zahm SH, Hines CI, Coble IB, Barone-Adesi F, Sloan I, Sandler DP, Blair A, Alavanja MC, Atrazine and cancer incidence among pesticide applicators in the agricultural health study (1994-2007). Environ Health Perspect. 2011;119:1253-1259
- Freire C, Lopez-Espinosa MJ, Fernandez M, Molina-Molina JM, Prada R, Olea N. Prenatal exposure to organochlorine pesticides and tsh status in newborns from southern spain. The Science of the total environment. 2011;409:3281-3287
- Freire C, Ramos R, Amaya E, Fernandez MF, Santiago-Fernandez P, Lopez-Espinosa MJ, Arrebola JP, Olea N. Newborn tsh concentration and its association with cognitive development in healthy boys. European journal of endocrinology / European Federation of Endocrine Societies, 2010;163:901-909
- Friesen MC, Davies HW, Teschke K, Ostrv AS, Hertzman C, Demers PA. Impact of the specificity of the exposure metric on exposure-response relationships. Epidemiology. 2007;18:88-94
- Frigerio R, Sanft KR, Grossardt BR, Peterson BJ, Elbaz A, Bower JH, Ahlskog JE, de Andrade M, Maraganore DM, Rocca WA. Chemical exposures and Parkinson"s disease: A population-based case-control study. Movement disorders: official journal of the Movement Disorder Society. 2006;21:1688-1692
- Fritschi L, Glass DC, Tabrizi JS, Leavy JE, Ambrosini GL. Occupational risk factors for prostate cancer and benign prostatic hyperplasia: A case-control study in Western Australia. Occupational and environmental medicine. 2007;64:60-65
- Gabel P, Jensen MS, Andersen HR, Baelum J, Thulstrup AM, Bonde JP, Toft G. The risk of cryptorchidism among sons of women working in horticulture in Denmark: A cohort study. Environmental health: a global access science source. 2011;10:100
- Gallagher RP, Macarthur AC, Lee TK, Weber TP, Leblanc A, Mark Elwood J, Borugian M, Abanto Z, Spinelli JJ. Plasma levels of polychlorinated biphenyls and risk of cutaneous malignant melanoma: A preliminary study. International journal of cancer. Journal international du cancer. 2011;128:1872-1880
- Ganesh B, Sushama S, Monika S, Suvarna P. A case-control study of risk factors for lung cancer in mumbai, India, Asian Pacific journal of cancer prevention: APICP. 2011;12:357-362

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

cancer in mumbai, India. Asian Pacific journal of cancer prevention: APJCP. 2011;12:357-362

- Garced S, Torres-Sanchez L, Cebrian ME, Claudio L, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and child growth during the first year of life. Environmental research. 2012;113:58-62
- Gascon M, Vrijheid M, Martinez D, Ballester F, Basterrechea M, Blarduni E, Esplugues A, Vizcaino E, Grimalt JO, Morales E, Sunyer J. Pre-natal exposure to dichlorodiphenyldichloroethylene and infant lower respiratory tract infections and wheeze. The European respiratory journal. 2012;39:1188-1196
- Gaspari L, Paris F, Jandel C, Kalfa N, Orsini M, Daures JP, Sultan C. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: A nested case-control study. Hum Reprod. 2011;26:3155-3162
- Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B. Well-water consumption and parkinson's disease in rural california. Environ Health Perspect. 2009;117:1912-1918
- Gatto NM, Longnecker MP, Press MF, Sullivan-Halley J, McKean-Cowdin R, Bernstein L. Serum organochlorines and breast cancer: A case-control study among african-american women. Cancer causes & control: CCC. 2007;18:29-39
- German D, Roy A, Shalat S, Buckley B, Gearing M, Levey A, Richardson J. A ddt metabolite is elevated in the serum of alzheimer's disease patients. Alzheimer's & Dementia. 2012;8:P496
- Gian S. Jhangri, Colin L. Soskolne, Giovanni Pagano, Gerardo Botte, Patrizia Di Cintio. Alcohol and tobacco variables in the assessment of internal validity in an
- Giannandrea F, Gandini L, Paoli D, Turci R, Figa-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:780-787
- Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figa-Talamanca I. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth defects research. Part A, Clinical and molecular teratology. 2010;88:241-250
- Giordano F, Dell'Orco V, Giannandrea F, Lauria L, Valente P, Figa-Talamanca I. Mortality in a cohort of pesticide applicators in an urban setting: Sixty years of follow-up. International journal of immunopathology and pharmacology. 2006;19:61-65
- Giwercman A, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, Lindh C, Pedersen HS, Ludwicki JK, Lesovoy V, Shvets M, Spano M, Manicardi GC, Bizzaro D, Bonefeld-Jorgensen EC, Bonde JP. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three european cohorts. Environmental Health Perspectives. 2006;114:1348-1353
- Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S. Immune cell counts and risks of respiratory infections among infants exposed pre- and postnatally to organochlorine compounds: A prospective study. Environmental health : a global access science source. 2008;7:62

Gold LS, Ward MH, Dosemeci M, De Roos AJ. Systemic autoimmune disease mortality and

EFSA supporting publication 2013:EN-497

### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Garced S, Torres-Sanchez L, Cebrian ME, Claudio L, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and child growth during the first year of life. Environmental research. 2012;113:58-62
- Gascon M, Vrijheid M, Martinez D, Ballester F, Basterrechea M, Blarduni E, Esplugues A, Vizcaino E, Grimalt JO, Morales E, Sunyer J. Pre-natal exposure to dichlorodiphenyldichloroethylene and infant lower respiratory tract infections and wheeze. The European respiratory journal. 2012;39:1188-1196
- Gaspari L, Paris F, Jandel C, Kalfa N, Orsini M, Daures JP, Sultan C. Prenatal environmental risk factors for genital malformations in a population of 1442 French male newborns: A nested case-control study. Hum Reprod. 2011;26:3155-3162
- Gatto NM, Cockburn M, Bronstein J, Manthripragada AD, Ritz B. Well-water consumption and parkinson's disease in rural california. Environ Health Perspect. 2009;117:1912-1918
- Gatto NM, Longnecker MP, Press MF, Sullivan-Halley J, McKean-Cowdin R, Bernstein L. Serum organochlorines and breast cancer: A case-control study among african-american women. Cancer causes & control: CCC. 2007;18:29-39
- German D, Roy A, Shalat S, Buckley B, Gearing M, Levey A, Richardson J. A ddt metabolite is elevated in the serum of alzheimer's disease patients. Alzheimer's & Dementia. 2012;8:P496
- Gian S. Jhangri, Colin L. Soskolne, Giovanni Pagano, Gerardo Botte, Patrizia Di Cintio. Alcohol and tobacco variables in the assessment of internal validity in an Giannandrea F, Gandini L, Paoli D, Turci R, Figa-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes. 2011;46:780-787
- Giordano F, Abballe A, De Felip E, di Domenico A, Ferro F, Grammatico P, Ingelido AM, Marra V, Marrocco G, Vallasciani S, Figa-Talamanca I. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth defects research. Part A, Clinical and molecular teratology. 2010;88:241-250
- Giordano F, Dell'Orco V, Giannandrea F, Lauria L, Valente P, Figa-Talamanca I. Mortality in a cohort of pesticide applicators in an urban setting: Sixty years of followup. International journal of immunopathology and pharmacology. 2006;19:61-65
- Giwercman A, Rignell-Hydbom A, Toft G, Rylander L, Hagmar L, Lindh C, Pedersen HS, Ludwicki JK, Lesovoy V, Shvets M, Spano M, Manicardi GC, Bizzaro D, Bonefeld- Jorgensen EC, Bonde JP. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: A study of Inuit and three european cohorts. Environmental Health Perspectives. 2006;114:1348-1353
- Glynn A, Thuvander A, Aune M, Johannisson A, Darnerud PO, Ronquist G, Cnattingius S. Immune cell counts and risks of respiratory infections among infants exposed preand postnatally to organochlorine compounds: A prospective study. Environmental health : a global access science source. 2008;7:62
- Gold LS, Ward MH, Dosemeci M, De Roos AJ. Systemic autoimmune disease mortality and occupational exposures. Arthritis and rheumatism. 2007;56:3189-3201

EFSA 支援出版 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupational exposures. Arthritis and rheumatism. 2007;56:3189-3201

- Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the agricultural health study. American journal of epidemiology. 2010;171:455-464
- Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of pcb congeners and chlorinated pesticides. Environ Health Perspect. 2011;119:319-325
- Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, Morse G, Carpenter DO. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native american men. Environ Health Perspect. 2009;117:1454-1460
- Grandjean P, Harari R, Barr DB, Debes F. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in ecuadorian school children. Pediatrics. 2006;117:e546-556
- Greenburg DL, Rusiecki J, Koutros S, Dosemeci M, Patel R, Hines CJ, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to captan in the agricultural health study. Cancer causes & control : CCC. 2008;19:1401-1407
- Guillette EA, Conard C, Lares F, Aguilar MG, McLachlan J, Guillette LJ. Altered breast development in young girls from an agricultural environment. Environmental Health Perspectives. 2005;114:471-475
- Gunnar Toft, Allan Flyvbjerg and Jens Peter Bonde. A GlobalAccess Science Source. Enviromental Health 2006; 5: 32 32
- Guodong D, Pei W, Ying T, Jun Z, Yu G, Xiaojin W, Rong S, Guoquan W, Xiaoming S. Organophosphate pesticide exposure and neurodevelopment in young shanghai children. Environmental science & technology. 2012;46:2911-2917
- H Yu, X Liu, K Kezios, O Kalantzi, YWang, M Petreas, J-S Park, P Cirillio, B Cohn, P Factor-Litvak. Prenatal organochlorine exposure maternal thyroid function and neuromotor development. Am J Epidemiol. 2011;173(Suppl):S1–S316
- Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the national health and nutrition examination survey, 1999-2002. Environ Health Perspect. 2007;115:1204-1209
- Ha MH, Lee DH, Son HK, Park SK, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999-2002. Journal of human hypertension. 2009;23:274-286
- Hadjigeorgiou GM, Stefanidis I, Dardiotis E, Aggellakis K, Sakkas GK, Xiromerisiou G, Konitsiotis S, Paterakis K, Poultsidi A, Tsimourtou V, Ralli S, Gourgoulianis K, Zintzaras E. Low rls prevalence and awareness in central greece: An epidemiological survey. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2007;14:1275-1280
- Han Y, Xia Y, Han J, Zhou J, Wang S, Zhu P, Zhao R, Jin N, Song L, Wang X. The relationship of 3-pba pyrethroids metabolite and male reproductive hormones among non-

EFSA supporting publication 2013:EN-497

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- Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, Levan TD. Pesticide use and thyroid disease among women in the agricultural health study. American journal of epidemiology. 2010;171:455-464
- Goncharov A, Pavuk M, Foushee HR, Carpenter DO. Blood pressure in relation to concentrations of pcb congeners and chlorinated pesticides. Environ Health Perspect. 2011;119:319-325
- Goncharov A, Rej R, Negoita S, Schymura M, Santiago-Rivera A, Morse G, Carpenter DO. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in native american men. Environ Health Perspect. 2009;117:1454-1460
- Grandjean P, Harari R, Barr DB, Debes F. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in ecuadorian school children. Pediatrics. 2006;117:e546-556
- Greenburg DL, Rusiecki J, Koutros S, Dosemeci M, Patel R, Hines CJ, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to captan in the agricultural health study. Cancer causes & control : CCC. 2008;19:1401-1407
- Guillette EA, Conard C, Lares F, Aguilar MG, McLachlan J, Guillette LJ. Altered breast development in young girls from an agricultural environment. Environmental Health Perspectives. 2005;114:471-475
- Gunnar Toft, Allan Flyvbjerg and Jens Peter Bonde. A Global Access Science Source. Enviromental Health 2006; 5: 32 32
- Guodong D, Pei W, Ying T, Jun Z, Yu G, Xiaojin W, Rong S, Guoquan W, Xiaoming S. Organophosphate pesticide exposure and neurodevelopment in young shanghai children. Environmental science & technology. 2012;46:2911-2917
- H Yu, X Liu, K Kezios, O Kalantzi, YWang, M Petreas, J-S Park, P Cirillio, B Cohn, P Factor- Litvak. Prenatal organochlorine exposure maternal thyroid function and neuromotor development. Am J Epidemiol. 2011;173(Suppl):S1-S316
- Ha MH, Lee DH, Jacobs DR. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: Results from the national health and nutrition examination survey, 1999-2002. Environ Health Perspect. 2007;115:1204-1209
- Ha MH, Lee DH, Son HK, Park SK, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999-2002. Journal of human hypertension. 2009;23:274-286
- Hadjigeorgiou GM, Stefanidis I, Dardiotis E, Aggellakis K, Sakkas GK, Xiromerisiou G, Konitsiotis S, Paterakis K, Poultsidi A, Tsimourtou V, Ralli S, Gourgoulianis K, Zintzaras
- E. Low rls prevalence and awareness in central greece: An epidemiological survey. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2007;14:1275-1280
- Han Y, Xia Y, Han J, Zhou J, Wang S, Zhu P, Zhao R, Jin N, Song L, Wang X. The relationship of 3-pba pyrethroids metabolite and male reproductive hormones among nonoccupational exposure males. Chemosphere. 2008;72:785-790

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupational exposure males. Chemosphere. 2008;72:785-790

- Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of parkinson's disease: A family-based case-control study. BMC neurology. 2008;8:6
- Handal AJ, Harlow SD, Breilh J, Lozoff B. Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. Epidemiology. 2008;19:851-859
- Handal AJ, Lozoff B, Breilh J, Harlow SD. Neurobehavioral development in children with potential exposure to pesticides. Epidemiology. 2007;18:312-320
- Harari R, Julvez J, Murata K, Barr D, Bellinger DC, Debes F, Grandjean P. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect. 2010;118:890-896
- Hardell L, Andersson SO, Carlberg M, Bohr L, van Bavel B, Lindstrom G, Bjornfoth H, Ginman C. Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:700-707
- Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. International journal of andrology. 2006;29:228-234
- Hardell L, Carlberg M, Hardell K, Bjornfoth H, Wickbom G, Ionescu M, van Bavel B, Lindstrom G. Decreased survival in pancreatic cancer patients with high concentrations of organochlorines in adipose tissue. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2007;61:659-664
- Hardell. Concentrations of organohalogen compounds and titres of antibodies to epstein-barr virus antigens and the risk for non-hodgkin lymphoma. Oncology Reports. 2009;21
- Harley KG, Marks AR, Bradman A, Barr DB, Eskenazi B. Ddt exposure, work in agriculture, and time to pregnancy among farmworkers in california. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:1335-1342
- Hashemi N, Mirsadraee M, Shakeri MT, Varasteh AR. Prevalence of work-related respiratory symptoms in iranian farmers. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2006;13:198-202
- Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, Welsh-Bohmer KA. Occupational exposure to pesticides increases the risk of incident ad: The cache county study. Neurology. 2010;74:1524-1530
- Herin F, Boutet-Robinet E, Levant A, Dulaurent S, Manika M, Galatry-Bouju F, Caron P, Soulat JM. Thyroid function tests in persons with occupational exposure to fipronil. Thyroid : official journal of the American Thyroid Association. 2011;21:701-706
- Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. Inhalation toxicology. 2008;20:839-849

Hernandez-Morales AL, Zonana-Nacach A, Zaragoza-Sandoval VM. [associated risk factors in

127

# EFSA supporting publication 2013:EN-497

- Hancock DB, Martin ER, Mayhew GM, Stajich JM, Jewett R, Stacy MA, Scott BL, Vance JM, Scott WK. Pesticide exposure and risk of parkinson's disease: A family-based casecontrol study. BMC neurology. 2008;8:6
- Handal AJ, Harlow SD, Breilh J, Lozoff B. Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. Epidemiology. 2008;19:851-859
- Handal AJ, Lozoff B, Breilh J, Harlow SD. Neurobehavioral development in children with potential exposure to pesticides. Epidemiology. 2007;18:312-320
- Harari R, Julvez J, Murata K, Barr D, Bellinger DC, Debes F, Grandjean P. Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. Environ Health Perspect. 2010;118:890-896
- Hardell L, Andersson SO, Carlberg M, Bohr L, van Bavel B, Lindstrom G, Bjornfoth H, Ginman C. Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:700-707
- Hardell L, Bavel B, Lindstrom G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. International journal of andrology. 2006;29:228-234
- Hardell L, Carlberg M, Hardell K, Bjornfoth H, Wickbom G, Ionescu M, van Bavel B, Lindstrom G. Decreased survival in pancreatic cancer patients with high concentrations of organochlorines in adipose tissue. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2007;61:659-664
- Hardell. Concentrations of organohalogen compounds and titres of antibodies to epsteinbarr virus antigens and the risk for non-hodgkin lymphoma. Oncology Reports. 2009;21
- Harley KG, Marks AR, Bradman A, Barr DB, Eskenazi B. Ddt exposure, work in agriculture, and time to pregnancy among farmworkers in california. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2008;50:1335-1342
- Hashemi N, Mirsadraee M, Shakeri MT, Varasteh AR. Prevalence of work-related respiratory symptoms in iranian farmers. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2006;13:198-202
- Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, Welsh-Bohmer KA. Occupational exposure to pesticides increases the risk of incident ad: The cache county study. Neurology. 2010;74:1524-1530
- Herin F, Boutet-Robinet E, Levant A, Dulaurent S, Manika M, Galatry-Bouju F, Caron P, Soulat JM. Thyroid function tests in persons with occupational exposure to fipronil. Thyroid: official journal of the American Thyroid Association. 2011;21:701-706
- Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. Inhalation toxicology. 2008;20:839-849
- Hernandez-Morales AL, Zonana-Nacach A, Zaragoza-Sandoval VM. [associated risk factors in acute leukemia in children. A cases and controls study]. Revista medica del Instituto Mexicano del Seguro Social. 2009;47:497-503

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

acute leukemia in children. A cases and controls study]. Revista medica del Instituto Mexicano del Seguro Social. 2009;47:497-503

- Hodgson S, Thomas L, Fattore E, Lind PM, Alfven T, Hellstrom L, Hakansson H, Carubelli G, Fanelli R, Jarup L. Bone mineral density changes in relation to environmental pcb exposure. Environ Health Perspect. 2008;116:1162-1166
- Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA, Demers PA, Blair A. Exposure to multiple pesticides and risk of non-hodgkin lymphoma in men from six canadian provinces. International journal of environmental research and public health. 2011;8:2320-2330
- Hoppin JA, Umbach DM, Kullman GJ, Henneberger PK, London SJ, Alavanja MC, Sandler DP. Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the agricultural health study. Occupational and environmental medicine. 2007;64:334-341
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the agricultural health study. American journal of respiratory and critical care medicine. 2008;177:11-18
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MC, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the agricultural health study. The European respiratory journal. 2009;34:1296-1303
- Hoppin JA, Umbach DM, London SJ, Lynch CF, Alavanja MC, Sandler DP. Pesticides and adult respiratory outcomes in the agricultural health study. Annals of the New York Academy of Sciences. 2006;1076:343-354
- Hoppin JA, Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP. Pesticide use and chronic bronchitis among farmers in the agricultural health study. American journal of industrial medicine. 2007;50:969-979
- Horton MK, Rundle A, Camann DE, Boyd Barr D, Rauh VA, Whyatt RM. Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. Pediatrics. 2011;127:e699-706
- Hossain F, Ali O, D'Souza UJ, Naing DK. Effects of pesticide use on semen quality among farmers in rural areas of sabah, malaysia. Journal of occupational health. 2010;52:353-360
- Hou L, Lee WJ, Rusiecki J, Hoppin JA, Blair A, Bonner MR, Lubin JH, Samanic C, Sandler DP, Dosemeci M, Alavanja MC. Pendimethalin exposure and cancer incidence among pesticide applicators. Epidemiology. 2006;17:302-307
- Huisman M.H.B., De Jong D.E., Van Doormaa P.T.C., Vermeulen R., Heederick D., Kromhout H., Schelhaas H.J., Van Der Kooi A.J., De VISSER M., Veldink J.H., Van Den Berg A.H. Exogenous risk factors in ALS: A population-based case-control study. Amyotrophic Lateral Sclerosis (2011) 12 SUPPL. 1 (23).
- Issaragrisil S, Kaufman DW, Anderson T, Chansung K, Leaverton PE, Shapiro S, Young NS. The epidemiology of aplastic anemia in thailand. Blood. 2006;107:1299-1307
- Itoh H, Iwasaki M, Hanaoka T, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S. Serum organochlorines and breast cancer risk in japanese women: A casecontrol study. Cancer causes & control : CCC. 2009;20:567-580

EFSA supporting publication 2013:EN-497

- Hodgson S, Thomas L, Fattore E, Lind PM, Alfven T, Hellstrom L, Hakansson H, Carubelli G, Fanelli R, Jarup L. Bone mineral density changes in relation to environmental pcb exposure. Environ Health Perspect. 2008;116:1162-1166
- Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA, Demers PA, Blair
- A. Exposure to multiple pesticides and risk of non-hodgkin lymphoma in men from six canadian provinces. International journal of environmental research and public health. 2011;8:2320-2330
- Hoppin JA, Umbach DM, Kullman GJ, Henneberger PK, London SJ, Alavanja MC, Sandler DP. Pesticides and other agricultural factors associated with self-reported farmer's lung among farm residents in the agricultural health study. Occupational and environmental medicine. 2007;64:334-341
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, Sandler DP. Pesticides and atopic and nonatopic asthma among farm women in the agricultural health study. American journal of respiratory and critical care medicine. 2008;177:11-18
- Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, Alavanja MC, Beane Freeman LE, Sandler DP. Pesticide use and adult-onset asthma among male farmers in the agricultural health study. The European respiratory journal. 2009;34:1296-1303
- Hoppin JA, Umbach DM, London SJ, Lynch CF, Alavanja MC, Sandler DP. Pesticides and adult respiratory outcomes in the agricultural health study. Annals of the New York Academy of Sciences. 2006;1076:343-354
- Hoppin JA, Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP. Pesticide use and chronic bronchitis among farmers in the agricultural health study. American journal of industrial medicine. 2007;50:969-979
- Horton MK, Rundle A, Camann DE, Boyd Barr D, Rauh VA, Whyatt RM. Impact of prenatal exposure to piperonyl butoxide and permethrin on 36-month neurodevelopment. Pediatrics. 2011;127:e699-706
- Hossain F, Ali O, D'Souza UJ, Naing DK. Effects of pesticide use on semen quality among farmers in rural areas of sabah, malaysia. Journal of occupational health. 2010;52:353-360
- Hou L, Lee WJ, Rusiecki J, Hoppin JA, Blair A, Bonner MR, Lubin JH, Samanic C, Sandler DP, Dosemeci M, Alavanja MC. Pendimethalin exposure and cancer incidence among pesticide applicators. Epidemiology. 2006;17:302-307
- Huisman M.H.B., De Jong D.E., Van Doormaa P.T.C., Vermeulen R., Heederick D., Kromhout H., Schelhaas H.J., Van Der Kooi A.J., De VISSER M., Veldink J.H., Van Den Berg A.H. Exogenous risk factors in ALS: A population-based case-control study. Amyotrophic Lateral Sclerosis (2011) 12 SUPPL. 1 (23).
- Issaragrisil S, Kaufman DW, Anderson T, Chansung K, Leaverton PE, Shapiro S, Young NS. The epidemiology of aplastic anemia in thailand. Blood. 2006;107:1299-1307
- Itoh H, Iwasaki M, Hanaoka T, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, Kusama R, Tsugane S. Serum organochlorines and breast cancer risk in japanese women: A casecontrol study. Cancer causes & control : CCC. 2009;20:567-580

<sup>128</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Iwasaki M, Inoue M, Sasazuki S, Kurahashi N, Itoh H, Usuda M, Tsugane S. Plasma organochlorine levels and subsequent risk of breast cancer among japanese women: A nested case-control study. The Science of the total environment. 2008;402:176-183
- J R Suarez-Lopez, J H Himes, D R Jacobs, Jr., B H Alexander, D Lazovich, M Gunnar. Secondary pesticide exposure is associated with head circumference and growth in children. Am J Epidemiol. 2012;175(11 Suppl):S1–S145
- Jansson C, Plato N, Johansson AL, Nyren O, Lagergren J. Airborne occupational exposures and risk of oesophageal and cardia adenocarcinoma. Occupational and environmental medicine. 2006;63:107-112
- Jeebhay MF, Baatjies R, Chang YS, Kim YK, Kim YY, Major V, Lopata AL. Risk factors for allergy due to the two-spotted spider mite (tetranychus urticae) among table grape farm workers. International archives of allergy and immunology. 2007;144:143-149
- Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, Wang S, Wang X. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in chinese men. Reprod Toxicol. 2011;31:171-176
- Jimenez-Jimenez FJ, de Toledo-Heras M, Alonso-Navarro H, Ayuso-Peralta L, Arevalo-Serrano J, Ballesteros-Barranco A, Puertas I, Jabbour-Wadih T, Barcenilla B. Environmental risk factors for essential tremor. European neurology. 2007;58:106-113
- Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland inuit. Diabetologia. 2008;51:1416-1422
- Jurewicz J, Hanke W, Makowiec-Dabrowska T. [low risk of reproductive disorders among female greenhouse workers--safe work conditions or health selection for the light work?]. Medycyna pracy. 2008;59:123-131
- Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. Epidemiology. 2012;23:689-698
- Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I. Maternal ddt exposures in relation to fetal and 5-year growth. Epidemiology. 2006;17:692-700
- Kallioniemi MK, Simola AJ, Kymalainen HR, Vesala HT, Louhelainen JK. Mental symptoms among Finnish farm entrepreneurs. Annals of agricultural and environmental medicine: AAEM. 2009;16:159-168
- Kamalesh Das, Chiranjib Nag and Mrinalkanti Ghosh. Familial, Environmental, and Occupational Risk Factors in Development of Amyotrophic Lateral Sclerosis. North American Journal of Medical Sciences 2012.
- Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed pesticide applicators in the agricultural health study. Human & experimental toxicology. 2007;26:243-250
- Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported parkinson's disease in the agricultural health study. American journal of epidemiology. 2007;165:364-374

EFSA supporting publication 2013:EN-497

- Iwasaki M, Inoue M, Sasazuki S, Kurahashi N, Itoh H, Usuda M, Tsugane S. Plasma organochlorine levels and subsequent risk of breast cancer among japanese women: A nested case-control study. The Science of the total environment. 2008;402:176-183
- J R Suarez-Lopez, J H Himes, D R Jacobs, Jr., B H Alexander, D Lazovich, M Gunnar. Secondary pesticide exposure is associated with head circumference and growth in children. Am J Epidemiol. 2012;175(11 Suppl):S1-S145
- Jansson C, Plato N, Johansson AL, Nyren O, Lagergren J. Airborne occupational exposures and risk of oesophageal and cardia adenocarcinoma. Occupational and environmental medicine. 2006;63:107-112
- Jeebhay MF, Baatjies R, Chang YS, Kim YK, Kim YY, Major V, Lopata AL. Risk factors for allergy due to the two-spotted spider mite (tetranychus urticae) among table grape farm workers. International archives of allergy and immunology. 2007;144:143-149
- Ji G, Xia Y, Gu A, Shi X, Long Y, Song L, Wang S, Wang X. Effects of non-occupational environmental exposure to pyrethroids on semen quality and sperm DNA integrity in chinese men. Reprod Toxicol. 2011;31:171-176
- Jimenez-Jimenez FJ, de Toledo-Heras M, Alonso-Navarro H, Ayuso-Peralta L, Arevalo- Serrano J, Ballesteros-Barranco A, Puertas I, Jabbour-Wadih T, Barcenilla B. Environmental risk factors for essential tremor. European neurology. 2007;58:106-113
- Jorgensen ME, Borch-Johnsen K, Bjerregaard P. A cross-sectional study of the association between persistent organic pollutants and glucose intolerance among Greenland inuit. Diabetologia. 2008;51:1416-1422
- Jurewicz J, Hanke W, Makowiec-Dabrowska T. [low risk of reproductive disorders among female greenhouse workers—safe work conditions or health selection for the light work?]. Medycyna pracy. 2008;59:123-131
- Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and schoolaged children. Epidemiology. 2012;23:689-698
- Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I. Maternal ddt exposures in relation to fetal and 5year growth. Epidemiology. 2006;17:692-700
- Kallioniemi MK, Simola AJ, Kymalainen HR, Vesala HT, Louhelainen JK. Mental symptoms among Finnish farm entrepreneurs. Annals of agricultural and environmental medicine: AAEM. 2009;16:159-168
- Kamalesh Das, Chiranjib Nag and Mrinalkanti Ghosh. Familial, Environmental, and Occupational Risk Factors in Development of Amyotrophic Lateral Sclerosis. North American Journal of Medical Sciences 2012.
- Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed pesticide applicators in the agricultural health study. Human & experimental toxicology. 2007;26:243-250
- Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja M, Blair A, Comyns K, Goldman S, Korell M, Langston J, Ross G, Sandler D. Pesticide exposure and self-reported parkinson's disease in the agricultural health study. American journal of epidemiology. 2007;165:364-374

<sup>129</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, Hoppin JA, Dosemeci M, Coble J, Lubin J, Blair A, Alavanja M. Cancer incidence among pesticide applicators exposed to trifluralin in the agricultural health study. Environmental research. 2008;107:271-276
- Karami S, Boffetta P, Rothman N, Hung RJ, Stewart T, Zaridze D, Navritalova M, Mates D, Janout V, Kollarova H, Bencko V, Szeszenia-Dabrowska N, Holcatova I, Mukeria A, Gromiec J, Chanock SJ, Brennan P, Chow WH, Moore LE. Renal cell carcinoma, occupational pesticide exposure and modification by glutathione s-transferase polymorphisms. Carcinogenesis. 2008;29:1567-1571
- Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH. Hodgkin lymphoma and pesticides exposure in men: A Canadian case-control study. Journal of agromedicine. 2012;17:30-39
- Kaufman DW, Anderson TE, Issaragrisil S. Risk factors for leukemia in Thailand. Annals of hematology. 2009;88:1079-1088
- Kelada SN, Checkoway H, Kardia SL, Carlson CS, Costa-Mallen P, Eaton DL, Firestone J, Powers KM, Swanson PD, Franklin GM, Longstreth WT, Jr., Weller TS, Afsharinejad Z, Costa LG. 5' and 3' region variability in the dopamine transporter gene (slc6a3), pesticide exposure and parkinson's disease risk: A hypothesis-generating study. Human molecular genetics. 2006;15:3055-3062
- Khanjani N, Sim MR. Maternal contamination with dichlorodiphenyltrichloroethane and reproductive outcomes in an Australian population. Environmental research. 2006;101:373-379
- Khanjani N, Sim MR. Reproductive outcomes of maternal contamination with cyclodiene insecticides, hexachlorobenzene and beta-benzene hexachloride. The Science of the total environment. 2006;368:557-564
- Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M. Gst polymorphisms, interaction with smoking and pesticide use, and risk for parkinson's disease in a japanese population. Parkinsonism & related disorders. 2010;16:447-452
- Koutros S, Andreotti G, Berndt SI, Hughes Barry K, Lubin JH, Hoppin JA, Kamel F, Sandler DP, Burdette LA, Yuenger J, Yeager M, Alavanja MC, Freeman LE. Xenobioticmetabolizing gene variants, pesticide use, and the risk of prostate cancer. Pharmacogenetics and genomics. 2011;21:615-623
- Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. Heterocyclic aromatic amine pesticide use and human cancer risk: Results from the u.S. Agricultural health study. International journal of cancer. Journal international du cancer. 2009;124:1206-1212
- Koutros S, Mahajan R, Zheng T, Hoppin JA, Ma X, Lynch CF, Blair A, Alavanja MC. Dichlorvos exposure and human cancer risk: Results from the agricultural health study. Cancer causes & control: CCC. 2008;19:59-65
- Kouznetsova M, Huang X, Ma J, Lessner L, Carpenter DO. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. Environmental Health

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, Hoppin JA, Dosemeci M, Coble J, Lubin J, Blair A, Alavanja M. Cancer incidence among pesticide applicators exposed to trifluralin in the agricultural health study. Environmental research. 2008;107:271-276
- Karami S, Boffetta P, Rothman N, Hung RJ, Stewart T, Zaridze D, Navritalova M, Mates D, Janout V, Kollarova H, Bencko V, Szeszenia-Dabrowska N, Holcatova I, Mukeria A, Gromiec J, Chanock SJ, Brennan P, Chow WH, Moore LE. Renal cell carcinoma, occupational pesticide exposure and modification by glutathione s-transferase polymorphisms. Carcinogenesis. 2008;29:1567-1571
- Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH. Hodgkin lymphoma and pesticides exposure in men: A Canadian case-control study. Journal of agromedicine. 2012;17:30-39
- Kaufman DW, Anderson TE, Issaragrisil S. Risk factors for leukemia in Thailand. Annals of hematology. 2009;88:1079-1088
- Kelada SN, Checkoway H, Kardia SL, Carlson CS, Costa-Mallen P, Eaton DL, Firestone J, Powers KM, Swanson PD, Franklin GM, Longstreth WT, Jr., Weller TS, Afsharinejad Z, Costa LG. 5' and 3' region variability in the dopamine transporter gene (slc6a3), pesticide exposure and parkinson's disease risk: A hypothesis-generating study. Human molecular genetics. 2006;15:3055-3062
- Khanjani N, Sim MR. Maternal contamination with dichlorodiphenyltrichloroethane and reproductive outcomes in an Australian population. Environmental research. 2006;101:373-379
- Khanjani N, Sim MR. Reproductive outcomes of maternal contamination with cyclodiene insecticides, hexachlorobenzene and beta-benzene hexachloride. The Science of the total environment. 2006;368:557-564
- Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, Tanaka K, Fukushima W, Sasaki S, Tsuboi Y, Yamada T, Oeda T, Miki T, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M. Gst polymorphisms, interaction with smoking and pesticide use, and risk for parkinson's disease in a japanese population. Parkinsonism & related disorders. 2010;16:447-452
- Koutros S, Andreotti G, Berndt SI, Hughes Barry K, Lubin JH, Hoppin JA, Kamel F, Sandler DP, Burdette LA, Yuenger J, Yeager M, Alavanja MC, Freeman LE. Xenobioticmetabolizing gene variants, pesticide use, and the risk of prostate cancer. Pharmacogenetics and genomics. 2011;21:615-623
- Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. Heterocyclic aromatic amine pesticide use and human cancer risk: Results from the u.S. Agricultural health study. International journal of cancer. Journal international du cancer. 2009;124:1206-1212
- Koutros S, Mahajan R, Zheng T, Hoppin JA, Ma X, Lynch CF, Blair A, Alavanja MC. Dichlorvos exposure and human cancer risk: Results from the agricultural health study. Cancer causes & control: CCC. 2008;19:59-65
- Kouznetsova M, Huang X, Ma J, Lessner L, Carpenter DO. Increased rate of hospitalization for diabetes and residential proximity of hazardous waste sites. Environmental Health Perspectives. 2006;115:75-79

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Pesticide epidemiology

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Perspectives. 2006;115:75-79

- Kumar V, Yadav CS, Singh S, Goel S, Ahmed RS, Gupta S, Grover RK, Banerjee BD. Cyp 1a1 polymorphism and organochlorine pesticides levels in the etiology of prostate cancer. Chemosphere. 2010;81:464-468
- L. Lind, D. Lee, D.R. Jacobs, S. Salihovic, B. vanBavel, P.M. Lind. Are persistent organic pollutants associated with obesity, the metabolic syndrome or both? Abstracts / Toxicology Letters 205S (2011) S60–S179
- Lacasana M, Lopez-Flores I, Rodriguez-Barranco M, Aguilar-Garduno C, Blanco-Munoz J, Perez-Mendez O, Gamboa R, Bassol S, Cebrian ME. Association between organophosphate pesticides exposure and thyroid hormones in floriculture workers. Toxicology and applied pharmacology. 2010;243:19-26
- Lacasana M, Vazquez-Grameix H, Borja-Aburto VH, Blanco-Munoz J, Romieu I, Aguilar-Garduno C, Garcia AM. Maternal and paternal occupational exposure to agricultural work and the risk of anencephaly. Occupational and environmental medicine. 2006;63:649-656
- Laden F, Bertrand KA, Altshul L, Aster JC, Korrick SA, Sagiv SK. Plasma organochlorine levels and risk of non-hodgkin lymphoma in the nurses' health study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010;19:1381-1384
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV, Alavanja MC. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the agricultural health study. Blood. 2009;113:6386-6391
- Langer P, Kocan A, Drobna B, Susienkova K, Radikova Z, Huckova M, Imrich R, Ksinantova L, Klimes I. Polychlorinated biphenyls and testosterone: Age and congener related correlation approach in heavily exposed males. Endocrine regulations. 2010;44:109-114
- Langer P, Kocan A, Tajtakova M, Koska J, Radikova Z, Ksinantova L, Imrich R, Huckova M, Drobna B, Gasperikova D, Sebokova E, Klimes I. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: Outcome of transgenerational transmission? Chemosphere. 2008;73:1145-1150
- Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Radikova Z, Koska J, Ksinantova L, Huckova M, Imrich R, Wimmerova S, Gasperikova D, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Fish from industrially polluted freshwater as the main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere. 2007;67:S379-385
- Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. Chemosphere. 2007;69:118-127
- Lauria L, Settimi L, Spinelli A, Figa-Talamanca I. Exposure to pesticides and time to pregnancy among female greenhouse workers. Reprod Toxicol. 2006;22:425-430
- Lee DH, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and gamma glutamyltransferase: Results from the national health and

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Kumar V, Yadav CS, Singh S, Goel S, Ahmed RS, Gupta S, Grover RK, Banerjee BD. Cyp 1a1 polymorphism and organochlorine pesticides levels in the etiology of prostate cancer. Chemosphere. 2010;81:464-468
- L. Lind, D. Lee, D.R. Jacobs, S. Salihovic, B. vanBavel, P.M. Lind. Are persistent organic pollutants associated with obesity, the metabolic syndrome or both? Abstracts / Toxicology Letters 2058 (2011) S60-S179
- Lacasana M, Lopez-Flores I, Rodriguez-Barranco M, Aguilar-Garduno C, Blanco-Munoz J, Perez-Mendez O, Gamboa R, Bassol S, Cebrian ME. Association between organophosphate pesticides exposure and thyroid hormones in floriculture workers. Toxicology and applied pharmacology. 2010;243:19-26
- Lacasana M, Vazquez-Grameix H, Borja-Aburto VH, Blanco-Munoz J, Romieu I, Aguilar- Garduno C, Garcia AM. Maternal and paternal occupational exposure to agricultural work and the risk of anencephaly. Occupational and environmental medicine. 2006;63:649-656
- Laden F, Bertrand KA, Altshul L, Aster JC, Korrick SA, Sagiv SK. Plasma organochlorine levels and risk of non-hodgkin lymphoma in the nurses' health study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010;19:1381-1384
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA, Rajkumar SV, Alavanja MC. Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the agricultural health study. Blood. 2009;113:6386-6391
- Langer P, Kocan A, Drobna B, Susienkova K, Radikova Z, Huckova M, Imrich R, Ksinantova L, Klimes I. Polychlorinated biphenyls and testosterone: Age and congener related correlation approach in heavily exposed males. Endocrine regulations. 2010;44:109-114
- Langer P, Kocan A, Tajtakova M, Koska J, Radikova Z, Ksinantova L, Imrich R, Huckova M, Drobna B, Gasperikova D, Sebokova E, Klimes I. Increased thyroid volume, prevalence of thyroid antibodies and impaired fasting glucose in young adults from organochlorine cocktail polluted area: Outcome of transgenerational transmission? Chemosphere. 2008;73:1145-1150
- Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Radikova Z, Koska J, Ksinantova L, Huckova M, Imrich R, Wimmerova S, Gasperikova D, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Fish from industrially polluted freshwater as the main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere. 2007;67:S379-385
- Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Sebokova E, Klimes I. Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. Chemosphere. 2007;69:118-127
- Lauria L, Settimi L, Spinelli A, Figa-Talamanca I. Exposure to pesticides and time to pregnancy among female greenhouse workers. Reprod Toxicol. 2006;22:425-430
- Lee DH, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and gamma glutamyltransferase: Results from the national health and examination survey 1999-2002. Clinical chemistry. 2006;52:1825-1827

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

examination survey 1999-2002. Clinical chemistry. 2006;52:1825-1827

- Lee DH, Jacobs DR, Jr., Steffes M. Association of organochlorine pesticides with peripheral neuropathy in patients with diabetes or impaired fasting glucose. Diabetes. 2008;57:3108-3111
- Lee DH, Jacobs DR, Kocher T. Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. Environ Health Perspect. 2008;116:1558-1562
- Lee DH, Jacobs DR, Porta M. Association of serum concentrations of persistent organic pollutants with the prevalence of learning disability and attention deficit disorder. Journal of epidemiology and community health. 2007;61:591-596
- Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetes care. 2007;30:622-628
- Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetologia. 2007;50:1841-1851
- Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr. A strong doseresponse relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. Diabetes care. 2006;29:1638-1644
- Lee DH, Lee IK, Steffes M, Jacobs DR, Jr. Extended analyses of the association between serum concentrations of persistent organic pollutants and diabetes. Diabetes care. 2007;30:1596-1598
- Lee DH, Lind L, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind PM. Associations of persistent organic pollutants with abdominal obesity in the elderly: The prospective investigation of the vasculature in uppsala seniors (pivus) study. Environment international. 2012;40:170-178
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Background exposure to persistent organic pollutants predicts stroke in the elderly. Environment international. 2012;47:115-120
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: The prospective investigation of the vasculature in Uppsala seniors (pivus) study. Diabetes care. 2011;34:1778-1784
- Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect. 2007;115:883-888
- Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose of some persistent organic pollutants predicts type 2 diabetes: A nested case-control study. Environ Health Perspect. 2010;118:1235-1242

Lee WJ, Alavanja MC, Hoppin JA, Rusiecki JA, Kamel F, Blair A, Sandler DP. Mortality

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Lee DH, Jacobs DR, Jr., Steffes M. Association of organochlorine pesticides with peripheral neuropathy in patients with diabetes or impaired fasting glucose. Diabetes. 2008;57:3108-3111
- Lee DH, Jacobs DR, Kocher T. Associations of serum concentrations of persistent organic pollutants with the prevalence of periodontal disease and subpopulations of white blood cells. Environ Health Perspect. 2008;116:1558-1562
- Lee DH, Jacobs DR, Porta M. Association of serum concentrations of persistent organic pollutants with the prevalence of learning disability and attention deficit disorder. Journal of epidemiology and community health. 2007;61:591-596
- Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetes care. 2007;30:622-628
- Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: Results from the national health and nutrition examination survey 1999-2002. Diabetologia. 2007;50:1841-1851
- Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR, Jr. A strong doseresponse relation between serum concentrations of persistent organic pollutants and diabetes: Results from the national health and examination survey 1999-2002. Diabetes care. 2006;29:1638-1644
- Lee DH, Lee IK, Steffes M, Jacobs DR, Jr. Extended analyses of the association between serum concentrations of persistent organic pollutants and diabetes. Diabetes care. 2007;30:1596-1598
- Lee DH, Lind L, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind PM. Associations of persistent organic pollutants with abdominal obesity in the elderly: The prospective investigation of the vasculature in uppsala seniors (pivus) study. Environment international. 2012;40:170- 178
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Background exposure to persistent organic pollutants predicts stroke in the elderly. Environment international. 2012;47:115-120
- Lee DH, Lind PM, Jacobs DR, Jr., Salihovic S, van Bavel B, Lind L. Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: The prospective investigation of the vasculature in Uppsala seniors (pivus) study. Diabetes care. 2011;34:1778-1784
- Lee DH, Steffes M, Jacobs DR. Positive associations of serum concentration of polychlorinated biphenyls or organochlorine pesticides with self-reported arthritis, especially rheumatoid type, in women. Environ Health Perspect. 2007;115:883-888
- Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. Low dose of some persistent organic pollutants predicts type 2 diabetes: A nested case-control study. Environ Health Perspect. 2010;118:1235-1242
- Lee WJ, Alavanja MC, Hoppin JA, Rusiecki JA, Kamel F, Blair A, Sandler DP. Mortality among pesticide applicators exposed to chlorpyrifos in the agricultural health study. Environ Health Perspect. 2007;115:528-534

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

among pesticide applicators exposed to chlorpyrifos in the agricultural health study. Environ Health Perspect. 2007;115:528-534

- Lee WJ, Baccarelli A, Tretiakova M, Gorbanev S, Lomtev A, Klimkina I, Tchibissov V, Averkina O, Dosemeci M. Pesticide exposure and lung cancer mortality in leningrad province in russia. Environment international. 2006;32:412-416
- Lee WJ, Purdue MP, Stewart P, Schenk M, De Roos AJ, Cerhan JR, Severson RK, Cozen W, Hartge P, Blair A. Asthma history, occupational exposure to pesticides and the risk of nonhodgkin's lymphoma. International journal of cancer. Journal international du cancer. 2006;118:3174-3176
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC. Pesticide use and colorectal cancer risk in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:339-346
- LeVan TD, Koh WP, Lee HP, Koh D, Yu MC, London SJ. Vapor, dust, and smoke exposure in relation to adult-onset asthma and chronic respiratory symptoms: The singapore chinese health study. American journal of epidemiology. 2006;163:1118-1128
- Li J, Lu Y, Shi Y, Wang T, Wang G, Luo W, Jiao W, Chen C, Yan F. Environmental pollution by persistent toxic substances and health risk in an industrial area of china. Journal of Environmental Sciences. 2011;23:1359-1367
- Li JY, Li H, Tao P, Lei FM. [serum organochlorines pesticides level of non-occupational exposure women and risk of breast cancer:A case-control study]. Wei sheng yan jiu = Journal of hygiene research. 2006;35:391-394
- Li JY, Wu DS, Yang F, Zeng HY, Lei FM, Zhou WD, Li H, Tao P. [study on serum organochlorines pesticides (ddts) level, cyp1a1 genetic polymorphism and risk of breast cancer: A case control study]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:217-222
- Lifeng T, Shoulin W, Junmin J, Xuezhao S, Yannan L, Qianli W, Longsheng C. Effects of fenvalerate exposure on semen quality among occupational workers. Contraception. 2006;73:92-96
- Lim JS, Son HK, Park SK, Jacobs DR, Jr., Lee DH. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond). 2011;35:744-747
- Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (pops) and carotid atherosclerosis in the elderly. Environ Health Perspect. 2012;120:38-43
- Liu B, Jung KH, Horton MK, Camann DE, Liu X, Reardon AM, Perzanowski MS, Zhang H, Perera FP, Whyatt RM, Miller RL. Prenatal exposure to pesticide ingredient piperonyl butoxide and childhood cough in an urban cohort. Environment international. 2012;48:156-161
- Lizardi PS, O'Rourke MK, Morris RJ. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of pediatric psychology. 2008;33:91-101
- Lo AC, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, Omar S, Hamilton SR, Greenson JK, Abbruzzese JL. Lifestyle, occupational, and reproductive

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Lee WJ, Baccarelli A, Tretiakova M, Gorbanev S, Lomtev A, Klimkina I, Tchibissov V, Averkina O, Dosemeci M. Pesticide exposure and lung cancer mortality in leningrad province in russia. Environment international. 2006;32:412-416
- Lee WJ, Purdue MP, Stewart P, Schenk M, De Roos AJ, Cerhan JR, Severson RK, Cozen W, Hartge P, Blair A. Asthma history, occupational exposure to pesticides and the risk of non- hodgkin's lymphoma. International journal of cancer. Journal international du cancer. 2006;118:3174-3176
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC. Pesticide use and colorectal cancer risk in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:339-346
- LeVan TD, Koh WP, Lee HP, Koh D, Yu MC, London SJ. Vapor, dust, and smoke exposure in relation to adult-onset asthma and chronic respiratory symptoms: The singapore chinese health study. American journal of epidemiology. 2006;163:1118-1128
- Li J, Lu Y, Shi Y, Wang T, Wang G, Luo W, Jiao W, Chen C, Yan F. Environmental pollution by persistent toxic substances and health risk in an industrial area of china. Journal of Environmental Sciences. 2011;23:1359-1367
- Li JY, Li H, Tao P, Lei FM. [serum organochlorines pesticides level of non-occupational exposure women and risk of breast cancer:A case-control study]. Wei sheng yan jiu = Journal of hygiene research. 2006;35:391-394
- Li JY, Wu DS, Yang F, Zeng HY, Lei FM, Zhou WD, Li H, Tao P. [study on serum organochlorines pesticides (ddts) level, cyplal genetic polymorphism and risk of breast cancer: A case control study]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:217-222
- Lifeng T, Shoulin W, Junmin J, Xuezhao S, Yannan L, Qianli W, Longsheng C. Effects of fenvalerate exposure on semen quality among occupational workers. Contraception. 2006;73:92-96
- Lim JS, Son HK, Park SK, Jacobs DR, Jr., Lee DH. Inverse associations between long-term weight change and serum concentrations of persistent organic pollutants. Int J Obes (Lond). 2011;35:744-747
- Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (pops) and carotid atherosclerosis in the elderly. Environ Health Perspect. 2012;120:38-43
- Liu B, Jung KH, Horton MK, Camann DE, Liu X, Reardon AM, Perzanowski MS, Zhang H, Perera FP, Whyatt RM, Miller RL. Prenatal exposure to pesticide ingredient piperonyl butoxide and childhood cough in an urban cohort. Environment international. 2012;48:156-161
- Lizardi PS, O'Rourke MK, Morris RJ. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of pediatric psychology. 2008;33:91-101
- Lo AC, Soliman AS, El-Ghawalby N, Abdel-Wahab M, Fathy O, Khaled HM, Omar S, Hamilton SR, Greenson JK, Abbruzzese JL. Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. Pancreas. 2007;35:120-129

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

factors in relation to pancreatic cancer risk. Pancreas. 2007;35:120-129

- Lo AC, Soliman AS, Khaled HM, Aboelyazid A, Greenson JK. Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. Diseases of the colon and rectum. 2010;53:830-837
- Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, Hernandez-Avila M. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(pchlorophenyl)ethylene (dde) in relation to anogenital distance in male newborns from chiapas, mexico. American journal of epidemiology. 2007;165:1015-1022
- Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Zock JP, Pollan M. Occupation, exposure to chemicals, sensitizing agents, and risk of multiple myeloma in Sweden. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:3123-3127
- Lopez-Espinosa MJ, Granada A, Carreno J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from southern spain and some related factors. Placenta. 2007;28:631-638
- Lopez-Espinosa MJ, Murcia M, Iniguez C, Vizcaino E, Llop S, Vioque J, Grimalt JO, Rebagliato M, Ballester F. Prenatal exposure to organochlorine compounds and birth size. Pediatrics. 2011;128:e127-134
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, Grimalt JO, Ballester F. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. Journal of exposure science & environmental epidemiology. 2010;20:579-588
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, Marco A, Rebagliato M, Grimalt JO, Ballester F. Association between thyroid hormone levels and 4,4'-dde concentrations in pregnant women (valencia, spain). Environmental research. 2009;109:479-485
- Louis ED, Factor-Litvak P, Parides M, Andrews L, Santella RM, Wolff MS. Organochlorine pesticide exposure in essential tremor: A case-control study using biological and occupational exposure assessments. Neurotoxicology. 2006;27:579-586
- Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. American journal of public health. 2011;101:63-70
- Lu JL. Comparison of pesticide exposure and physical examination, neurological assessment, and laboratory findings between full-time and part-time vegetable farmers in the Philippines. Environmental health and preventive medicine. 2009;14:345-352
- Lu JL. Occupational safety of farmers in the vegetable industry. International journal of occupational safety and ergonomics: JOSE. 2011;17:445-453
- Luis D Boada, Manuel Zumbado, Luis Alberto Henríquez-Hernández, Maira Almeida-González, Eva E Álvarez-León, Lluis Serra-Majem and Octavio P Luzardo. Complex organochlorine pesticide mixtures as determinant factor for breast cancer risk: a populationbased case–control study in theCanary Islands (Spain). Environmental Health 2012, 11:28

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Lo AC, Soliman AS, Khaled HM, Aboelyazid A, Greenson JK. Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. Diseases of the colon and rectum. 2010;53:830-837
- Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, Hernandez-Avila M. In utero exposure to the antiandrogen 1, 1-dichloro-2, 2-bis(pchlorophenyl)ethylene (dde) in relation to anogenital distance in male newborns from chiapas, mexico. American journal of epidemiology. 2007;165:1015-1022
- Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Zock JP, Pollan M. Occupation, exposure to chemicals, sensitizing agents, and risk of multiple myeloma in Sweden. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17:3123-3127
- Lopez-Espinosa MJ, Granada A, Carreno J, Salvatierra M, Olea-Serrano F, Olea N. Organochlorine pesticides in placentas from southern spain and some related factors. Placenta. 2007;28:631-638
- Lopez-Espinosa MJ, Murcia M, Iniguez C, Vizcaino E, Llop S, Vioque J, Grimalt JO, Rebagliato M, Ballester F. Prenatal exposure to organochlorine compounds and birth size. Pediatrics. 2011;128:e127-134
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, Grimalt JO, Ballester F. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. Journal of exposure science & environmental epidemiology. 2010;20:579-588
- Lopez-Espinosa MJ, Vizcaino E, Murcia M, Llop S, Espada M, Seco V, Marco A, Rebagliato M, Grimalt JO, Ballester F. Association between thyroid hormone levels and 4,4'dde concentrations in pregnant women (valencia, spain). Environmental research. 2009;109:479-485
- Louis ED, Factor-Litvak P, Parides M, Andrews L, Santella RM, Wolff MS. Organochlorine pesticide exposure in essential tremor: A case-control study using biological and occupational exposure assessments. Neurotoxicology. 2006;27:579-586
- Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. American journal of public health. 2011;101:63-70
- Lu JL. Comparison of pesticide exposure and physical examination, neurological assessment, and laboratory findings between full-time and part-time vegetable farmers in the Philippines. Environmental health and preventive medicine. 2009;14:345-352
- Lu JL. Occupational safety of farmers in the vegetable industry. International journal of occupational safety and ergonomics: JOSE. 2011;17:445-453
- Luis D Boada, Manuel Zumbado, Luis Alberto Henríquez-Hernández, Maira Almeida- González, Eva E Álvarez-León, Lluis Serra-Majem and Octavio P Luzardo. Complex organochlorine pesticide mixtures as determinant factor for breast cancer risk: a population- based case-control study in theCanary Islands (Spain). Environmental Health 2012, 11:28

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Lv L, Lin G, Gao X, Wu C, Dai J, Yang Y, Zou H, Sun H, Gu M, Chen X, Fu H, Bao L. Casecontrol study of risk factors of myelodysplastic syndromes according to world health organization classification in a chinese population. American journal of hematology. 2011;86:163-169

- Lv L, Lin GW, Wang XQ, Bao LM, Zou HJ. [a case-control study of risk factors for myelodysplastic syndromes]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2007;25:705-709
- Lynch SM, Mahajan R, Beane Freeman LE, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to butylate in the agricultural health study (ahs). Environmental research. 2009;109:860-868
- Lynch SM, Rusiecki JA, Blair A, Dosemeci M, Lubin J, Sandler D, Hoppin JA, Lynch CF, Alavanja MCR. Cancer incidence among pesticide applicators exposed to cyanazine in the agricultural health study. Environmental Health Perspectives. 2006;114:1248-1252
- MacCarthy A, Bunch KJ, Fear NT, King JC, Vincent TJ, Murphy MF. Paternal occupation and neuroblastoma: A case-control study based on cancer registry data for Great Britain 1962-1999. British journal of cancer. 2010;102:615-619
- MacFarlane E, Simpson P, Benke G, Sim MR. Suicide in australian pesticide-exposed workers. Occup Med (Lond). 2011;61:259-264
- Mackenzie Ross SJ, Brewin CR, Curran HV, Furlong CE, Abraham-Smith KM, Harrison V. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicology and teratology. 2010;32:452-459
- Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect. 2007;115:1780-1786
- Mahajan R, Blair A, Coble J, Lynch CF, Hoppin JA, Sandler DP, Alavanja MC. Carbaryl exposure and incident cancer in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:1799-1805
- Mahajan R, Bonner MR, Hoppin JA, Alavanja MCR. Phorate exposure and incidence of cancer in the agricultural health study. Environmental Health Perspectives. 2006;114:1205-1209
- Maluf E, Hamerschlak N, Cavalcanti AB, Junior AA, Eluf-Neto J, Falcao RP, Lorand-Metze IG, Goldenberg D, Santana CL, Rodrigues Dde O, Passos LN, Rosenfeld LG, Pitta M, Loggetto S, Ribeiro AA, Velloso ED, Kondo AT, Coelho EO, Pintao MC, de Souza HM, Borbolla JR, Pasquini R. Incidence and risk factors of aplastic anemia in latin american countries: The latin case-control study. Haematologica. 2009;94:1220-1226
- Manfo FPT, Moundipa PF, Déchaud H, Tchana AlN, Nantia EA, Zabot M-T, Pugeat M. Effect of agropesticides use on male reproductive function: A study on farmers in djutitsa (cameroon). Environmental Toxicology. 2012;27:423-432
- Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and parkinson disease. Epidemiology. 2010;21:87-94

EFSA supporting publication 2013:EN-497

#### 135

- Lv L, Lin G, Gao X, Wu C, Dai J, Yang Y, Zou H, Sun H, Gu M, Chen X, Fu H, Bao L. Casecontrol study of risk factors of myelodysplastic syndromes according to world health organization classification in a chinese population. American journal of hematology. 2011;86:163-169
- Lv L, Lin GW, Wang XQ, Bao LM, Zou HJ. [a case-control study of risk factors for myelodysplastic syndromes]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2007;25:705-709
- Lynch SM, Mahajan R, Beane Freeman LE, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to butylate in the agricultural health study (ahs). Environmental research. 2009;109:860-868
- Lynch SM, Rusiecki JA, Blair A, Dosemeci M, Lubin J, Sandler D, Hoppin JA, Lynch CF, Alavanja MCR. Cancer incidence among pesticide applicators exposed to cyanazine in the agricultural health study. Environmental Health Perspectives. 2006;114:1248-1252
- MacCarthy A, Bunch KJ, Fear NT, King JC, Vincent TJ, Murphy MF. Paternal occupation and neuroblastoma: A case-control study based on cancer registry data for Great Britain 1962-1999. British journal of cancer. 2010;102:615-619
- MacFarlane E, Simpson P, Benke G, Sim MR. Suicide in australian pesticide-exposed workers. Occup Med (Lond). 2011;61:259-264
- Mackenzie Ross SJ, Brewin CR, Curran HV, Furlong CE, Abraham-Smith KM, Harrison V. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicology and teratology. 2010;32:452-459
- Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene MK. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect. 2007;115:1780-1786
- Mahajan R, Blair A, Coble J, Lynch CF, Hoppin JA, Sandler DP, Alavanja MC. Carbaryl exposure and incident cancer in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;121:1799-1805
- Mahajan R, Bonner MR, Hoppin JA, Alavanja MCR. Phorate exposure and incidence of cancer in the agricultural health study. Environmental Health Perspectives. 2006;114:1205-1209
- Maluf E, Hamerschlak N, Cavalcanti AB, Junior AA, Eluf-Neto J, Falcao RP, Lorand-Metze IG, Goldenberg D, Santana CL, Rodrigues Dde O, Passos LN, Rosenfeld LG, Pitta M, Loggetto S, Ribeiro AA, Velloso ED, Kondo AT, Coelho EO, Pintao MC, de Souza HM, Borbolla JR, Pasquini R. Incidence and risk factors of aplastic anemia in latin american countries: The latin case-control study. Haematologica. 2009;94:1220-1226
- Manfo FPT, Moundipa PF, Déchaud H, Tchana AlN, Nantia EA, Zabot M-T, Pugeat M. Effect of agropesticides use on male reproductive function: A study on farmers in djutitsa (cameroon). Environmental Toxicology. 2012;27:423-432
- Manthripragada AD, Costello S, Cockburn MG, Bronstein JM, Ritz B. Paraoxonase 1, agricultural organophosphate exposure, and parkinson disease. Epidemiology. 2010;21:87-94

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Marina Krstevska Konstantinova. Organochlorine pesticides in Macedonain girls

- Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. Organophosphate pesticide exposure and attention in young mexican-american children: The chamacos study. Environ Health Perspect. 2010;118:1768-1774
- Marzouk D.A., El Gaafary M.M., El Damaty S.I., Sabbour S.M., Mecky F.A.S., Saker M., Sayed A.M., Fahim H.I., Anwar W.A.. Breast cancer and hormonal intake among Egyptian females. European Journal of Oncology (2009) 14:1 (37-51).
- Matmurodov R.J., Khalimova K.M., Raimova M.M. Polymorphism of the genes GSTM1, GSTT1, and environmental factors in the development of Parkinson's disease among representatives of the Uzbek nationality. European Journal of Neurology (2011) 18 SUPPL. 2 (501).
- McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ. Environmental exposure to polychlorinated biphenyls and p,p'-dde and sperm sex-chromosome disomy. Environ Health Perspect. 2012;120:535-540
- McDuffie HH, Quail J, Ghosh S, Pahwa P. Host factors, occupation, and testicular cancer in saskatchewan, canada: 1979-2002. Journal of agricultural safety and health. 2007;13:247-258
- McElroy JA, Gangnon RE, Newcomb PA, Kanarek MS, Anderson HA, Brook JV, Trentham-Dietz A, Remington PL. Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in wisconsin. Journal of exposure science & environmental epidemiology. 2007;17:207-214
- McGlynn KA, Abnet CC, Zhang M, Sun XD, Fan JH, O'Brien TR, Wei WQ, Ortiz-Conde BA, Dawsey SM, Weber JP, Taylor PR, Katki H, Mark SD, Qiao YL. Serum concentrations of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (ddt) and 1,1-dichloro-2,2-bis(pchlorophenyl)ethylene (dde) and risk of primary liver cancer. Journal of the National Cancer Institute. 2006;98:1005-1010
- McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of the National Cancer Institute. 2008;100:663-671
- McHugh MK, Kachroo S, Liu M, D'Amelio AM, Jr., Dong Q, Hong WK, Greisinger AJ, Spitz MR, Etzel CJ. Assessing environmental and occupational risk factors for lung cancer in mexican-americans. Cancer causes & control: CCC. 2010;21:2157-2164
- Meeker JD, Altshul L, Hauser R. Serum pcbs, p,p'-dde and hcb predict thyroid hormone levels in men. Environmental research. 2007;104:296-304
- Meeker JD, Barr DB, Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Hum Reprod. 2008;23:1932-1940
- Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol. 2009;27:155-160
- Meeker JD, Barr DB, Hauser R. Thyroid hormones in relation to urinary metabolites of nonpersistent insecticides in men of reproductive age. Reprod Toxicol. 2006;22:437-442
- Meeker JD, Ravi SR, Barr DB, Hauser R. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod Toxicol. 2008;25:184-191

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

## Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Marina Krstevska Konstantinova. Organochlorine pesticides in Macedonain girls Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, Calderon N, Eskenazi B. Organophosphate pesticide exposure and attention in young mexican-american children: The chamacos study. Environ Health Perspect. 2010;118:1768-1774
- Marzouk D. A., El Gaafary M. M., El Damaty S. I., Sabbour S. M., Mecky F. A. S., Saker M., Sayed A. M., Fahim H. I., Anwar W. A.. Breast cancer and hormonal intake among Egyptian females. European Journal of Oncology (2009) 14:1 (37-51).
- Matmurodov R. J., Khalimova K.M., Raimova M.M. Polymorphism of the genes GSTM1, GSTT1, and environmental factors in the development of Parkinson's disease among representatives of the Uzbek nationality. European Journal of Neurology (2011) 18 SUPPL. 2 (501).
- McAuliffe ME, Williams PL, Korrick SA, Altshul LM, Perry MJ. Environmental exposure to polychlorinated biphenyls and p,p'-dde and sperm sex-chromosome disomy. Environ Health Perspect. 2012;120:535-540
- McDuffie HH, Quail J, Ghosh S, Pahwa P. Host factors, occupation, and testicular cancer in saskatchewan, canada: 1979-2002. Journal of agricultural safety and health. 2007;13:247- 258
- McElroy JA, Gangnon RE, Newcomb PA, Kanarek MS, Anderson HA, Brook JV, Trentham- Dietz A, Remington PL. Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in wisconsin. Journal of exposure science & environmental epidemiology. 2007;17:207-214
- McGlynn KA, Abnet CC, Zhang M, Sun XD, Fan JH, O'Brien TR, Wei WQ, Ortiz-Conde BA, Dawsey SM, Weber JP, Taylor PR, Katki H, Mark SD, Qiao YL. Serum concentrations of 1, 1, 1trichloro-2, 2-bis(p-chlorophenyl)ethane (ddt) and 1, 1-dichloro-2, 2-bis(pchlorophenyl)ethylene (dde) and risk of primary liver cancer. Journal of the National Cancer Institute. 2006;98:1005-1010
- McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, Erickson RL. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of the National Cancer Institute. 2008;100:663-671
- McHugh MK, Kachroo S, Liu M, D'Amelio AM, Jr., Dong Q, Hong WK, Greisinger AJ, Spitz MR, Etzel CJ. Assessing environmental and occupational risk factors for lung cancer in mexican-americans. Cancer causes & control: CCC. 2010;21:2157-2164
- Meeker JD, Altshul L, Hauser R. Serum pcbs, p,p'-dde and hcb predict thyroid hormone levels in men. Environmental research. 2007;104:296-304
- Meeker JD, Barr DB, Hauser R. Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Hum Reprod. 2008;23:1932-1940
- Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol. 2009;27:155-160
- Meeker JD, Barr DB, Hauser R. Thyroid hormones in relation to urinary metabolites of nonpersistent insecticides in men of reproductive age. Reprod Toxicol. 2006;22:437-442
- Meeker JD, Ravi SR, Barr DB, Hauser R. Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod Toxicol. 2008;25:184-191

EFSA supporting publication 2013:EN-497

<sup>136</sup> 

- Meeker JD, Ryan L, Barr DB, Hauser R. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 2006;17:61-68
- Melkonian S, Argos M, Pierce BL, Chen Y, Islam T, Ahmed A, Syed EH, Parvez F, Graziano J, Rathouz PJ, Ahsan H. A prospective study of the synergistic effects of arsenic exposure and smoking, sun exposure, fertilizer use, and pesticide use on risk of premalignant skin lesions in Bangladeshi men. American journal of epidemiology. 2011;173:183-191
- Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, Fochs S, Sunyer J. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect. 2011;119:272-278
- Menegaux F, Baruchel A, Bertrand Y, Lescoeur B, Leverger G, Nelken B, Sommelet D, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood acute leukaemia. Occupational and environmental medicine. 2006;63:131-134
- Merletti F, Richiardi L, Bertoni F, Ahrens W, Buemi A, Costa-Santos C, Eriksson M, Guenel P, Kaerlev L, Jockel KH, Llopis-Gonzalez A, Merler E, Miranda A, Morales-Suarez-Varela MM, Olsson H, Fletcher T, Olsen J. Occupational factors and risk of adult bone sarcomas: A multicentric case-control study in europe. International journal of cancer. Journal international du cancer. 2006;118:721-727
- Messaros BM, Rossano MG, Liu G, Diamond MP, Friderici K, Nummy-Jernigan K, Daly D, Puscheck E, Paneth N, Wirth JJ. Negative effects of serum p,p'-dde on sperm parameters and modification by genetic polymorphisms. Environmental research. 2009;109:457-464
- Meyer A, Alexandre PC, Chrisman Jde R, Markowitz SB, Koifman RJ, Koifman S. Esophageal cancer among brazilian agricultural workers: Case-control study based on death certificates. International journal of hygiene and environmental health. 2011;214:151-155
- Meyer KJ, Reif JS, Veeramachaneni DNR, Luben TJ, Mosley BS, Nuckols JR. Agricultural pesticide use and hypospadias in eastern arkansas. Environmental Health Perspectives. 2006;114:1589-1595
- Meyer TE, Coker AL, Sanderson M, Symanski E. A case-control study of farming and prostate cancer in african-american and caucasian men. Occupational and environmental medicine. 2007;64:155-160
- Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: An overview and some results of the italian multicenter case-control study on hematolymphopoietic malignancies. Annals of the New York Academy of Sciences. 2006;1076:366-377
- Mills KT, Blair A, Freeman LE, Sandler DP, Hoppin JA. Pesticides and myocardial infarction incidence and mortality among male pesticide applicators in the agricultural health study. American journal of epidemiology. 2009;170:892-900
- Mills PK, Yang RC. Agricultural exposures and gastric cancer risk in Hispanic farm workers in california. Environmental research. 2007;104:282-289
- Min JY, Cho JS, Lee KJ, Park JB, Park SG, Kim JY, Min KB. Potential role for organochlorine pesticides in the prevalence of peripheral arterial diseases in obese persons: Results from the national health and nutrition examination survey 1999-2004. Atherosclerosis. 2011;218:200-206
- Miyake Y, Tanaka K, Masuzaki Y, Sato N, Ikeda Y, Chisaki Y, Arakawa M. Organochlorine concentrations in breast milk and prevalence of allergic disorders in japanese women.

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Pesticide epidemiology
- Meeker JD, Ryan L, Barr DB, Hauser R. Exposure to nonpersistent insecticides and male reproductive hormones. Epidemiology. 2006;17:61-68
- Melkonian S, Argos M, Pierce BL, Chen Y, Islam T, Ahmed A, Syed EH, Parvez F, Graziano J, Rathouz PJ, Ahsan H. A prospective study of the synergistic effects of arsenic exposure and smoking, sun exposure, fertilizer use, and pesticide use on risk of premalignant skin lesions in Bangladeshi men. American journal of epidemiology. 2011;173:183-191
- Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, Fochs S, Sunyer J. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect. 2011;119:272-278
- Menegaux F, Baruchel A, Bertrand Y, Lescoeur B, Leverger G, Nelken B, Sommelet D, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood acute leukaemia. Occupational and environmental medicine. 2006;63:131-134
- Merletti F, Richiardi L, Bertoni F, Ahrens W, Buemi A, Costa-Santos C, Eriksson M, Guenel P, Kaerlev L, Jockel KH, Llopis-Gonzalez A, Merler E, Miranda A, Morales-Suarez-Varela MM, Olsson H, Fletcher T, Olsen J. Occupational factors and risk of adult bone sarcomas: A multicentric case-control study in europe. International journal of cancer. Journal international du cancer. 2006;118:721-727
- Messaros BM, Rossano MG, Liu G, Diamond MP, Friderici K, Nummy-Jernigan K, Daly D, Puscheck E, Paneth N, Wirth JJ. Negative effects of serum p,p'-dde on sperm parameters and modification by genetic polymorphisms. Environmental research. 2009;109:457-464
- Meyer A, Alexandre PC, Chrisman Jde R, Markowitz SB, Koifman RJ, Koifman S. Esophageal cancer among brazilian agricultural workers: Case-control study based on death certificates. International journal of hygiene and environmental health. 2011;214:151-155
- Meyer KJ, Reif JS, Veeramachaneni DNR, Luben TJ, Mosley BS, Nuckols JR. Agricultural pesticide use and hypospadias in eastern arkansas. Environmental Health Perspectives. 2006;114:1589-1595
- Meyer TE, Coker AL, Sanderson M, Symanski E. A case-control study of farming and prostate cancer in african-american and caucasian men. Occupational and environmental medicine. 2007;64:155-160
- Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: An overview and some results of the italian multicenter case-control study on hematolymphopoietic malignancies. Annals of the New York Academy of Sciences. 2006;1076:366-377
- Mills KT, Blair A, Freeman LE, Sandler DP, Hoppin JA. Pesticides and myocardial infarction incidence and mortality among male pesticide applicators in the agricultural health study. American journal of epidemiology. 2009;170:892-900
- Mills PK, Yang RC. Agricultural exposures and gastric cancer risk in Hispanic farm workers in california. Environmental research. 2007;104:282-289
- Min JY, Cho JS, Lee KJ, Park JB, Park SG, Kim JY, Min KB. Potential role for organochlorine pesticides in the prevalence of peripheral arterial diseases in obese persons: Results from the national health and nutrition examination survey 1999-2004. Atherosclerosis. 2011;218:200- 206
- Miyake Y, Tanaka K, Masuzaki Y, Sato N, Ikeda Y, Chisaki Y, Arakawa M. Organochlorine concentrations in breast milk and prevalence of allergic disorders in japanese women. Chemosphere. 2011;85:374-378

EFSA supporting publication 2013:EN-497

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EFSA 支援出版 2013:EN-497

Pesticide epidemiology

Chemosphere. 2011;85:374-378

- Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP, Weiderpass E, Partanen T. Parental occupational exposure to pesticides and the risk of childhood leukemia in costa rica. Scandinavian Journal of Work, Environment & Health. 2007;33:293-303
- Montgomery MP, Kamel F, Saldana TM, Alavanja MC, Sandler DP. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993-2003. American journal of epidemiology. 2008;167:1235-1246
- Morahan JM, Pamphlett R. Amyotrophic lateral sclerosis and exposure to environmental toxins: An Australian case-control study. Neuroepidemiology. 2006;27:130-135
- Mozzachio AM, Rusiecki JA, Hoppin JA, Mahajan R, Patel R, Beane-Freeman L, Alavanja MC. Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study. Environmental research. 2008;108:400-403
- Mueller BA, Kuehn CM, Shapiro-Mendoza CK, Tomashek KM. Fetal deaths and proximity to hazardous waste sites in washington state. Environ Health Perspect. 2007;115:776-780
- Multigner L, Kadhel P, Pascal M, Huc-Terki F, Kercret H, Massart C, Janky E, Auger J, Jegou B. Parallel assessment of male reproductive function in workers and wild rats exposed to pesticides in banana plantations in guadeloupe. Environmental health: a global access science source. 2008;7:40
- Multigner L, Ndong JR, Giusti A, Romana M, Delacroix-Maillard H, Cordier S, Jegou B, Thome JP, Blanchet P. Chlordecone exposure and risk of prostate cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010;28:3457-3462
- N. Murgia, M. Dell'Omo, G. Muzi, G. Brugnami, M. Biancalana,
- Nagayama J, Kohno H, Kunisue T, Kataoka K, Shimomura H, Tanabe S, Konishi S. Concentrations of organochlorine pollutants in mothers who gave birth to neonates with congenital hypothyroidism. Chemosphere. 2007;68:972-976
- Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, Yanagawa T, Fukushige J, Watanabe T. Immunologic effects of perinatal exposure to dioxins, pcbs and organochlorine pesticides in japanese infants. Chemosphere. 2007;67:S393-398
- Naidoo S, London L, Burdorf A, Naidoo R, Kromhout H. Spontaneous miscarriages and infant deaths among female farmers in rural south africa. Scand J Work Environ Health. 2011;37:227-236
- Narendra M, Kavitha G, Helah Kiranmai A, Raghava Rao N, Varadacharyulu NC. Chronic exposure to pyrethroid-based allethrin and prallethrin mosquito repellents alters plasma biochemical profile. Chemosphere. 2008;73:360-364
- Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environmental science & technology. 2011;45:1680-1687
- Neundorfer B. . Solvents and PARKINSON'S disease. Padiatrische Praxis (2008) 72:3 (508-510).
- Nicolas Lebas, Louise Nadon, Mounia Rhazi, Hugues Richard, Marie Desy, Marie-Elise Parent. Exposure to occupational and domestic pesticides, and prostate cancer risk:

- Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP, Weiderpass E, Partanen T. Parental occupational exposure to pesticides and the risk of childhood leukemia in costa rica. Scandinavian Journal of Work, Environment & Health. 2007;33:293-303
- Montgomery MP, Kamel F, Saldana TM, Alavanja MC, Sandler DP. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural health study, 1993- 2003. American journal of epidemiology. 2008;167:1235-1246
- Morahan JM, Pamphlett R. Amyotrophic lateral sclerosis and exposure to environmental toxins: An Australian case-control study. Neuroepidemiology. 2006;27:130-135
- Mozzachio AM, Rusiecki JA, Hoppin JA, Mahajan R, Patel R, Beane-Freeman L, Alavanja MC. Chlorothalonil exposure and cancer incidence among pesticide applicator participants in the agricultural health study. Environmental research. 2008;108:400-403
- Mueller BA, Kuehn CM, Shapiro-Mendoza CK, Tomashek KM. Fetal deaths and proximity to hazardous waste sites in washington state. Environ Health Perspect. 2007;115:776-780
- Multigner L, Kadhel P, Pascal M, Huc-Terki F, Kercret H, Massart C, Janky E, Auger J, Jegou B. Parallel assessment of male reproductive function in workers and wild rats exposed to pesticides in banana plantations in guadeloupe. Environmental health: a global access science source. 2008;7:40
- Multigner L, Ndong JR, Giusti A, Romana M, Delacroix-Maillard H, Cordier S, Jegou B, Thome JP, Blanchet P. Chlordecone exposure and risk of prostate cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2010;28:3457-3462
- N. Murgia, M. Dell"Omo, G. Muzi, G. Brugnami, M. Biancalana, Nagayama J, Kohno H, Kunisue T, Kataoka K, Shimomura H, Tanabe S, Konishi S. Concentrations of organochlorine pollutants in mothers who gave birth to neonates with congenital hypothyroidism. Chemosphere. 2007;68:972-976
- Nagayama J, Tsuji H, Iida T, Nakagawa R, Matsueda T, Hirakawa H, Yanagawa T, Fukushige J, Watanabe T. Immunologic effects of perinatal exposure to dioxins, pcbs and organochlorine pesticides in japanese infants. Chemosphere. 2007;67:S393-398
- Naidoo S, London L, Burdorf A, Naidoo R, Kromhout H. Spontaneous miscarriages and infant deaths among female farmers in rural south africa. Scand J Work Environ Health. 2011;37:227-236
- Narendra M, Kavitha G, Helah Kiranmai A, Raghava Rao N, Varadacharyulu NC. Chronic exposure to pyrethroid-based allethrin and prallethrin mosquito repellents alters plasma biochemical profile. Chemosphere. 2008;73:360-364
- Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environmental science & technology. 2011;45:1680-1687
- Neundorfer B. . Solvents and PARKINSON'S disease. Padiatrische Praxis (2008) 72:3 (508-510).
- Nicolas Lebas, Louise Nadon, Mounia Rhazi, Hugues Richard, Marie Desy, Marie-Elise Parent. Exposure to occupational and domestic pesticides, and prostate cancer risk: preliminary findings from a case-control study in Montreal, Canada.

EFSA supporting publication 2013:EN-497

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preliminary findings from a case-control study in Montreal, Canada.

- Nicolle-Mir L. Exposure to pesticides and thyroid diseases. Environmement, Risques et Sante (2010) 9:5 (381-382).
- Nicolle-Mir L. Occupational factors and Parkinson's disease. Environmement, Risques et Sante (2010) 9:5 (382-383).
- Nordby KC, Irgens LM, Kristensen P. Immunological exposures in Norwegian agriculture and pre-eclampsia. Paediatric and perinatal epidemiology. 2006;20:462-470
- Norlaily H, Azidah AK, Asrenee AR, Rohayah H, Juwita S. Proportion of dementia and its associated factors among elderly patients attending outpatient clinics of universiti sains malaysia hospital. The Medical journal of Malaysia. 2009;64:140-145
- Ochoa-Acuna H, Frankenberger J, Hahn L, Carbajo C. Drinking-water herbicide exposure in indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Perspect. 2009;117:1619-1624
- Ociepa-Zawal M, Rubis B, Wawrzynczak D, Wachowiak R, Trzeciak WH. Accumulation of environmental estrogens in adipose tissue of breast cancer patients. Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering. 2010;45:305-312
- Onishchenko GG, Mamaev IA, Guseinov GK. [impact of the area burden of agrochemicals on tuberculosis morbidity and mortality]. Problemy tuberkuleza i boleznei legkikh. 2006:30-33
- Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X, Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: Results of a french case-control study. Occupational and environmental medicine. 2009;66:291-298
- Orsi L, Troussard X, Monnereau A, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Clavel J. Occupation and lymphoid malignancies: Results from a french case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:1339-1350
- Ostrea EM, Jr., Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012;33:669-675
- Ozen S, Darcan S, Bayindir P, Karasulu E, Simsek DG, Gurler T. Effects of pesticides used in agriculture on the development of precocious puberty. Environmental monitoring and assessment. 2012;184:4223-4232
- P. Monica Lind, Samira Salihovic, Bert van Bavel, Lars Lind. Circulating levels of pp-DDE and hypertension. Abstracts / Toxicology Letters 211S (2012) S43–S216
- Padmaja R. Jonnalagadda\*, A. Y. E. Prasad, Katla Ashok Reddy, Challa Suresh, M. Vishnu, Vardhana Rao, Goparaju Ramya and D. Raghunatha Rao. Biochemical alterations of certain health parameters in cotton growing farmers exposed to organophosphorous and pyrethroid insecticides. African Journal of Biotechnology Vol. 9(49), pp. 8369-8377, 6 December, 2010

EFSA supporting publication 2013:EN-497

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### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Nicolle-Mir L. Exposure to pesticides and thyroid diseases. Environmement, Risques et Sante (2010) 9:5 (381-382).
- Nicolle-Mir L. Occupational factors and Parkinson's disease. Environmement, Risques et Sante (2010) 9:5 (382-383).
- Nordby KC, Irgens LM, Kristensen P. Immunological exposures in Norwegian agriculture and pre-eclampsia. Paediatric and perinatal epidemiology. 2006;20:462-470
- Norlaily H, Azidah AK, Asrenee AR, Rohayah H, Juwita S. Proportion of dementia and its associated factors among elderly patients attending outpatient clinics of universiti sains malaysia hospital. The Medical journal of Malaysia. 2009;64:140-145
- Ochoa-Acuna H, Frankenberger J, Hahn L, Carbajo C. Drinking-water herbicide exposure in indiana and prevalence of small-for-gestational-age and preterm delivery. Environ Health Perspect. 2009;117:1619-1624
- Ociepa-Zawal M, Rubis B, Wawrzynczak D, Wachowiak R, Trzeciak WH. Accumulation of environmental estrogens in adipose tissue of breast cancer patients. Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering. 2010;45:305-312
- Onishchenko GG, Mamaev IA, Guseinov GK. [impact of the area burden of agrochemicals on tuberculosis morbidity and mortality]. Problemy tuberkuleza i boleznei legkikh. 2006:30-33
- Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X, Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: Results of a french casecontrol study. Occupational and environmental medicine. 2009;66:291-298
- Orsi L, Troussard X, Monnereau A, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Clavel J. Occupation and lymphoid malignancies: Results from a french case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:1339-1350
- Ostrea EM, Jr., Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012;33:669-675
- Ozen S, Darcan S, Bayindir P, Karasulu E, Simsek DG, Gurler T. Effects of pesticides used in agriculture on the development of precocious puberty. Environmental monitoring and assessment. 2012;184:4223-4232
- P. Monica Lind, Samira Salihovic , Bert van Bavel, Lars Lind. Circulating levels of pp-DDE and hypertension. Abstracts / Toxicology Letters 211S (2012) S43-S216
- Padmaja R. Jonnalagadda\*, A. Y. E. Prasad, Katla Ashok Reddy, Challa Suresh, M. Vishnu, Vardhana Rao, Goparaju Ramya and D. Raghunatha Rao. Biochemical alterations of certain health parameters in cotton growing farmers exposed to organophosphorous and pyrethroid insecticides. African Journal of Biotechnology Vol. 9(49), pp. 8369-8377, 6 December, 2010

EFSA 支援出版 2013:EN-497

<sup>139</sup> 

- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McDuffie HH, McLaughlin JR. Multiple myeloma and exposure to pesticides: A Canadian case-control study. Journal of agromedicine. 2012;17:40-50
- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR. Soft-tissue sarcoma and pesticides exposure in men: Results of a Canadian case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:1279-1286
- Pahwa P, Karunanayake CP, Spinelli JJ, Dosman JA, McDuffie HH. Ethnicity and incidence of Hodgkin lymphoma in canadian population. BMC cancer. 2009;9:141
- Pahwa P, McDuffie HH, Dosman JA, McLaughlin JR, Spinelli JJ, Robson D, Fincham S. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:264-274
- Pamphlett R. Exposure to environmental toxins and the risk of sporadic motor neuron disease: An expanded Australian case-control study. European journal of neurology: the official journal of the European Federation of Neurological Societies. 2012;19:1343-1348
- Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: An analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect. 2009;117:488-494
- Pan IJ, Daniels JL, Herring AH, Rogan WJ, Siega-Riz AM, Goldman BD, Sjodin A. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: An analysis of the pregnancy, infection, and nutrition babies study. Paediatric and perinatal epidemiology. 2010;24:262-271
- Pant N, Kumar R, Mathur N, Srivastava SP, Saxena DK, Gujrati VR. Chlorinated pesticide concentration in semen of fertile and infertile men and correlation with sperm quality. Environmental toxicology and pharmacology. 2007;23:135-139
- Park SK, Kang D, Beane-Freeman L, Blair A, Hoppin JA, Sandler DP, Lynch CF, Knott C, Gwak J, Alavanja M. Cancer incidence among paraquat exposed applicators in the agricultural health study: Prospective cohort study. International journal of occupational and environmental health. 2009;15:274-281
- Park SK, Son HK, Lee SK, Kang JH, Chang YS, Jacobs DR, Lee DH. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among nondiabetic adults. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2010;43:1-8
- Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J, Sarto G, Howard BV. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the women's health initiative observational study. Arthritis care & research. 2011;63:184-194
- Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (ewas) on type 2 diabetes mellitus. PloS one. 2010;5:e10746

Pathak R, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Banerjee BD.

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# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McDuffie HH, McLaughlin JR. Multiple myeloma and exposure to pesticides: A Canadian case-control study. Journal of agromedicine. 2012;17:40-50
- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR. Soft-tissue sarcoma and pesticides exposure in men: Results of a Canadian case-control study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:1279-1286
- Pahwa P, Karunanayake CP, Spinelli JJ, Dosman JA, McDuffie HH. Ethnicity and incidence of Hodgkin lymphoma in canadian population. BMC cancer. 2009;9:141
- Pahwa P, McDuffie HH, Dosman JA, McLaughlin JR, Spinelli JJ, Robson D, Fincham S. Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:264-274
- Pamphlett R. Exposure to environmental toxins and the risk of sporadic motor neuron disease: An expanded Australian case-control study. European journal of neurology: the official journal of the European Federation of Neurological Societies. 2012;19:1343-1348
- Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: An analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect. 2009;117:488-494
- Pan IJ, Daniels JL, Herring AH, Rogan WJ, Siega-Riz AM, Goldman BD, Sjodin A. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: An analysis of the pregnancy, infection, and nutrition babies study. Paediatric and perinatal epidemiology. 2010;24:262- 271
- Pant N, Kumar R, Mathur N, Srivastava SP, Saxena DK, Gujrati VR. Chlorinated pesticide concentration in semen of fertile and infertile men and correlation with sperm quality. Environmental toxicology and pharmacology. 2007;23:135-139
- Park SK, Kang D, Beane-Freeman L, Blair A, Hoppin JA, Sandler DP, Lynch CF, Knott C, Gwak J, Alavanja M. Cancer incidence among paraquat exposed applicators in the agricultural health study: Prospective cohort study. International journal of occupational and environmental health. 2009;15:274-281
- Park SK, Son HK, Lee SK, Kang JH, Chang YS, Jacobs DR, Lee DH. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among nondiabetic adults. Journal of preventive medicine and public health = Yebang Uihakhoe chi. 2010;43:1-8
- Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J, Sarto G, Howard BV. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the women's health initiative observational study. Arthritis care & research. 2011;63:184-194
- Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (ewas) on type 2 diabetes mellitus. PloS one. 2010;5:e10746
- Pathak R, Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Banerjee BD. Maternal and cord blood levels of organochlorine pesticides: Association with preterm labor. Clinical biochemistry. 2009;42:746-749

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Maternal and cord blood levels of organochlorine pesticides: Association with preterm labor. Clinical biochemistry. 2009;42:746-749

- Pathak R, Mustafa M, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Association between recurrent miscarriages and organochlorine pesticide levels. Clinical biochemistry. 2010;43:131-135
- Pathak R, Mustafa MD, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Intra uterine growth retardation: Association with organochlorine pesticide residue levels and oxidative stress markers. Reprod Toxicol. 2011;31:534-539
- Patil JA, Patil AJ, Sontakke AV, Govindwar SP. Occupational pesticides exposure of sprayers of grape gardens in western Maharashtra (india): Effects on liver and kidney function. Journal of basic and clinical physiology and pharmacology. 2009;20:335-355
- Pearce MS, Hammal DM, Dorak MT, McNally RJ, Parker L. Paternal occupational exposure to pesticides or herbicides as risk factors for cancer in children and young adults: A casecontrol study from the north of england. Arch Environ Occup Health. 2006;61:138-144
- Pekmezovic T, Suvajdzic Vukovic N, Kisic D, Grgurevic A, Bogdanovic A, Gotic M, Bakrac M, Brkic N. A case-control study of myelodysplastic syndromes in belgrade (serbia montenegro). Annals of hematology. 2006;85:514-519
- Pereira D, Garrett C. [risk factors for Parkinson disease: An epidemiologic study]. Acta medica portuguesa. 2010;23:15-24
- Perez-Herrera N, Polanco-Minaya H, Salazar-Arredondo E, Solis-Heredia MJ, Hernandez-Ochoa I, Rojas-Garcia E, Alvarado-Mejia J, Borja-Aburto VH, Quintanilla-Vega B. Pon1q192r genetic polymorphism modifies organophosphorous pesticide effects on semen quality and DNA integrity in agricultural workers from southern mexico. Toxicology and applied pharmacology. 2008;230:261-268
- Perry MJ, Ouyang F, Korrick SA, Venners SA, Chen C, Xu X, Lasley BL, Wang X. A prospective study of serum ddt and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age. American journal of epidemiology. 2006;164:1056-1064
- Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, Barr DB, Xu X. Organophosphorous pesticide exposures and sperm quality. Reprod Toxicol. 2011;31:75-79
- Persson EC, Graubard BI, Evans AA, London WT, Weber JP, LeBlanc A, Chen G, Lin W, McGlynn KA. Dichlorodiphenyltrichloroethane and risk of hepatocellular carcinoma. International journal of cancer. Journal international du cancer. 2012;131:2078-2084
- Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, Jorgensen PJ, Budtz-Jorgensen E, Grandjean P. Impact of dietary exposure to food contaminants on the risk of parkinson's disease. Neurotoxicology. 2008;29:584-590
- Petit C, Blangiardo M, Richardson S, Coquet F, Chevrier C, Cordier S. Association of environmental insecticide exposure and fetal growth with a bayesian model including multiple exposure sources: The pelagie mother-child cohort. American journal of epidemiology. 2012;175:1182-1190
- Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R, Cordier S. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort

EFSA supporting publication 2013:EN-497

- Pathak R, Mustafa M, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Association between recurrent miscarriages and organochlorine pesticide levels. Clinical biochemistry. 2010;43:131-135
- Pathak R, Mustafa MD, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD. Intra uterine growth retardation: Association with organochlorine pesticide residue levels and oxidative stress markers. Reprod Toxicol. 2011;31:534-539
- Patil JA, Patil AJ, Sontakke AV, Govindwar SP. Occupational pesticides exposure of sprayers of grape gardens in western Maharashtra (india): Effects on liver and kidney function. Journal of basic and clinical physiology and pharmacology. 2009;20:335-355
- Pearce MS, Hammal DM, Dorak MT, McNally RJ, Parker L. Paternal occupational exposure to pesticides or herbicides as risk factors for cancer in children and young adults: A case- control study from the north of england. Arch Environ Occup Health. 2006;61:138-144
- Pekmezovic T, Suvajdzic Vukovic N, Kisic D, Grgurevic A, Bogdanovic A, Gotic M, Bakrac M, Brkic N. A case-control study of myelodysplastic syndromes in belgrade (serbia montenegro). Annals of hematology. 2006;85:514-519
- Pereira D, Garrett C. [risk factors for Parkinson disease: An epidemiologic study]. Acta medica portuguesa. 2010;23:15-24
- Perez-Herrera N, Polanco-Minaya H, Salazar-Arredondo E, Solis-Heredia MJ, Hernandez- Ochoa I, Rojas-Garcia E, Alvarado-Mejia J, Borja-Aburto VH, Quintanilla-Vega B. Ponlq192r genetic polymorphism modifies organophosphorous pesticide effects on semen quality and DNA integrity in agricultural workers from southern mexico. Toxicology and applied pharmacology. 2008;230:261-268
- Perry MJ, Ouyang F, Korrick SA, Venners SA, Chen C, Xu X, Lasley BL, Wang X. A prospective study of serum ddt and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age. American journal of epidemiology. 2006;164:1056-1064
- Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, Barr DB, Xu X. Organophosphorous pesticide exposures and sperm quality. Reprod Toxicol. 2011;31:75-79
- Persson EC, Graubard BI, Evans AA, London WT, Weber JP, LeBlanc A, Chen G, Lin W, McGlynn KA. Dichlorodiphenyltrichloroethane and risk of hepatocellular carcinoma. International journal of cancer. Journal international du cancer. 2012;131:2078-2084
- Petersen MS, Halling J, Bech S, Wermuth L, Weihe P, Nielsen F, Jorgensen PJ, Budtz-Jorgensen E, Grandjean P. Impact of dietary exposure to food contaminants on the risk of parkinson's disease. Neurotoxicology. 2008;29:584-590
- Petit C, Blangiardo M, Richardson S, Coquet F, Chevrier C, Cordier S. Association of environmental insecticide exposure and fetal growth with a bayesian model including multiple exposure sources: The pelagie mother-child cohort. American journal of epidemiology. 2012;175:1182-1190
- Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R, Cordier S. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort study in Brittany, France. Environmental health: a global access science source. 2010;9:71

<sup>141</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

study in Brittany, France. Environmental health: a global access science source. 2010;9:71

- Philibert A, Schwartz H, Mergler D. An exploratory study of diabetes in a first nation community with respect to serum concentrations of p,p'-dde and pcbs and fish consumption. International journal of environmental research and public health. 2009;6:3179-3189
- Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environmental research. 2007;105:364-369
- Polsky JY, Aronson KJ, Heaton JP, Adams MA. Pesticides and polychlorinated biphenyls as potential risk factors for erectile dysfunction. Journal of andrology. 2007;28:28-37
- Pombo-de-Oliveira MS, Koifman S. Infant acute leukemia and maternal exposures during pregnancy. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:2336-2341
- Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, Ferro A, Ingelido AM, Maggi A, Panici PB, De Felip E. Endometriosis and organochlorinated environmental pollutants: A case-control study on italian women of reproductive age. Environ Health Perspect. 2009;117:1070-1075
- Postuma RB, Montplaisir JY, Pelletier A, Dauvilliers Y, Oertel W, Iranzo A, Ferini-Strambi L, Arnulf I, Hogl B, Manni R, Miyamoto T, Mayer G, Stiasny-Kolster K, Puligheddu M, Ju Y, Jennum P, Sonka K, Santamaria J, Fantini ML, Zucconi M, Leu-Semenescu S, Frauscher B, Terzaghi M, Miyamoto M, Unger MM, Cochen De Cock V, Wolfson C. Environmental risk factors for rem sleep behavior disorder: A multicenter case-control study. Neurology. 2012;79:428-434
- Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, Kaufman D. Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific journal of public health / Asia-Pacific Academic Consortium for Public Health. 2011;23:369-377
- Prochazka M, Feychting M, Ahlbom A, Edwards CG, Nise G, Plato N, Schwartzbaum JA, Forssen UM. Occupational exposures and risk of acoustic neuroma. Occupational and environmental medicine. 2010;67:766-771
- Provost D, Cantagrel A, Lebailly P, Jaffre A, Loyant V, Loiseau H, Vital A, Brochard P, Baldi I. Brain tumours and exposure to pesticides: A case-control study in southwestern France. Occupational and environmental medicine. 2007;64:509-514
- Puertas R, Lopez-Espinosa MJ, Cruz F, Ramos R, Freire C, Perez-Garcia M, Abril A, Julvez J, Salvatierra M, Campoy C, Olea N. Prenatal exposure to mirex impairs neurodevelopment at age of 4 years. Neurotoxicology. 2010;31:154-160
- Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, Barr DB, Blair A, Rothman N, McGlynn KA. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. Environ Health Perspect. 2009;117:1514-1519
- Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;120:642-649
- Qiu XQ, Zhong QA, Zeng XY, Li YH, Nie SF. [a case-control study on congenital heart diseases with methylenetetrahydrofolate reductase gene, cystathionine beta-synthase gene,

142

- Philibert A, Schwartz H, Mergler D. An exploratory study of diabetes in a first nation community with respect to serum concentrations of p,p'-dde and pcbs and fish consumption. International journal of environmental research and public health. 2009;6:3179-3189
- Pierik FH, Klebanoff MA, Brock JW, Longnecker MP. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environmental research. 2007;105:364-369
- Polsky JY, Aronson KJ, Heaton JP, Adams MA. Pesticides and polychlorinated biphenyls as potential risk factors for erectile dysfunction. Journal of andrology. 2007;28:28-37
- Pombo-de-Oliveira MS, Koifman S. Infant acute leukemia and maternal exposures during pregnancy. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15:2336-2341
- Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, Ferro A, Ingelido AM, Maggi A, Panici PB, De Felip E. Endometriosis and organochlorinated environmental pollutants: A case-control study on italian women of reproductive age. Environ Health Perspect. 2009;117:1070-1075
- Postuma RB, Montplaisir JY, Pelletier A, Dauvilliers Y, Oertel W, Iranzo A, Ferini-Strambi L, Arnulf I, Hogl B, Manni R, Miyamoto T, Mayer G, Stiasny-Kolster K, Puligheddu M, Ju Y, Jennum P, Sonka K, Santamaria J, Fantini ML, Zucconi M, Leu-Semenescu S, Frauscher B, Terzaghi M, Miyamoto M, Unger MM, Cochen De Cock V, Wolfson C. Environmental risk factors for rem sleep behavior disorder: A multicenter casecontrol study. Neurology. 2012;79:428-434
- Prihartono N, Kriebel D, Woskie S, Thetkhathuek A, Sripaung N, Padungtod C, Kaufman D. Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific journal of public health / Asia-Pacific Academic Consortium for Public Health. 2011;23:369-377
- Prochazka M, Feychting M, Ahlbom A, Edwards CG, Nise G, Plato N, Schwartzbaum JA, Forssen UM. Occupational exposures and risk of acoustic neuroma. Occupational and environmental medicine. 2010;67:766-771
- Provost D, Cantagrel A, Lebailly P, Jaffre A, Loyant V, Loiseau H, Vital A, Brochard P, Baldi I. Brain tumours and exposure to pesticides: A case-control study in southwestern France. Occupational and environmental medicine. 2007;64:509-514
- Puertas R, Lopez-Espinosa MJ, Cruz F, Ramos R, Freire C, Perez-Garcia M, Abril A, Julvez J, Salvatierra M, Campoy C, Olea N. Prenatal exposure to mirex impairs neurodevelopment at age of 4 years. Neurotoxicology. 2010;31:154-160
- Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, Barr DB, Blair A, Rothman N, McGlynn KA. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. Environ Health Perspect. 2009;117:1514-1519
- Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the agricultural health study. International journal of cancer. Journal international du cancer. 2007;120:642-649
- Qiu XQ, Zhong QA, Zeng XY, Li YH, Nie SF. [a case-control study on congenital heart diseases with methylenetetrahydrofolate reductase gene, cystathionine beta-synthase gene, and environmental factors]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:260-263

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

and environmental factors]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi. 2006;27:260-263

- Quaranta MG, Porpora MG, Mattioli B, Giordani L, Libri I, Ingelido AM, Cerenzia P, Di Felice A, Abballe A, De Felip E, Viora M. Impaired nk-cell-mediated cytotoxic activity and cytokine production in patients with endometriosis: A possible role for pcbs and dde. Life sciences. 2006;79:491-498
- Quiros-Alcala L, Alkon AD, Boyce WT, Lippert S, Davis NV, Bradman A, Barr DB, Eskenazi B. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. Neurotoxicology. 2011;32:646-655
- Qureshi MM, Hayden D, Urbinelli L, Ferrante K, Newhall K, Myers D, Hilgenberg S, Smart R, Brown RH, Cudkowicz ME. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (als). Amyotrophic lateral sclerosis: official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2006;7:173-182
- R. Alarcon, M. Requena, A.F. Hernández, T. Parrón. The relationship between breast cancer and pesticide exposure in regions with differing pesticide use levels. Abstracts / Toxicology Letters 205S (2011) S180–S300
- Ragab M, Elzayadi AR, Hamdy H, Badran H, Shawky S, Emara S. 692 non viral risk factors in development of hcc among egyptian chronic liver disease patients. Journal of Hepatology. 2012;56:S274
- Rastogi SK, Singh VK, Kesavachandran C, Jyoti, Siddiqui MK, Mathur N, Bharti RS. Monitoring of plasma butyrylcholinesterase activity and hematological parameters in pesticide sprayers. Indian journal of occupational and environmental medicine. 2008;12:29-32
- Rau AT, Coutinho A, Avabratha KS, Rau AR, Warrier RP. Pesticide (endosulfan) levels in the bone marrow of children with hematological malignancies. Indian pediatrics. 2012;49:113-117
- Rauch SA, Braun JM, Barr DB, Calafat AM, Khoury J, Montesano AM, Yolton K, Lanphear BP. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012;120:1055-1060
- Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, Whyatt R. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 2011;119:1196-1201
- Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006;118:e1845-1859
- Recio-Vega R, Ocampo-Gomez G, Borja-Aburto VH, Moran-Martinez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: Association between sperm parameters and urinary pesticide levels. Journal of applied toxicology: JAT. 2008;28:674-680
- Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proceedings of the National Academy of Sciences of the United States of America. 2011;108:12770-

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Quaranta MG, Porpora MG, Mattioli B, Giordani L, Libri I, Ingelido AM, Cerenzia P, Di Felice A, Abballe A, De Felip E, Viora M. Impaired nk-cell-mediated cytotoxic activity and cytokine production in patients with endometriosis: A possible role for pcbs and dde. Life sciences. 2006;79:491-498
- Quiros-Alcala L, Alkon AD, Boyce WT, Lippert S, Davis NV, Bradman A, Barr DB, Eskenazi B. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. Neurotoxicology. 2011;32:646-655
- Qureshi MM, Hayden D, Urbinelli L, Ferrante K, Newhall K, Myers D, Hilgenberg S, Smart R, Brown RH, Cudkowicz ME. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (als). Amyotrophic lateral sclerosis: official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2006;7:173-182
- R. Alarcon, M. Requena, A.F. Hernández, T. Parrón. The relationship between breast cancer and pesticide exposure in regions with differing pesticide use levels. Abstracts / Toxicology Letters 205S (2011) S180-S300
- Ragab M, Elzayadi AR, Hamdy H, Badran H, Shawky S, Emara S. 692 non viral risk factors in development of hcc among egyptian chronic liver disease patients. Journal of Hepatology. 2012;56:S274
- Rastogi SK, Singh VK, Kesavachandran C, Jyoti, Siddiqui MK, Mathur N, Bharti RS. Monitoring of plasma butyrylcholinesterase activity and hematological parameters in pesticide sprayers. Indian journal of occupational and environmental medicine. 2008;12:29-32
- Rau AT, Coutinho A, Avabratha KS, Rau AR, Warrier RP. Pesticide (endosulfan) levels in the bone marrow of children with hematological malignancies. Indian pediatrics. 2012;49:113- 117
- Rauch SA, Braun JM, Barr DB, Calafat AM, Khoury J, Montesano AM, Yolton K, Lanphear BP. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012;120:1055-1060
- Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, Whyatt R. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 2011;119:1196-1201
- Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006;118:e1845-1859
- Recio-Vega R, Ocampo-Gomez G, Borja-Aburto VH, Moran-Martinez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: Association between sperm parameters and urinary pesticide levels. Journal of applied toxicology: JAT. 2008;28:674-680
- Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proceedings of the National Academy of Sciences of the United States of America. 2011;108:12770-12775

EFSA 支援出版 2013:EN-497

EFSA supporting publication 2013:EN-497

<sup>143</sup> 

12775

- Ribas-Fito N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. Prenatal exposure to 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-dde) in relation to child growth. International journal of epidemiology. 2006;35:853-858
- Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. Environ Health Perspect. 2007;115:447-450
- Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, Sunyer J. In utero exposure to background concentrations of ddt and cognitive functioning among preschoolers. American journal of epidemiology. 2006;164:955-962
- Richardson DB, Terschuren C, Hoffmann W. Occupational risk factors for non-hodgkin's lymphoma: A population-based case-control study in northern Germany. American journal of industrial medicine. 2008;51:258-268
- Richardson JR, Shalat SL, Buckley B, Winnik B, O'Suilleabhain P, Diaz-Arrastia R, Reisch J, German DC. Elevated serum pesticide levels and risk of parkinson disease. Archives of neurology. 2009;66:870-875
- Rignell-Hydbom A, Elfving M, Ivarsson SA, Lindh C, Jonsson BA, Olofsson P, Rylander L. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants in relation to risk of type 1 diabetes. PloS one. 2010;5:e11281
- Rignell-Hydbom A, Lidfeldt J, Kiviranta H, Rantakokko P, Samsioe G, Agardh CD, Rylander L. Exposure to p,p'-dde: A risk factor for type 2 diabetes. PloS one. 2009;4:e7503
- Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. Human & experimental toxicology. 2007;26:447-452
- Rignell-Hydbom A, Skerfving S, Lundh T, Lindh CH, Elmstahl S, Bjellerup P, Junsson BA, Strumberg U, Akesson A. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. Environmental research. 2009;109:991-996
- Riu E, Monso E, Marin A, Magarolas R, Radon K, Morera J, Andreo F, Nowak D. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. American journal of rhinology. 2008;22:361-364
- Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California central valley. Environ Health Perspect. 2007;115:1482-1489
- Rocheleau CM, Romitti PA, Sanderson WT, Sun L, Lawson CC, Waters MA, Stewart PA, Olney RS, Reefhuis J. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth defects research. Part A, Clinical and molecular teratology. 2011;91:927-936
- Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral performance of adult and adolescent agricultural workers. Neurotoxicology. 2007;28:374-380
- Rojas-Garcia AE, Medina-Diaz IM, Robledo-Marenco Mde L, Barron-Vivanco BS, Giron-Perez MI, Velazquez-Fernandez JB, Gonzalez-Arias CA, Albores-Medina A, Quintanilla-

144

- Ribas-Fito N, Gladen BC, Brock JW, Klebanoff MA, Longnecker MP. Prenatal exposure to 1,1dichloro-2,2-bis (p-chlorophenyl)ethylene (p,p'-dde) in relation to child growth. International journal of epidemiology. 2006;35:853-858
- Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy and children's social behavior at 4 years of age. Environ Health Perspect. 2007;115:447-450
- Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, Sunyer J. In utero exposure to background concentrations of ddt and cognitive functioning among preschoolers. American journal of epidemiology. 2006;164:955-962
- Richardson DB, Terschuren C, Hoffmann W. Occupational risk factors for non-hodgkin's lymphoma: A population-based case-control study in northern Germany. American journal of industrial medicine. 2008;51:258-268
- Richardson JR, Shalat SL, Buckley B, Winnik B, O'Suilleabhain P, Diaz-Arrastia R, Reisch J, German DC. Elevated serum pesticide levels and risk of parkinson disease. Archives of neurology. 2009;66:870-875
- Rignell-Hydbom A, Elfving M, Ivarsson SA, Lindh C, Jonsson BA, Olofsson P, Rylander L. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants in relation to risk of type 1 diabetes. PloS one. 2010;5:e11281
- Rignell-Hydbom A, Lidfeldt J, Kiviranta H, Rantakokko P, Samsioe G, Agardh CD, Rylander L. Exposure to p,p'-dde: A risk factor for type 2 diabetes. PloS one. 2009;4:e7503
- Rignell-Hydbom A, Rylander L, Hagmar L. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. Human & experimental toxicology. 2007;26:447-452
- Rignell-Hydbom A, Skerfving S, Lundh T, Lindh CH, Elmstahl S, Bjellerup P, Junsson BA, Strumberg U, Akesson A. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. Environmental research. 2009;109:991-996
- Riu E, Monso E, Marin A, Magarolas R, Radon K, Morera J, Andreo F, Nowak D. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. American journal of rhinology. 2008;22:361-364
- Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California central valley. Environ Health Perspect. 2007;115:1482-1489
- Rocheleau CM, Romitti PA, Sanderson WT, Sun L, Lawson CC, Waters MA, Stewart PA, Olney RS, Reefhuis J. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth defects research. Part A, Clinical and molecular teratology. 2011;91:927-936
- Rohlman DS, Lasarev M, Anger WK, Scherer J, Stupfel J, McCauley L. Neurobehavioral performance of adult and adolescent agricultural workers. Neurotoxicology. 2007;28:374-380
- Rojas-Garcia AE, Medina-Diaz IM, Robledo-Marenco Mde L, Barron-Vivanco BS, Giron- Perez MI, Velazquez-Fernandez JB, Gonzalez-Arias CA, Albores-Medina A, Quintanilla-Vega B, Ostrosky-Wegman P, Rojas-Garcia MC, Perez-Herrera NE, Lopez-Flores JF. Hematological, biochemical effects, and self-reported symptoms in pesticide retailers. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:517-521

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

Vega B, Ostrosky-Wegman P, Rojas-Garcia MC, Perez-Herrera NE, Lopez-Flores JF. Hematological, biochemical effects, and self-reported symptoms in pesticide retailers. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2011;53:517-521

- Roldan-Tapia L, Nieto-Escamez FA, del Aguila EM, Laynez F, Parron T, Sanchez-Santed F. Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicology and teratology. 2006;28:694-703
- Romero Ramos R, Romero Gutierrez G, Abortes Monroy I, Medina Sanchez HG. [risk factors associated to female infertility]. Ginecologia y obstetricia de Mexico. 2008;76:717-721
- Rosano A, Gemelli V, Giovannelli C, Paciotti G, Sabatucci A, Spagnolo A. [fertility changes in women working in greenhouses]. La Medicina del lavoro. 2009;100:448-454
- Rossman MD, Thompson B, Frederick M, Iannuzzi MC, Rybicki BA, Pander JP, Newman LS, Rose C, Magira E, Monos D. Hla and environmental interactions in sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders. 2008;25:125-132
- Rosso AL, Hovinga ME, Rorke-Adams LB, Spector LG, Bunin GR. A case-control study of childhood brain tumors and fathers' hobbies: A children's oncology group study. Cancer causes & control: CCC. 2008;19:1201-1207
- Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and nonagricultural hispanic workers. Environmental Health Perspectives. 2006;114:691-696
- Rubin CH, Lanier A, Kieszak S, Brock JW, Koller KR, Strosnider H, Needham L, Zahm S, Harpster A. Breast cancer among alaska native women potentially exposed to environmental organochlorine chemicals. International journal of circumpolar health. 2006;65:18-27
- Rudant J, Menegaux F, Leverger G, Baruchel A, Nelken B, Bertrand Y, Patte C, Pacquement H, Verite C, Robert A, Michel G, Margueritte G, Gandemer V, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood hematopoietic malignancies: The escale study (sfce). Environ Health Perspect. 2007;115:1787-1793
- Ruder AM, Carreon T, Butler MA, Calvert GM, Davis-King KE, Waters MA, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. Exposure to farm crops, livestock, and farm tasks and risk of glioma: The upper midwest health study. American journal of epidemiology. 2009;169:1479-1491
- Ruder AM, Waters MA, Carreon T, Butler MA, Davis-King KE, Calvert GM, Schulte PA, Ward EM, Connally LB, Lu J, Wall D, Zivkovich Z, Heineman EF, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of primary intracranial gliomas in farm and rural residents. Journal of agricultural safety and health. 2006;12:255-274
- Ruder AM, Yiin JH. Mortality of us pentachlorophenol production workers through 2005. Chemosphere. 2011;83:851-861
- Rugbjerg K, Harris MA, Shen H, Marion SA, Tsui JK, Teschke K. Pesticide exposure and risk of Parkinson's disease--a population-based case-control study evaluating the potential for recall bias. Scand J Work Environ Health. 2011;37:427-436

Rull RP, Gunier R, Von Behren J, Hertz A, Crouse V, Buffler PA, Reynolds P. Residential

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Roldan-Tapia L, Nieto-Escamez FA, del Aguila EM, Laynez F, Parron T, Sanchez-Santed F. Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicology and teratology. 2006;28:694-703
- Romero Ramos R, Romero Gutierrez G, Abortes Monroy I, Medina Sanchez HG. [risk factors associated to female infertility]. Ginecologia y obstetricia de Mexico. 2008;76:717-721
- Rosano A, Gemelli V, Giovannelli C, Paciotti G, Sabatucci A, Spagnolo A. [fertility changes in women working in greenhouses]. La Medicina del lavoro. 2009;100:448-454
- Rossman MD, Thompson B, Frederick M, Iannuzzi MC, Rybicki BA, Pander JP, Newman LS, Rose C, Magira E, Monos D. Hla and environmental interactions in sarcoidosis. Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG / World Association of Sarcoidosis and Other Granulomatous Disorders. 2008;25:125-132
- Rosso AL, Hovinga ME, Rorke-Adams LB, Spector LG, Bunin GR. A case-control study of childhood brain tumors and fathers' hobbies: A children's oncology group study. Cancer causes & control: CCC. 2008;19:1201-1207
- Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and nonagricultural hispanic workers. Environmental Health Perspectives. 2006;114:691-696
- Rubin CH, Lanier A, Kieszak S, Brock JW, Koller KR, Strosnider H, Needham L, Zahm S, Harpster A. Breast cancer among alaska native women potentially exposed to environmental organochlorine chemicals. International journal of circumpolar health. 2006;65:18-27
- Rudant J, Menegaux F, Leverger G, Baruchel A, Nelken B, Bertrand Y, Patte C, Pacquement H, Verite C, Robert A, Michel G, Margueritte G, Gandemer V, Hemon D, Clavel J. Household exposure to pesticides and risk of childhood hematopoietic malignancies: The escale study (sfce). Environ Health Perspect. 2007;115:1787-1793
- Ruder AM, Carreon T, Butler MA, Calvert GM, Davis-King KE, Waters MA, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. Exposure to farm crops, livestock, and farm tasks and risk of glioma: The upper midwest health study. American journal of epidemiology. 2009;169:1479-1491
- Ruder AM, Waters MA, Carreon T, Butler MA, Davis-King KE, Calvert GM, Schulte PA, Ward EM, Connally LB, Lu J, Wall D, Zivkovich Z, Heineman EF, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of primary intracranial gliomas in farm and rural residents. Journal of agricultural safety and health. 2006;12:255-274
- Ruder AM, Yiin JH. Mortality of us pentachlorophenol production workers through 2005. Chemosphere. 2011;83:851-861
- Rugbjerg K, Harris MA, Shen H, Marion SA, Tsui JK, Teschke K. Pesticide exposure and risk of Parkinson"s disease--a population-based case-control study evaluating the potential for recall bias. Scand J Work Environ Health. 2011;37:427-436
- Rull RP, Gunier R, Von Behren J, Hertz A, Crouse V, Buffler PA, Reynolds P. Residential proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environmental research. 2009;109:891-899

EFSA 支援出版 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environmental research. 2009;109:891-899

- Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. American journal of epidemiology. 2006;163:743-753
- Rusiecki JA, Hou L, Lee WJ, Blair A, Dosemeci M, Lubin JH, Bonner M, Samanic C, Hoppin JA, Sandler DP, Alavanja MC. Cancer incidence among pesticide applicators exposed to metolachlor in the agricultural health study. International journal of cancer. Journal international du cancer. 2006;118:3118-3123
- Rusiecki JA, Patel R, Koutros S, Beane-Freeman L, Landgren O, Bonner MR, Coble J, Lubin J, Blair A, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to permethrin in the agricultural health study. Environ Health Perspect. 2009;117:581-586
- Rylander L, Wallin E, Jonssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between cb-153 and p,p'-dde and hormone levels in serum in middle-aged and elderly men. Chemosphere. 2006;65:375-381
- Sagiv SK, Nugent JK, Brazelton TB, Choi AL, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and measures of behavior in infancy using the neonatal behavioral assessment scale (nbas). Environ Health Perspect. 2008;116:666-673
- Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. Environ Health Perspect. 2012;120:904-909
- Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. American journal of epidemiology. 2010;171:593-601
- Sagiv SK, Tolbert PE, Altshul LM, Korrick SA. Organochlorine exposures during pregnancy and infant size at birth. Epidemiology. 2007;18:120-129
- Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: A case-control study in Lebanon. Journal of epidemiology and community health. 2006;60:256-261
- Salameh PR, Waked M, Baldi I, Brochard P, Saleh BA. Chronic bronchitis and pesticide exposure: A case-control study in Lebanon. European journal of epidemiology. 2006;21:681-688
- Saldana TM, Basso O, Baird DD, Hoppin JA, Weinberg CR, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and hypertensive disorders during pregnancy. Environ Health Perspect. 2009:117:1393-1396
- Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. Diabetes care. 2007;30:529-534
- Samanic C, Rusiecki J, Dosemeci M, Hou L, Hoppin JA, Sandler DP, Lubin J, Blair A, Alavanja MCR. Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study. Environmental Health Perspectives. 2006;114:1521-1526
- Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD. Occupational exposure to pesticides and risk of adult brain tumors. American journal of epidemiology.

EFSA supporting publication 2013:EN-497

- Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. American journal of epidemiology. 2006;163:743-753
- Rusiecki JA, Hou L, Lee WJ, Blair A, Dosemeci M, Lubin JH, Bonner M, Samanic C, Hoppin JA, Sandler DP, Alavanja MC. Cancer incidence among pesticide applicators exposed to metolachlor in the agricultural health study. International journal of cancer. Journal international du cancer. 2006;118:3118-3123
- Rusiecki JA, Patel R, Koutros S, Beane-Freeman L, Landgren O, Bonner MR, Coble J, Lubin J, Blair A, Hoppin JA, Alavanja MC. Cancer incidence among pesticide applicators exposed to permethrin in the agricultural health study. Environ Health Perspect. 2009;117:581-586
- Rylander L, Wallin E, Jonssson BA, Stridsberg M, Erfurth EM, Hagmar L. Associations between cb-153 and p,p'-dde and hormone levels in serum in middle-aged and elderly men. Chemosphere. 2006;65:375-381
- Sagiv SK, Nugent JK, Brazelton TB, Choi AL, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and measures of behavior in infancy using the neonatal behavioral assessment scale (nbas). Environ Health Perspect. 2008;116:666-673
- Sagiv SK, Thurston SW, Bellinger DC, Altshul LM, Korrick SA. Neuropsychological measures of attention and impulse control among 8-year-old children exposed prenatally to organochlorines. Environ Health Perspect. 2012;120:904-909
- Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. American journal of epidemiology. 2010;171:593-601
- Sagiv SK, Tolbert PE, Altshul LM, Korrick SA. Organochlorine exposures during pregnancy and infant size at birth. Epidemiology. 2007;18:120-129
- Salameh P, Waked M, Baldi I, Brochard P, Saleh BA. Respiratory diseases and pesticide exposure: A case-control study in Lebanon. Journal of epidemiology and community health. 2006;60:256-261
- Salameh PR, Waked M, Baldi I, Brochard P, Saleh BA. Chronic bronchitis and pesticide exposure: A case-control study in Lebanon. European journal of epidemiology. 2006;21:681-688
- Saldana TM, Basso O, Baird DD, Hoppin JA, Weinberg CR, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and hypertensive disorders during pregnancy. Environ Health Perspect. 2009;117:1393-1396
- Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, Alavanja MC, Sandler DP. Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. Diabetes care. 2007;30:529-534
- Samanic C, Rusiecki J, Dosemeci M, Hou L, Hoppin JA, Sandler DP, Lubin J, Blair A, Alavanja MCR. Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study. Environmental Health Perspectives. 2006;114:1521-1526
- Samanic CM, De Roos AJ, Stewart PA, Rajaraman P, Waters MA, Inskip PD. Occupational exposure to pesticides and risk of adult brain tumors. American journal of epidemiology. 2008;167:976-985

<sup>146</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

Pesticide epidemiology

2008:167:976-985

- Sanchez A.T., Olivera R.M.P., Sanchez G.M.D.O., Dorantes G.L.. Pemphigus vulgaris. Epidemiological study and analysis of possible risk factors of mortality. Dermatologia Revista Mexicana (2006) 50:2 (50-53).
- Santibanez M, Alguacil J, de la Hera MG, Navarrete-Munoz EM, Llorca J, Aragones N, Kauppinen T, Vioque J. Occupational exposures and risk of stomach cancer by histological type. Occupational and environmental medicine. 2012;69:268-275
- Santibanez M, Vioque J, Alguacil J, de la Hera MG, Moreno-Osset E, Carrato A, Porta M, Kauppinen T. Occupational exposures and risk of pancreatic cancer. European journal of epidemiology, 2010;25:721-730
- Sanyal J, Chakraborty DP, Sarkar B, Banerjee TK, Mukherjee SC, Ray BC, Rao VR. Environmental and familial risk factors of Parkinson's disease: Case-control study. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 2010:37:637-642
- Sartor SG, Eluf-Neto J, Travier N, Wunsch Filho V, Arcuri AS, Kowalski LP, Boffetta P. [occupational risks for laryngeal cancer: A case-control study]. Cadernos de saude publica. 2007;23:1473-1481
- Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, Hoppin JA. Maternal pesticide use and birth weight in the agricultural health study. Journal of agromedicine. 2010;15:127-136
- Sawada N, Iwasaki M, Inoue M, Itoh H, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: A nested casecontrol study. Environ Health Perspect. 2010;118:659-665
- Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p.p'- dde, and other toxicants in akwesasne mohawk youth. Environ Health Perspect. 2008;116:806-813
- Schell LM, Gallo MV, Ravenscroft J, DeCaprio AP. Persistent organic pollutants and antithyroid peroxidase levels in akwesasne mohawk young adults. Environmental research. 2009:109:86-92
- Schmeisser N, Behrens T, Mester B, Gottlieb A, Langner I, Ahrens W. Local cluster of germ cell cancer in a cohort of male automotive workers in germany not explained by previous or concurrent activities and exposures in farming and forestry. Cancer epidemiology, 2011;35:73-77
- Schmeisser N, Kaerlev L, Bourdon-Raverdy N, Ganry O, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Zambon P, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Ahrens W. Occupational exposure to pesticides and bile tract carcinoma in men: Results from a european multicenter case-control study. Cancer causes & control: CCC. 2010;21:1493-1502
- Schreinemachers DM. Perturbation of lipids and glucose metabolism associated with previous 2,4-d exposure: A cross-sectional study of nhanes iii data, 1988-1994. Environmental health: a global access science source. 2010;9:11
- Semchuk KM, Rosenberg AM, McDuffie HH, Cessna AJ, Pahwa P, Irvine DG. Antinuclear antibodies and bromoxynil exposure in a rural sample. Journal of toxicology and

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Sanchez A.T., Olivera R.M.P., Sanchez G.M.D.O., Dorantes G.L., Pemphigus vulgaris, Epidemiological study and analysis of possible risk factors of mortality. Dermatologia Revista Mexicana (2006) 50:2 (50-53).
- Santibanez M, Alguacil J, de la Hera MG, Navarrete-Munoz EM, Llorca J, Aragones N, Kauppinen T, Vioque J. Occupational exposures and risk of stomach cancer by histological type. Occupational and environmental medicine. 2012;69:268-275
- Santibanez M, Vioque J, Alguacil J, de la Hera MG, Moreno-Osset E, Carrato A, Porta M, Kauppinen T. Occupational exposures and risk of pancreatic cancer. European journal of epidemiology. 2010;25:721-730
- Sanyal J, Chakraborty DP, Sarkar B, Banerjee TK, Mukherjee SC, Ray BC, Rao VR. Environmental and familial risk factors of Parkinson"s disease: Case-control study. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques, 2010;37:637-642
- Sartor SG, Eluf-Neto J, Travier N, Wunsch Filho V, Arcuri AS, Kowalski LP, Boffetta P. [occupational risks for larvngeal cancer: A case-control study]. Cadernos de saude publica. 2007;23:1473-1481
- Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, Hoppin JA. Maternal pesticide use and birth weight in the agricultural health study. Journal of agromedicine. 2010;15:127-136
- Sawada N, Iwasaki M, Inoue M, Itoh H, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: A nested case- control study. Environ Health Perspect. 2010;118:659-665
- Schell LM, Gallo MV, Denham M, Ravenscroft J, DeCaprio AP, Carpenter DO. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p, p' - dde, and other toxicants in akwesasne mohawk youth. Environ Health Perspect. 2008;116:806-813
- Schell LM, Gallo MV, Ravenscroft J, DeCaprio AP. Persistent organic pollutants and antithyroid peroxidase levels in akwesasne mohawk young adults. Environmental research. 2009;109:86-92
- Schmeisser N. Behrens T. Mester B. Gottlieb A. Langner I. Ahrens W. Local cluster of germ cell cancer in a cohort of male automotive workers in germany not explained by previous or concurrent activities and exposures in farming and forestry. Cancer epidemiology. 2011;35:73-77
- Schmeisser N, Kaerlev L, Bourdon-Raverdy N, Ganry O, Llopis-Gonzalez A, Guenel P, Hardell L, Merletti F, Zambon P, Morales-Suarez-Varela M, Olsen J, Olsson H, Vyberg M, Ahrens W. Occupational exposure to pesticides and bile tract carcinoma in men: Results from a european multicenter case-control study. Cancer causes & control: CCC. 2010;21:1493-1502
- Schreinemachers DM. Perturbation of lipids and glucose metabolism associated with previous 2, 4-d exposure: A cross-sectional study of nhanes iii data, 1988-1994. Environmental health: a global access science source, 2010;9:11
- Semchuk KM, Rosenberg AM, McDuffie HH, Cessna AJ, Pahwa P, Irvine DG, Antinuclear antibodies and bromoxynil exposure in a rural sample. Journal of toxicology and environmental health. Part A. 2007;70:638-657

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

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Pesticide epidemiology

environmental health. Part A. 2007;70:638-657

- Settimi L, Spinelli A, Lauria L, Miceli G, Pupp N, Angotzi G, Fedi A, Donati S, Miligi L, Osborn J, Figa-Talamanca I. Spontaneous abortion and maternal work in greenhouses. American journal of industrial medicine. 2008;51:290-295
- Seyed Jalal Emam, Maryam Salehcheh, Mohammad Hossein Haghighizadeh, Seyed Mohammad Hosein Mousavi Jazayeri. Occupational exposure to pesticides among farmers. Pak J Med Sci 2012;28(1):120-123
- Sharma E, Mustafa M, Pathak R, Guleria K, Ahmed RS, Vaid NB, Banerjee BD. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. European journal of obstetrics, gynecology, and reproductive biology. 2012;161:163-169
- Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in cyp1a1, gstmi, gstt1 genes and organochlorine pesticides in the etiology of hypospadias. Human & experimental toxicology. 2011;30:1464-1474
- Shim YK, Mlynarek SP, van Wijngaarden E. Parental exposure to pesticides and childhood brain cancer: U.S. Atlantic coast childhood brain cancer study. Environ Health Perspect. 2009;117:1002-1006
- Shirangi A, Fritschi L, Holman CD. Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. Occupational and environmental medicine. 2008;65:719-725
- Siddharth M, Datta SK, Bansal S, Mustafa M, Banerjee BD, Kalra OP, Tripathi AK. Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. Journal of biochemical and molecular toxicology. 2012;26:241-247
- Silva SR, Martins JL, Seixas S, Silva DC, Lemos SP, Lemos PV. [congenital defects and exposure to pesticides in sao francisco valley]. Revista brasileira de ginecologia e obstetricia : revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia. 2011;33:20-26
- Skeie GO, Muller B, Haugarvoll K, Larsen JP, Tysnes OB. Differential effect of environmental risk factors on postural instability gait difficulties and tremor dominant Parkinson's disease. Movement disorders: official journal of the Movement Disorder Society. 2010;25:1847-1852
- Slager RE, Poole JA, LeVan TD, Sandler DP, Alavanja MC, Hoppin JA. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the agricultural health study. Occupational and environmental medicine. 2009;66:718-724
- Slager RE, Simpson SL, Levan TD, Poole JA, Sandler DP, Hoppin JA. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. Journal of toxicology and environmental health. Part A. 2010;73:1382-1393
- Slater ME, Linabery AM, Spector LG, Johnson KJ, Hilden JM, Heerema NA, Robison LL, Ross JA. Maternal exposure to household chemicals and risk of infant leukemia: A report from the children's oncology group. Cancer causes & control : CCC. 2011;22:1197-1204
- Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors. Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Settimi L, Spinelli A, Lauria L, Miceli G, Pupp N, Angotzi G, Fedi A, Donati S, Miligi L, Osborn J, Figa-Talamanca I. Spontaneous abortion and maternal work in greenhouses. American journal of industrial medicine. 2008;51:290-295
- Seyed Jalal Emam, Maryam Salehcheh, Mohammad Hossein Haghighizadeh, Seyed Mohammad Hosein Mousavi Jazayeri. Occupational exposure to pesticides among farmers. Pak J Med Sci 2012;28(1):120-123
- Sharma E, Mustafa M, Pathak R, Guleria K, Ahmed RS, Vaid NB, Banerjee BD. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. European journal of obstetrics, gynecology, and reproductive biology. 2012;161:163-169
- Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in cyplal, gstmi, gsttl genes and organochlorine pesticides in the etiology of hypospadias. Human & experimental toxicology. 2011;30:1464-1474
- Shim YK, Mlynarek SP, van Wijngaarden E. Parental exposure to pesticides and childhood brain cancer: U.S. Atlantic coast childhood brain cancer study. Environ Health Perspect. 2009;117:1002-1006
- Shirangi A, Fritschi L, Holman CD. Maternal occupational exposures and risk of spontaneous abortion in veterinary practice. Occupational and environmental medicine. 2008;65:719-725
- Siddharth M, Datta SK, Bansal S, Mustafa M, Banerjee BD, Kalra OP, Tripathi AK. Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. Journal of biochemical and molecular toxicology. 2012;26:241-247
- Silva SR, Martins JL, Seixas S, Silva DC, Lemos SP, Lemos PV. [congenital defects and exposure to pesticides in sao francisco valley]. Revista brasileira de ginecologia e obstetrician: revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia. 2011;33:20-26
- Skeie GO, Muller B, Haugarvoll K, Larsen JP, Tysnes OB. Differential effect of environmental risk factors on postural instability gait difficulties and tremor dominant Parkinson"s disease. Movement disorders: official journal of the Movement Disorder Society. 2010;25:1847-1852
- Slager RE, Poole JA, LeVan TD, Sandler DP, Alavanja MC, Hoppin JA. Rhinitis associated with pesticide exposure among commercial pesticide applicators in the agricultural health study. Occupational and environmental medicine. 2009;66:718-724
- Slager RE, Simpson SL, Levan TD, Poole JA, Sandler DP, Hoppin JA. Rhinitis associated with pesticide use among private pesticide applicators in the agricultural health study. Journal of toxicology and environmental health. Part A. 2010;73:1382-1393
- Slater ME, Linabery AM, Spector LG, Johnson KJ, Hilden JM, Heerema NA, Robison LL, Ross JA. Maternal exposure to household chemicals and risk of infant leukemia: A report from the children's oncology group. Cancer causes & control : CCC. 2011;22:1197-1204
- Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, Grimalt JO, Sunyer J. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr. 2008;97:1465-1469

<sup>148</sup> 

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Pesticide epidemiology

vears. Acta Paediatr. 2008;97:1465-1469

- Snijder CA, Roeleveld N, Te Velde E, Steegers EA, Raat H, Hofman A, Jaddoe VW, Burdorf A. Occupational exposure to chemicals and fetal growth: The generation r study. Hum Reprod. 2012;27:910-920
- Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27:1510-1517
- Soldin OP, Nsouli-Maktabi H, Genkinger JM, Loffredo CA, Ortega-Garcia JA, Colantino D, Barr DB, Luban NL, Shad AT, Nelson D. Pediatric acute lymphoblastic leukemia and exposure to pesticides. Therapeutic drug monitoring. 2009;31:495-501
- Solomon C, Poole J, Palmer KT, Peveler R, Coggon D. Neuropsychiatric symptoms in past users of sheep dip and other pesticides. Occupational and environmental medicine. 2007:64:259-266
- Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, Jacobs DR, Jr., Lee DH. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in korea. Environment international, 2010;36:410-414
- Spinelli JJ, Ng CH, Weber JP, Connors JM, Gascovne RD, Lai AS, Brooks-Wilson AR, Le ND, Berry BR, Gallagher RP. Organochlorines and risk of non-hodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;121:2767-2775
- Spix C, Schulze-Rath R, Kaatsch P, Blettner M, Case-control study on risk factors for leukaemia and brain tumours in children under 5 years in germany. Klinische Padiatrie. 2009;221:362-368
- Stallones L. Suicide and potential occupational exposure to pesticides, colorado 1990-1999. Journal of agromedicine. 2006;11:107-112
- Starks SE, Gerr F, Kamel F, Lvnch CF, Jones MP, Alavania MC, Sandler DP, Hoppin JA. Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the agricultural health study. Neurotoxicology and teratology. 2012;34:168-176
- Starks SE, Hoppin JA, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Gerr F. Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the agricultural health study. Environ Health Perspect. 2012;120:515-520
- Steerenberg P. van Amelsvoort L. Colosio C. Corsini E. Fustinoni S. Vergieva T. Zaikov C. Pennanen S, Liesivuori J, Van Loveren H, Toxicological evaluation of the immune function of pesticide workers, a european wide assessment. Human & experimental toxicology, 2008;27:701-707
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal pcb exposure and intelligence (iq) in 9-year-old children. Environ Health Perspect. 2008;116:1416-1422
- Strom SS, Yamamura Y, Flores-Sandoval FN, Pettaway CA, Lopez DS. Prostate cancer in mexican-americans: Identification of risk factors. The Prostate. 2008;68:563-570
- Strom SS, Yamamura Y, Kantarijian HM, Cortes-Franco JE. Obesity, weight gain, and risk of chronic myeloid leukemia. Cancer epidemiology, biomarkers & prevention: a publication of

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

- Pesticide epidemiology
- Snijder CA, Roeleveld N, Te Velde E, Steegers EA, Raat H, Hofman A, Jaddoe VW, Burdorf A. Occupational exposure to chemicals and fetal growth: The generation r study. Hum Reprod. 2012;27:910-920
- Snijder CA, Vlot IJ, Burdorf A, Obermann-Borst SA, Helbing WA, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and parental occupational exposure to chemicals. Hum Reprod. 2012;27:1510-1517
- Soldin OP, Nsouli-Maktabi H, Genkinger JM, Loffredo CA, Ortega-Garcia JA, Colantino D, Barr DB, Luban NL, Shad AT, Nelson D. Pediatric acute lymphoblastic leukemia and exposure to pesticides. Therapeutic drug monitoring. 2009;31:495-501
- Solomon C, Poole J, Palmer KT, Peveler R, Coggon D. Neuropsychiatric symptoms in past users of sheep dip and other pesticides. Occupational and environmental medicine. 2007;64:259-266
- Son HK, Kim SA, Kang JH, Chang YS, Park SK, Lee SK, Jacobs DR, Jr., Lee DH. Strong associations between low-dose organochlorine pesticides and type 2 diabetes in korea. Environment international. 2010;36:410-414
- Spinelli II, Ng CH, Weber IP, Connors IM, Gascovne RD, Lai AS, Brooks-Wilson AR, Le ND, Berry BR, Gallagher RP. Organochlorines and risk of non-hodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;121:2767-2775
- Spix C, Schulze-Rath R, Kaatsch P, Blettner M. Case-control study on risk factors for leukaemia and brain tumours in children under 5 years in germany. Klinische Padiatrie. 2009;221:362-368
- Stallones L. Suicide and potential occupational exposure to pesticides, colorado 1990-1999. Journal of agromedicine. 2006;11:107-112
- Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Hoppin JA. Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the agricultural health study. Neurotoxicology and teratology. 2012;34:168-176
- Starks SE, Hoppin JA, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Gerr F. Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the agricultural health study. Environ Health Perspect. 2012;120:515-520
- Steerenberg P, van Amelsvoort L, Colosio C, Corsini E, Fustinoni S, Vergieva T, Zaikov C, Pennanen S, Liesivuori J, Van Loveren H. Toxicological evaluation of the immune function of pesticide workers, a european wide assessment. Human & experimental toxicology. 2008;27:701-707
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal pcb exposure and intelligence (iq) in 9-year-old children. Environ Health Perspect. 2008;116:1416-1422
- Strom SS, Yamamura Y, Flores-Sandoval FN, Pettaway CA, Lopez DS. Prostate cancer in mexican-americans: Identification of risk factors. The Prostate. 2008;68:563-570
- Strom SS, Yamamura Y, Kantarijian HM, Cortes-Franco IE, Obesity, weight gain, and risk of chronic myeloid leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 2009;18:1501-1506

EFSA supporting publication 2013:EN-497

<sup>149</sup> 

the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18:1501-1506

- Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spano M, Toft G, Bonde JP, Jonsson BA, Rignell-Hydbom A, Rylander L, Giwercman A, Pedersen HS, Bonefeld-Jorgensen EC, Ludwicki JK, Lesovoy V, Sakkas D, Bizzaro D. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of cb-153 and p,p'dde in european and inuit populations. Reproduction. 2006;132:949-958
- Stuetz W, McGready R, Cho T, Prapamontol T, Biesalski HK, Stepniewska K, Nosten F. Relation of ddt residues to plasma retinol, alpha-tocopherol, and beta-carotene during pregnancy and malaria infection: A case-control study in karen women in northern thailand. The Science of the total environment. 2006;363:78-86
- Su Y, Dai Y, Lin Y, Gao X, Han Y, Zhao B. Serum organochlorine pesticide residues and risk of gallstone disease: A case-control study in xiamen. Annals of epidemiology. 2012;22:592-597
- Subahir MN, Shah SA, Zainuddin ZM. Risk factors for prostate cancer in universiti kebangsaan Malaysia medical centre: A case-control study. Asian Pacific journal of cancer prevention: APJCP. 2009;10:1015-1020
- Sunyer J, Alvarez-Pedrerol M, To-Figueras J, Ribas-Fito N, Grimalt JO, Herrero C. Urinary porphyrin excretion in children is associated with exposure to organochlorine compounds. Environ Health Perspect. 2008;116:1407-1410
- Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de Cid R, Anto JM, Torrent M. Early life environment, neurodevelopment and the interrelation with atopy. Environmental research. 2010;110:733-738
- Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goni F, Basterrechea M, Vrijheid M, Guerra S, Anto JM. Dde in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology. 2010;21:729-735
- Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2006;36:1236-1241
- Sutoluk Z, Kekec Z, Daglioglu N, Hant I. Association of chronic pesticide exposure with serum cholinesterase levels and pulmonary functions. Archives of Environmental & Occupational Health. 2011;66:95-99
- Swaen G, van Amelsvoort L, Boers D, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Pennanen S, Liesivuori J, Colosio C, van Loveren H. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: Results from the europit field study. Human & experimental toxicology. 2008;27:715-720
- Swan SH. Semen quality in fertile us men in relation to geographical area and pesticide exposure. International journal of andrology. 2006;29:62-68; discussion 105-108
- Tadevosyan N.S., Tadevosyan A.E., Petrosyan M.S. Pesticides application in agricultural of Armenia and their impact on reproductive function in humans. THE NEW ARMENIAN MEDICAL JOURNALVOI. 3 (2009), N 2, 41 - 48

Tagiyeva N, Devereux G, Semple S, Sherriff A, Henderson J, Elias P, Ayres JG. Parental

EFSA supporting publication 2013:EN-497

# Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Pesticide epidemiology
- Stronati A, Manicardi GC, Cecati M, Bordicchia M, Ferrante L, Spano M, Toft G, Bonde JP, Jonsson BA, Rignell-Hydbom A, Rylander L, Giwercman A, Pedersen HS, Bonefeld-Jorgensen EC, Ludwicki JK, Lesovoy V, Sakkas D, Bizzaro D. Relationships between sperm DNA fragmentation, sperm apoptotic markers and serum levels of cb-153 and p,p'- dde in european and inuit populations. Reproduction. 2006;132:949-958
- Stuetz W, McGready R, Cho T, Prapamontol T, Biesalski HK, Stepniewska K, Nosten F. Relation of ddt residues to plasma retinol, alpha-tocopherol, and beta-carotene during pregnancy and malaria infection: A case-control study in karen women in northern thailand. The Science of the total environment. 2006;363:78-86
- Su Y, Dai Y, Lin Y, Gao X, Han Y, Zhao B. Serum organochlorine pesticide residues and risk of gallstone disease: A case-control study in xiamen. Annals of epidemiology. 2012;22:592-597
- Subahir MN, Shah SA, Zainuddin ZM. Risk factors for prostate cancer in universiti kebangsaan Malaysia medical centre: A case-control study. Asian Pacific journal of cancer prevention: APJCP. 2009;10:1015-1020
- Sunyer J, Alvarez-Pedrerol M, To-Figueras J, Ribas-Fito N, Grimalt JO, Herrero C. Urinary porphyrin excretion in children is associated with exposure to organochlorine compounds. Environ Health Perspect. 2008;116:1407-1410
- Sunyer J, Basagana X, Gonzalez JR, Julvez J, Guerra S, Bustamante M, de Cid R, Anto JM, Torrent M. Early life environment, neurodevelopment and the interrelation with atopy. Environmental research. 2010;110:733-738
- Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goni F, Basterrechea M, Vrijheid M, Guerra S, Anto JM. Dde in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology. 2010;21:729-735
- Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, Anto JM, Grimalt JO. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2006;36:1236-1241
- Sutoluk Z, Kekec Z, Daglioglu N, Hant I. Association of chronic pesticide exposure with serum cholinesterase levels and pulmonary functions. Archives of Environmental & Occupational Health. 2011;66:95-99
- Swaen G, van Amelsvoort L, Boers D, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Pennanen S, Liesivuori J, Colosio C, van Loveren H. Occupational exposure to ethylenebisdithiocarbamates in agriculture and allergy: Results from the europit field study. Human & experimental toxicology. 2008;27:715-720
- Swan SH. Semen quality in fertile us men in relation to geographical area and pesticide exposure. International journal of andrology. 2006;29:62-68; discussion 105-108
- Tadevosyan N.S., Tadevosyan A.E., Petrosyan M.S. Pesticides application in agricultural of Armenia and their impact on reproductive function in humans. THE NEW ARMENIAN MEDICAL JOURNALVOL. 3 (2009), N 2, 41 - 48
- Tagiyeva N, Devereux G, Semple S, Sherriff A, Henderson J, Elias P, Ayres JG. Parental occupation is a risk factor for childhood wheeze and asthma. The European respiratory journal. 2010;35:987-993

EFSA 支援出版 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

occupation is a risk factor for childhood wheeze and asthma. The European respiratory journal. 2010;35:987-993

- Tan X.H. Risk factors for Parkinson disease and the path analysis: One-to-one paired design. Neural Regeneration Research (2007) 2:2 (117-120)
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011;119:866-872
- Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW. Occupation and risk of Parkinsonism: A multicenter case-control study. Archives of neurology. 2009;66:1106-1113
- Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. Reported residential pesticide use and breast cancer risk on long island, New York. American journal of epidemiology. 2007;165:643-651
- Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. Epidemiological study of high cancer among rural agricultural community of punjab in northern india. International journal of environmental research and public health. 2008;5:399-407
- Tiido T, Rignell-Hydbom A, Jönsson BAG, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvyezday V, Spano M, Manicardi G-C, Bizzaro D, Bonefeld-Jørgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A. Impact of pcb and p, p'-dde contaminants on human sperm y:X chromosome ratio: Studies in three european populations and the inuit population in greenland. Environmental Health Perspectives. 2005;114:718-724
- Toft G, Axmon A, Lindh CH, Giwercman A, Bonde JP. Menstrual cycle characteristics in european and inuit women exposed to persistent organochlorine pollutants. Hum Reprod. 2008;23:193-200
- Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, Lindh CH, Pedersen HS, Ludwicki JK, Lesovoy V, Hagmar L, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Thulstrup AM, Bonde JP. Semen quality and exposure to persistent organochlorine pollutants. Epidemiology. 2006;17:450-458
- Toft G, Thulstrup AM, Jonsson BA, Pedersen HS, Ludwicki JK, Zvezday V, Bonde JP. Fetal loss and maternal serum levels of 2,2',4,4',5,5'-hexachlorbiphenyl (cb-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'dde) exposure: A cohort study in greenland and two european populations. Environmental health: a global access science source. 2010;9:22
- Ton TG, Longstreth WT, Jr., Koepsell TD. Environmental toxins and risk of narcolepsy among people with hla dqb1\*0602. Environmental research. 2010;110:565-570
- Tondel M, Lindh J, Jonsson P, Vrethem M, Persson B. Occupational determinants of cryptogenic polyneuropathy. Neuroepidemiology. 2006;26:187-194
- Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen Hernandez M, Garcia-Hernandez RM, Del Rio-Garcia C, Wolff MS, Lopez-Carrillo L. In utero p,p'dde exposure and infant neurodevelopment: A perinatal cohort in mexico. Environ Health Perspect. 2007;115:435-439

EFSA supporting publication 2013:EN-497

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### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Tan X.H. Risk factors for Parkinson disease and the path analysis: One-to-one paired design. Neural Regeneration Research (2007) 2:2 (117-120)
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson"s disease. Environ Health Perspect. 2011;119:866-872
- Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, Bressman S, Deligtisch A, Marras C, Lyons KE, Bhudhikanok GS, Roucoux DF, Meng C, Abbott RD, Langston JW. Occupation and risk of Parkinsonism: A multicenter case-control study. Archives of neurology. 2009;66:1106-1113
- Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. Reported residential pesticide use and breast cancer risk on long island, New York. American journal of epidemiology. 2007;165:643-651
- Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. Epidemiological study of high cancer among rural agricultural community of punjab in northern india. International journal of environmental research and public health. 2008;5:399-407
- Tiido T, Rignell-Hydbom A, Jönsson BAG, Giwercman YL, Pedersen HS, Wojtyniak B, Ludwicki JK, Lesovoy V, Zvyezday V, Spano M, Manicardi G-C, Bizzaro D, Bonefeld- Jørgensen EC, Toft G, Bonde JP, Rylander L, Hagmar L, Giwercman A. Impact of pcb and p, p' dde contaminants on human sperm y:X chromosome ratio: Studies in three european populations and the inuit population in greenland. Environmental Health Perspectives. 2005;114:718-724
- Toft G, Axmon A, Lindh CH, Giwercman A, Bonde JP. Menstrual cycle characteristics in european and inuit women exposed to persistent organochlorine pollutants. Hum Reprod. 2008;23:193-200
- Toft G, Rignell-Hydbom A, Tyrkiel E, Shvets M, Giwercman A, Lindh CH, Pedersen HS, Ludwicki JK, Lesovoy V, Hagmar L, Spano M, Manicardi GC, Bonefeld-Jorgensen EC, Thulstrup AM, Bonde JP. Semen quality and exposure to persistent organochlorine pollutants. Epidemiology. 2006;17:450-458
- Toft G, Thulstrup AM, Jonsson BA, Pedersen HS, Ludwicki JK, Zvezday V, Bonde JP. Fetal loss and maternal serum levels of 2, 2', 4, 4', 5, 5'-hexachlorbiphenyl (cb-153) and 1,1-dichloro- 2,2-bis(p-chlorophenyl)ethylene (p,p'-dde) exposure: A cohort study in greenland and two european populations. Environmental health: a global access science source. 2010;9:22
- Ton TG, Longstreth WT, Jr., Koepsell TD. Environmental toxins and risk of narcolepsy among people with hla dqb1\*0602. Environmental research. 2010;110:565-570
- Tondel M, Lindh J, Jonsson P, Vrethem M, Persson B. Occupational determinants of cryptogenic polyneuropathy. Neuroepidemiology. 2006;26:187-194
- Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen Hernandez M, Garcia-Hernandez RM, Del Rio-Garcia C, Wolff MS, Lopez-Carrillo L. In utero p,p'dde exposure and infant neurodevelopment: A perinatal cohort in mexico. Environ Health Perspect. 2007;115:435-439

EFSA 支援出版 2013:EN-497

<sup>151</sup> 

- Torres-Sanchez L, Schnaas L, Cebrian ME, Hernandez Mdel C, Valencia EO, Garcia Hernandez RM, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and neurodevelopment: A follow-up from 12 to 30 months of age. Neurotoxicology. 2009;30:1162-1165
- Torres-Sanchez L, Zepeda M, Cebrian ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, Lopez-Carrillo L. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Annals of the New York Academy of Sciences. 2008;1140:155-162
- Trabert B, Longnecker MP, Brock JW, Klebanoff MA, McGlynn KA. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ Health Perspect. 2012;120:478-482
- Tsai J, Kaye WE, Bove FJ. Wilms' tumor and exposures to residential and occupational hazardous chemicals. International journal of hygiene and environmental health. 2006;209:57-64
- Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Van Trong P, Nhung NT. Impacts of pesticide use on semen characteristics among rice farmers in kienxuong district, thaibinh province, vietnam. The Southeast Asian journal of tropical medicine and public health. 2007;38:569-575
- Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. Environ Health Perspect. 2009;117:1076-1082
- Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and dde in adults. Environ Health Perspect. 2007;115:1197-1203
- Twum C, Wei Y. The association between urinary concentrations of dichlorophenol pesticides and obesity in children. Reviews on environmental health. 2011;26:215-219
- Ubaidullaeva KM. [the clinical and functional features of chronic obstructive lung disease in patients with organic chlorine pesticides in blood]. Problemy tuberkuleza i boleznei legkikh. 2006:21-23
- Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, Drobna B, Trnovec T, Susienkova K, Labudova V, Gasperikova D, Langer P, Klimes I. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia. 2010;53:899-906
- Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Mdrl gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16:1172-1177
- Vajdic CM, Fritschi L, Grulich AE, Kaldor JM, Benke G, Kricker A, Hughes AM, Turner JJ, Milliken S, Goumas C, Armstrong BK. Atopy, exposure to pesticides and risk of nonhodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;120:2271-2274
- Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP, Hoppin JA. Chronic bronchitis among nonsmoking farm women in the agricultural health

- Torres-Sanchez L, Schnaas L, Cebrian ME, Hernandez Mdel C, Valencia EO, Garcia Hernandez RM, Lopez-Carrillo L. Prenatal dichlorodiphenyldichloroethylene (dde) exposure and neurodevelopment: A follow-up from 12 to 30 months of age. Neurotoxicology. 2009;30:1162-1165
- Torres-Sanchez L, Zepeda M, Cebrian ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, Lopez-Carrillo L. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Annals of the New York Academy of Sciences. 2008;1140:155-162
- Trabert B, Longnecker MP, Brock JW, Klebanoff MA, McGlynn KA. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ Health Perspect. 2012;120:478-482
- Tsai J, Kaye WE, Bove FJ. Wilms' tumor and exposures to residential and occupational hazardous chemicals. International journal of hygiene and environmental health. 2006;209:57-64
- Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Van Trong P, Nhung NT. Impacts of pesticide use on semen characteristics among rice farmers in kienxuong district, thaibinh province, vietnam. The Southeast Asian journal of tropical medicine and public health. 2007;38:569-575
- Turyk M, Anderson H, Knobeloch L, Imm P, Persky V. Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. Environ Health Perspect. 2009;117:1076-1082
- Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and dde in adults. Environ Health Perspect. 2007;115:1197-1203
- Twum C, Wei Y. The association between urinary concentrations of dichlorophenol pesticides and obesity in children. Reviews on environmental health. 2011;26:215-219
- Ubaidullaeva KM. [the clinical and functional features of chronic obstructive lung disease in patients with organic chlorine pesticides in blood]. Problemy tuberkuleza i boleznei legkikh. 2006:21-23
- Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, Drobna B, Trnovec T, Susienkova K, Labudova V, Gasperikova D, Langer P, Klimes I. High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia. 2010;53:899-906
- Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Mdr1 gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16:1172-1177
- Vajdic CM, Fritschi L, Grulich AE, Kaldor JM, Benke G, Kricker A, Hughes AM, Turner JJ, Milliken S, Goumas C, Armstrong BK. Atopy, exposure to pesticides and risk of nonhodgkin lymphoma. International journal of cancer. Journal international du cancer. 2007;120:2271-2274
- Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, Sandler DP, Hoppin JA. Chronic bronchitis among nonsmoking farm women in the agricultural health study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:574-583

EFSA supporting publication 2013:EN-497

<sup>152</sup> 

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

study. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2007;49:574-583

- Valikhani M, Kavusi S, Chams-Davatchi C, Daneshpazhooh M, Barzegari M, Ghiasi M, Abedini R. Pemphigus and associated environmental factors: A case-control study. Clinical and experimental dermatology. 2007;32:256-260
- Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. Prenatal concentrations of polychlorinated biphenyls, dde, and ddt and overweight in children: A prospective birth cohort study. Environ Health Perspect. 2012;120:451-457
- van Amelsvoort L, Mohren D, Slangen J, Swaen G, Corsini E, Fustinoni S, Vergieva T, Bosetti C. Liesivuori J. Tarkowski M. Colosio C. van Loveren H. Immune effects and exposure to ethylenebisdithiocarbamate pesticides in re-entry workers in the netherlands. Human & experimental toxicology. 2008;27:693-699
- van Balen E, Font R, Cavalle N, Font L, Garcia-Villanueva M, Benavente Y, Brennan P, de Sanjose S. Exposure to non-arsenic pesticides is associated with lymphoma among farmers in spain. Occupational and environmental medicine. 2006;63:663-668
- van Bemmel DM, Visvanathan K, Beane Freeman LE, Coble J, Hoppin JA, Alavanja MC. Sethyl-n,n-dipropylthiocarbamate exposure and cancer incidence among male pesticide applicators in the agricultural health study: A prospective cohort. Environ Health Perspect. 2008;116:1541-1546
- Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, Schoeters G, Desager K. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 2009;117:122-126
- Vidal JS, Vidailhet M, Derkinderen P, de Gaillarbois TD, Tzourio C, Alperovitch A. Risk factors for progressive supranuclear palsy: A case-control study in France. Journal of neurology, neurosurgery, and psychiatry. 2009;80:1271-1274
- Vidal JS, Vidailhet M, Elbaz A, Derkinderen P, Tzourio C, Alperovitch A. Risk factors of multiple system atrophy: A case-control study in french patients. Movement disorders: official journal of the Movement Disorder Society. 2008;23:797-803
- Viel JF, Floret N, Deconinck E, Focant JF, De Pauw E, Cahn JY, Increased risk of non-hodgkin lymphoma and serum organochlorine concentrations among neighbors of a municipal solid waste incinerator. Environment international. 2011;37:449-453
- Villarejo D, McCurdy SA. The California agricultural workers health survey. Journal of agricultural safety and health. 2008;14:135-146
- Villeneuve S, Cyr D, Lynge E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Fevotte J, Guenel P. Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: A case-control study in europe. Occupational and environmental medicine. 2010;67:837-844
- Vlajinac HD, Sipetic SB, Maksimovic JM, Marinkovic JM, Dzoljic ED, Ratkov IS, Kostic VS. Environmental factors and Parkinson's disease: A case-control study in belgrade, serbia. The International journal of neuroscience. 2010;120:361-367
- Waggoner JK, Kullman GJ, Henneberger PK, Umbach DM, Blair A, Alavanja MC, Kamel F, Lynch CF, Knott C, London SJ, Hines CJ, Thomas KW, Sandler DP, Lubin JH, Beane
- EFSA supporting publication 2013:EN-497

- Valikhani M, Kavusi S, Chams-Davatchi C, Daneshpazhooh M, Barzegari M, Ghiasi M, Abedini R. Pemphigus and associated environmental factors: A case-control study. Clinical and experimental dermatology. 2007;32:256-260
- Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. Prenatal concentrations of polychlorinated biphenyls, dde, and ddt and overweight in children: A prospective birth cohort study. Environ Health Perspect. 2012;120:451-457
- van Amelsvoort L, Mohren D, Slangen J, Swaen G, Corsini E, Fustinoni S, Vergieva T, Bosetti C, Liesivuori J, Tarkowski M, Colosio C, van Loveren H. Immune effects and exposure to ethylenebisdithiocarbamate pesticides in re-entry workers in the netherlands. Human & experimental toxicology. 2008;27:693-699
- van Balen E, Font R, Cavalle N, Font L, Garcia-Villanueva M, Benavente Y, Brennan P, de Sanjose S. Exposure to non-arsenic pesticides is associated with lymphoma among farmers in spain. Occupational and environmental medicine. 2006;63:663-668
- van Bemmel DM, Visvanathan K, Beane Freeman LE, Coble J, Hoppin JA, Alavanja MC, S- ethyln,n-dipropylthiocarbamate exposure and cancer incidence among male pesticide applicators in the agricultural health study: A prospective cohort. Environ Health Perspect. 2008;116:1541-1546
- Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, Schoeters G, Desager K. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect. 2009;117:122-126
- Vidal JS, Vidailhet M, Derkinderen P, de Gaillarbois TD, Tzourio C, Alperovitch A. Risk factors for progressive supranuclear palsy: A case-control study in France. Journal of neurology, neurosurgery, and psychiatry. 2009;80:1271-1274
- Vidal JS, Vidailhet M, Elbaz A, Derkinderen P, Tzourio C, Alperovitch A. Risk factors of multiple system atrophy: A case-control study in french patients. Movement disorders: official journal of the Movement Disorder Society. 2008;23:797-803
- Viel JF, Floret N, Deconinck E, Focant JF, De Pauw E, Cahn JY. Increased risk of nonhodgkin lymphoma and serum organochlorine concentrations among neighbors of a municipal solid waste incinerator. Environment international. 2011;37:449-453
- Villarejo D, McCurdy SA. The California agricultural workers health survey. Journal of agricultural safety and health. 2008;14:135-146
- Villeneuve S, Cyr D, Lynge E, Orsi L, Sabroe S, Merletti F, Gorini G, Morales-Suarez-Varela M, Ahrens W, Baumgardt-Elms C, Kaerlev L, Eriksson M, Hardell L, Fevotte J, Guenel P. Occupation and occupational exposure to endocrine disrupting chemicals in male breast cancer: A case-control study in europe. Occupational and environmental medicine. 2010;67:837-844
- Vlajinac HD, Sipetic SB, Maksimovic JM, Marinkovic JM, Dzoljic ED, Ratkov IS, Kostic VS. Environmental factors and Parkinson"s disease: A case-control study in belgrade, serbia. The International journal of neuroscience. 2010;120:361-367
- Waggoner IK, Kullman GI, Henneberger PK, Umbach DM, Blair A, Alavanja MC, Kamel F, Lynch CF, Knott C, London SJ, Hines CJ, Thomas KW, Sandler DP, Lubin IH, Beane Freeman LE, Hoppin JA. Mortality in the agricultural health study, 1993-2007. American journal of epidemiology. 2011;173:71-83

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

EFSA 支援出版 2013:EN-497

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I Pesticide epidemiology

Freeman LE, Hoppin JA. Mortality in the agricultural health study, 1993-2007. American journal of epidemiology. 2011;173:71-83

- Walker KM, Carozza S, Cooper S, Elgethun K. Childhood cancer in texas counties with moderate to intense agricultural activity. Journal of agricultural safety and health. 2007;13:9-24
- Waller SA, Paul K, Peterson SE, Hitti JE. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in washington state. American journal of obstetrics and gynecology. 2010;202:241 e241-246
- Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. European journal of epidemiology. 2011;26:547-555
- Wang J, Zhu Y, Cai X, Yu J, Yang X, Cheng J. Abnormal glucose regulation in pyrethroid pesticide factory workers. Chemosphere. 2011;82:1080-1082
- Wang P, Tian Y, Wang XJ, Gao Y, Shi R, Wang GQ, Hu GH, Shen XM. Organophosphate pesticide exposure and perinatal outcomes in shanghai, china. Environment international. 2012;42:100-104
- Wanigasuriya KP, Peiris-John RJ, Wickremasinghe R. Chronic kidney disease of unknown aetiology in Sri Lanka: Is cadmium a likely cause? BMC nephrology. 2011;12:32
- Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ Health Perspect. 2009;117:1007-1013
- Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, Altshul L, Ascherio A. Persistent organochlorine pesticides in serum and risk of parkinson disease. Neurology. 2010;74:1055-1061
- Weisskopf MG, Morozova N, O'Reilly EJ, McCullough ML, Calle EE, Thun MJ, Ascherio A. Prospective study of chemical exposures and amyotrophic lateral sclerosis. Journal of neurology, neurosurgery, and psychiatry. 2009;80:558-561
- Weselak M, Arbuckle TE, Wigle DT, Krewski D. In utero pesticide exposure and childhood morbidity. Environmental research. 2007;103:79-86
- Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D. Pre- and post-conception pesticide exposure and the risk of birth defects in an ontario farm population. Reprod Toxicol. 2008;25:472-480
- Wickerham EL, Lozoff B, Shao J, Kaciroti N, Xia Y, Meeker JD. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment international. 2012;47:80-85
- Wohlfahrt-Veje C, Andersen HR, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Smaller genitals at school age in boys whose mothers were exposed to non-persistent pesticides in early pregnancy. International journal of andrology. 2012;35:265-272
- Wohlfahrt-Veje C, Andersen HR, Schmidt IM, Aksglaede L, Sorensen K, Juul A, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Early breast development in girls after prenatal exposure to non-persistent pesticides. International journal of andrology. 2012;35:273-282
- Wohlfahrt-Veje C, Main KM, Schmidt IM, Boas M, Jensen TK, Grandjean P, Skakkebaek NE, Andersen HR. Lower birth weight and increased body fat at school age in children

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Walker KM, Carozza S, Cooper S, Elgethun K. Childhood cancer in texas counties with moderate to intense agricultural activity. Journal of agricultural safety and health. 2007;13:9-24
- Waller SA, Paul K, Peterson SE, Hitti JE. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in washington state. American journal of obstetrics and gynecology. 2010;202:241 e241-246
- Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. European journal of epidemiology. 2011;26:547-555
- Wang J, Zhu Y, Cai X, Yu J, Yang X, Cheng J. Abnormal glucose regulation in pyrethroid pesticide factory workers. Chemosphere. 2011;82:1080-1082
- Wang P, Tian Y, Wang XJ, Gao Y, Shi R, Wang GQ, Hu GH, Shen XM. Organophosphate pesticide exposure and perinatal outcomes in shanghai, china. Environment international. 2012;42:100-104
- Wanigasuriya KP, Peiris-John RJ, Wickremasinghe R. Chronic kidney disease of unknown aetiology in Sri Lanka: Is cadmium a likely cause? BMC nephrology. 2011;12:32
- Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ Health Perspect. 2009;117:1007-1013
- Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, Altshul L, Ascherio A. Persistent organochlorine pesticides in serum and risk of parkinson disease. Neurology. 2010;74:1055-1061
- Weisskopf MG, Morozova N, O'Reilly EJ, McCullough ML, Calle EE, Thun MJ, Ascherio A. Prospective study of chemical exposures and amyotrophic lateral sclerosis. Journal of neurology, neurosurgery, and psychiatry. 2009;80:558-561
- Weselak M, Arbuckle TE, Wigle DT, Krewski D. In utero pesticide exposure and childhood morbidity. Environmental research. 2007;103:79-86
- Weselak M, Arbuckle TE, Wigle DT, Walker MC, Krewski D. Pre- and post-conception pesticide exposure and the risk of birth defects in an ontario farm population. Reprod Toxicol. 2008;25:472-480
- Wickerham EL, Lozoff B, Shao J, Kaciroti N, Xia Y, Meeker JD. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment international. 2012;47:80-85
- Wohlfahrt-Veje C, Andersen HR, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Smaller genitals at school age in boys whose mothers were exposed to non-persistent pesticides in early pregnancy. International journal of andrology. 2012;35:265-272
- Wohlfahrt-Veje C, Andersen HR, Schmidt IM, Aksglaede L, Sorensen K, Juul A, Jensen TK, Grandjean P, Skakkebaek NE, Main KM. Early breast development in girls after prenatal exposure to non-persistent pesticides. International journal of andrology. 2012;35:273-282
- Wohlfahrt-Veje C, Main KM, Schmidt IM, Boas M, Jensen TK, Grandjean P, Skakkebaek NE, Andersen HR. Lower birth weight and increased body fat at school age in children prenatally exposed to modern pesticides: A prospective study. Environmental health : a global access science source. 2011;10:79

EFSA supporting publication 2013:EN-497

The present document has been produced and adopted by the bodies identified above as author(s). This task has been carried out exclusively by the author(s) in the context of a contract between the European Food Safety Authority and the author(s), awarded following a tender procedure. The present document is published complying with the transparency principle to which the Authority is subject. It may not be considered as an output adopted by the Authority. The European Food Safety Authority reserves its rights, view and position as regards the issues addressed and the conclusions reached in the present document, without prejudice to the rights of the authors.

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I Pesticide epidemiology

prenatally exposed to modern pesticides: A prospective study. Environmental health : a global access science source. 2011;10:79

- Wohlfahrt-Veje C., Main K.M., Schmidt I.M., Jensen T.K., Grandjean P., Skakkebaek N.E., Andersen H.R. Effects of prenatal exposure to modern pesticides on birth weight, growth and body composition in childhood; Interactions with maternal smoking and PON1 genepolymorphisms. Hormone Research in Paediatrics (2011) 76 SUPPL. 2 (232-233).
- Wojtyniak BJ, Rabczenko D, Jonsson BA, Zvezday V, Pedersen HS, Rylander L, Toft G, Ludwicki JK, Goralczyk K, Lesovaya A, Hagmar L, Bonde JP. Association of maternal serum concentrations of 2,2', 4,4'5,5'-hexachlorobiphenyl (cb-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-dde) levels with birth weight, gestational age and preterm births in inuit and european populations. Environmental health : a global access science source. 2010;9:56
- Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. Environmental exposures and puberty in inner-city girls. Environmental research. 2008;107:393-400
- Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, Wetmur J. Prenatal pesticide and pcb exposures and birth outcomes. Pediatric research. 2007;61:243-250
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of acute myeloid leukemia in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:112-128
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of non-hodgkin lymphoid neoplasms in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:129-146
- Wu P.-L., Dai B.-T., Yu Z.-H., Yu J., Xian Y., Su Y.-C. Dependablity investigation of the risk factors of childhood leukaemia. Chinese Journal of Evidence-Based Medicine (2010) 10:9 (1037-1040).
- Wu T, Bhanegaonkar AJ, Flowers JW. Blood concentrations of selected volatile organic compounds and neurobehavioral performance in a population-based sample. Arch Environ Occup Health. 2006;61:17-25
- Xia Y, Han Y, Wu B, Wang S, Gu A, Lu N, Bo J, Song L, Jin N, Wang X. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertility and sterility. 2008;89:1743-1750
- Xu JX, Hoshida Y, Yang WI, Inohara H, Kubo T, Kim GE, Yoon JH, Kojya S, Bandoh N, Harabuchi Y, Tsutsumi K, Koizuka I, Jia XS, Kirihata M, Tsukuma H, Aozasa K. Life-style and environmental factors in the development of nasal nk/t-cell lymphoma: A case-control study in east asia. International journal of cancer. Journal international du cancer. 2007;120:406–410
- Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR. Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in u.S. Adults. Environ Health Perspect. 2010;118:60-66
- Xu X, Nembhard WN, Kan H, Kearney G, Zhang ZJ, Talbott EO. Urinary trichlorophenol levels and increased risk of attention deficit hyperactivity disorder among us school-aged children. Occupational and environmental medicine. 2011;68:557-561

EFSA supporting publication 2013:EN-497

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Pesticide epidemiology
- Wohlfahrt-Veje C., Main K.M., Schmidt I.M., Jensen T.K., Grandjean P., Skakkebaek N.E., Andersen H.R. Effects of prenatal exposure to modern pesticides on birth weight, growth and body composition in childhood; Interactions with maternal smoking and PON1 gene- polymorphisms. Hormone Research in Paediatrics (2011) 76 SUPPL. 2 (232-233).
- Wojtyniak BJ, Rabczenko D, Jonsson BA, Zvezday V, Pedersen HS, Rylander L, Toft G, Ludwicki JK, Goralczyk K, Lesovaya A, Hagmar L, Bonde JP. Association of maternal serum concentrations of 2, 2', 4, 4'5, 5'-hexachlorobiphenyl (cb-153) and 1, 1-dichloro-2, 2-bis (p-chlorophenyl)-ethylene (p, p'-dde) levels with birth weight, gestational age and preterm births in inuit and european populations. Environmental health : a global access science source. 2010;9:56
- Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu Z, Berkowitz G, Larson S, Forman J. Environmental exposures and puberty in inner-city girls. Environmental research. 2008;107:393-400
- Wolff MS, Engel S, Berkowitz G, Teitelbaum S, Siskind J, Barr DB, Wetmur J. Prenatal pesticide and pcb exposures and birth outcomes. Pediatric research. 2007;61:243-250
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of acute myeloid leukemia in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:112-128
- Wong O, Harris F, Armstrong TW, Hua F. A hospital-based case-control study of non-hodgkin lymphoid neoplasms in shanghai: Analysis of environmental and occupational risk factors by subtypes of the who classification. Chemico-biological interactions. 2010;184:129-146
- Wu P.-L., Dai B.-T., Yu Z.-H., Yu J., Xian Y., Su Y.-C. Dependablity investigation of the risk factors of childhood leukaemia. Chinese Journal of Evidence-Based Medicine (2010) 10:9 (1037-1040).
- Wu T, Bhanegaonkar AJ, Flowers JW. Blood concentrations of selected volatile organic compounds and neurobehavioral performance in a population-based sample. Arch Environ Occup Health. 2006;61:17-25
- Xia Y, Han Y, Wu B, Wang S, Gu A, Lu N, Bo J, Song L, Jin N, Wang X. The relation between urinary metabolite of pyrethroid insecticides and semen quality in humans. Fertility and sterility. 2008;89:1743-1750
- Xu JX, Hoshida Y, Yang WI, Inohara H, Kubo T, Kim GE, Yoon JH, Kojya S, Bandoh N, Harabuchi Y, Tsutsumi K, Koizuka I, Jia XS, Kirihata M, Tsukuma H, Aozasa K. Life-style and environmental factors in the development of nasal nk/t-cell lymphoma: A case-control study in east asia. International journal of cancer. Journal international du cancer. 2007;120:406-410
- Xu X, Dailey AB, Talbott EO, Ilacqua VA, Kearney G, Asal NR. Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in u.S. Adults. Environ Health Perspect. 2010;118:60-66
- Xu X, Nembhard WN, Kan H, Kearney G, Zhang ZJ, Talbott EO. Urinary trichlorophenol levels and increased risk of attention deficit hyperactivity disorder among us school-aged children. Occupational and environmental medicine. 2011;68:557-561

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#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I Pesticide epidemiology

- Yang JH, Lee YM, Bae SG, Jacobs DR, Jr., Lee DH. Associations between organochlorine pesticides and vitamin d deficiency in the u.S. Population. PloS one. 2012;7:e30093
- Yang Yang, Zeng Li-Xia, Yan Hong. Analysis of risk factors of birth defects in Shaanxi Province. Journal of XI'an Jiaotong University (Medical Sciences) 2011; Vol. 32 No. 1
- Yiin JH, Ruder AM, Stewart PA, Waters MA, Carreon T, Butler MA, Calvert GM, Davis-King KE, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of pesticide applicators and risk of glioma. Environmental health: a global access science source. 2012;11:39
- Yucra S, Gasco M, Rubio J, Gonzales GF. Semen quality in peruvian pesticide applicators: Association between urinary organophosphate metabolites and semen parameters. Environmental health: a global access science source. 2008;7:59
- Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in peruvian pesticide sprayers. International journal of occupational and environmental health. 2006;12:355-361
- Zakerinia M, Namdari M, Amirghofran S. The relationship between exposure to pesticides and the occurrence of lymphoid neoplasm. Iranian Red Crescent medical journal. 2012;14:337-344
- Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. Muscle invasive bladder cancer in upper egypt: The shift in risk factors and tumor characteristics. BMC cancer. 2008;8:250
- Zhang Y, Zhu S, Gao Y, Wang XJ, Chen T, Yang Y, Wang GQ, Hu GH, Shi R, Jin P, Tian Y. [a case-control study on correlation of pesticide exposure with childhood acute leukemia]. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2011;45:41-46
- Zhu JL, Hjollund NH, Andersen AM, Olsen J. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: A study within the danish national birth cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:347-352
- Zota AR, Aschengrau A, Rudel RA, Brody JG. Self-reported chemicals exposure, beliefs about disease causation, and risk of breast cancer in the cape cod breast cancer and environment study: A case-control study. Environmental health: a global access science source. 2010;9:40
- Zschiedrich K, Konig IR, Bruggemann N, Kock N, Kasten M, Leenders KL, Kostic V, Vieregge P, Ziegler A, Klein C, Lohmann K. Mdr1 variants and risk of parkinson disease. Association with pesticide exposure? Journal of neurology. 2009;256:115-120

#### Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

- Yang JH, Lee YM, Bae SG, Jacobs DR, Jr., Lee DH. Associations between organochlorine pesticides and vitamin d deficiency in the u.S. Population. PloS one. 2012;7:e30093
- Yang Yang, Zeng Li-Xia, Yan Hong. Analysis of risk factors of birth defects in Shaanxi Province. Journal of XI"an Jiaotong University (Medical Sciences) 2011; Vol. 32 No. 1
- Yiin JH, Ruder AM, Stewart PA, Waters MA, Carreon T, Butler MA, Calvert GM, Davis-King KE, Schulte PA, Mandel JS, Morton RF, Reding DJ, Rosenman KD. The upper midwest health study: A case-control study of pesticide applicators and risk of glioma. Environmental health: a global access science source. 2012;11:39
- Yucra S, Gasco M, Rubio J, Gonzales GF. Semen quality in peruvian pesticide applicators: Association between urinary organophosphate metabolites and semen parameters. Environmental health: a global access science source. 2008;7:59
  - Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in peruvian pesticide sprayers. International journal of occupational and environmental health. 2006;12:355-361
  - Zakerinia M, Namdari M, Amirghofran S. The relationship between exposure to pesticides and the occurrence of lymphoid neoplasm. Iranian Red Crescent medical journal. 2012;14:337-344
  - Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. Muscle invasive bladder cancer in upper egypt: The shift in risk factors and tumor characteristics. BMC cancer. 2008;8:250
  - Zhang Y, Zhu S, Gao Y, Wang XJ, Chen T, Yang Y, Wang GQ, Hu GH, Shi R, Jin P, Tian Y. [a case-control study on correlation of pesticide exposure with childhood acute leukemia]. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2011;45:41-46
  - Zhu JL, Hjollund NH, Andersen AM, Olsen J. Occupational exposure to pesticides and pregnancy outcomes in gardeners and farmers: A study within the danish national birth cohort. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2006;48:347-352
  - Zota AR, Aschengrau A, Rudel RA, Brody JG. Self-reported chemicals exposure, beliefs about disease causation, and risk of breast cancer in the cape cod breast cancer and environment study: A case-control study. Environmental health: a global access science source. 2010;9:40
  - Zschiedrich K, Konig IR, Bruggemann N, Kock N, Kasten M, Leenders KL, Kostic V, Vieregge P, Ziegler A, Klein C, Lohmann K. Mdr1 variants and risk of parkinson disease. Association with pesticide exposure? Journal of neurology. 2009;256:115-120

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#### **GLOSSARY AND ABBREVIATIONS**

AHS: Agricultural Health Study

Beta estimate: coefficient of linear regression

Bias: A systemic inaccuracy in data due to the characteristics of the process employed in the creation, collection, manipulation and presentation of the data or due to faulty sample design of the estimating technique

Biomarker: A measurable substance or characteristic in the human body that can be used to monitor the presence of a chemical in the body, biological responses, or adverse health effects. Biomarkers of exposure are used to assess the amount of a chemical that is present within the body.

Blinded outcome assessment: Individuals who assess the exposure are blinded to the health outcome status of the participants.

CARDIA: The "Coronary Artery Risk Development In Young Adults" study, a multi-center, population-based study.

Case-control study: A type of observational study in which two existing groups differing in outcome are identified and compared on the basis of some supposed causal attribute. Case-control studies are retrospective, as the exposure status is assessed retrospectively.

Case reports: Detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of individual patients.

Case series: descriptive study that tracks patients with a known exposure given similar treatment or examines their medical records for exposure and outcome. These studies lack control groups.

Center-specific analysis: Analysis per centre in studies, which have participants, recruited from more than one centre.

CHAMACOS: The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS). A prospective birth cohort aimed at studying the association of pesticides and other environmental agents on the health of pregnant women and their children living in the Salinas Valley, California.

CI: Confidence Interval

Cohort study: A longitudinal/prospective study, which analyses risk factors and follows a group of people who do not have the disease until participants develop the disease(s) of interest

Confounders: Extraneous variables in a statistical model that correlate (positively or negatively) with both the dependent variable (exposure) and the independent variable (outcome)

Cross-sectional study: A study that involves observation of all of participants at one specific point in time, exposure and outcome are measured in the same time point.

Ecological study: Studies in which the unit of observation is the population or community. Disease rates and exposures are measured in each of a series of populations and their relation is examined.

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#### 用語解説と略語

AHS:農業健康調査

ベータ推定値:線形回帰の係数

バイアス:データの作成、収集、操作及び表示に採用されたプロセスの特性に起因する、または推定 手法の誤ったサンプルデザインに起因する、データの体系的な不正確さ。

バイオマーカー:体内の化学物質の存在、生物学的反応、または健康への有害影響を監視するために 使用できる、人体内の測定可能な物質または特性。ばく露のバイオマーカーは、体内に存在す る化学物質の量を評価するために使用される。

盲検影響評価:ばく露を評価する個人は、参加者の健康状態を盲検化する。

- CARDIA (カーディア): "Coronary Artery Risk Development In Young Adults (若年成人の冠状動 脈リスク発症) "研究、多施設、集団ベースの研究。
- 症例対照研究 (Case-control study):観察研究の一種で、結果の異なる2つの既存のグループを特 定し、何らかの因果関係に基づいて比較する研究。症例対照研究は、ばく露状況を後ろ向きに 評価する後ろ向き研究である。
- 症例報告:個々の患者の症状、徴候、診断、治療、経過観察の詳細な報告。
- 症例集積:同様の治療を受けたばく露が知られている患者を追跡したり、ばく露と影響について医療 記録を調べたりする記述研究。これらの研究には対照群がない。
- センターごとの分析:複数のセンターから募集した参加者がいる研究におけるセンターごとの分析。
- CHAMACOS (チャマコス): Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)。カリフォルニア州サリナスバレーに住む妊婦とその子供の健康に及ぼす農薬やそ の他の環境要因の関連を調べることを目的とした前向き出生コホート。

CI:信頼区間

- コホート研究:リスク因子を分析し、調査対象疾患を発症するまで疾患を持たない人のグループを追 跡調査する縦断的/前向き研究。
- 交絡因子 (Confounders): 従属変数(ばく露)と独立変数(影響)の両方と相関する(正または負の)統計モデルの外部変数。

横断研究:ある特定の時点での参加者全員の観察を行い、ばく露と影響を同じ時点で測定する研究。

生態学的研究:観察の単位が集団または共同体である研究。疾病率とばく露が各集団集積で測定さ れ、それらの関係が調査される。

効果(2変数/連続変数):影響は2変数法(二項対立、例:がん(はい/いいえ))または連続変数法 (例:収縮期血圧(120mmHg))である。

効果推定値/サイズ:関連の強さの尺度

ESCALE:全国登録ベースの症例対照研究「Etude sur les cancers de l'enfant」試験

- ファンネルプロット:システマティックレビューやメタアナリシスにおける出版バイアスの有無を確 認するために設計されたグラフ
- 不均一性:メタアナリシスは、類似した研究のグループから複合的な効果を推定するために使用され る。しかし、治療効果の個々の推定値は偶然によって変動する;ある程度の変動は予想され

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Effect (binary/continuous): Outcome is binary (dichotomous, e.g. cancer (yes/no)) or continuous (e.g. systolic blood pressure (120mmHg)).

Effect estimate/ size: A measure of the strength of association

ESCALE: The "Etude sur les cancers de l'enfant" study, a national registry-based case-control study

Funnel plots: graph designed to check the existence of publication bias in systematic reviews and meta-analyses

Heterogeneity: meta-analysis is used to estimate a combined effect from a group of similar studies. However, the individual estimates of treatment effect will vary by chance; some variation is expected. The question is whether there is more variation than would be expected by chance alone. When this excessive variation occurs, it is called heterogeneity

#### HR: Hazard Ratio

 $I^2$ : measure of the consistency between trials in a meta-analysis, it is a measurement of heterogeneity and takes values form 0 (no heterogeneity) to 1 (extreme heterogeneity)

INUENDO: "INUENDO—Biopersistent organochlorines in diet and human fertility" Epidemiological studies of time to pregnancy and semen quality in Inuit and European populations", a European project on fertility that was supported by the European Commission to the 5th Framework Programme Quality of Life and Management of Living Resources, Key Action 4 on Environment and Health (Contract no. QLK4-CT-2001-00202) (http://www.inuendo.dk).

IRR: Incidence rate ratio

IQR: Interquartile Range

JEM :Job Exposure Matrix

MD: Mean Difference

Meta-analysis: The process or technique of synthesizing research results by using various statistical methods to retrieve, select, and combine results from previous separate but related studies.

Multiple testing: Testing many hypotheses, which are not a priori defined or based on a priori hypothesis.

Misclassification: Bias in an estimate arising from measurement error

Multivariable models: Statistical models with more than one dependent variable. These models typically adjust for a number of confounders the analysis of interest.

Nested case-control study: In a nested case-control study, cases of a disease that occur in a defined cohort are identified and, for each, a specified number of matched controls is selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case

NHANES: National Health and Nutrition Examination Survey

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Observational study: an observational study draws inferences about the possible effect of a treatment on subjects, where the assignment of subjects into a treated group versus a control group is outside the control of the investigator

#### OR: Odds ratio

Pooled effect estimate: Summary effect estimate of the meta-analysis, the result of meta-analysis

POPs: Persistent Organic Pollutants

Prospective study: An epidemiologic study in which the groups of individuals (cohorts) are selected on the bases of factors that are to be examined for possible effects on some outcome

Publication bias: Bias arisen from the tendency for <u>researchers</u>, editors, and pharmaceutical companies to handle the reporting of experimental results that are *positive* (i.e. showing a <u>significant</u> finding) differently from results that are <u>negative</u> (i.e. supporting the <u>null hypothesis</u>) or inconclusive.

Recall bias: Systematic errors due to differences in accuracy or completeness of recall to memory of past events or experiences.

Residual confounding: Residual confounding occurs when a confounder has not been adequately adjusted for in the analysis (usually because the confounder is not known)

Retrospective study: an epidemiologic study in which participating individuals are classified as either having some outcome (cases) or lacking it (controls); the outcome may be a specific disease, and the persons' histories are examined for specific factors that might be associated with that outcome

Reverse causality: Reverse causality refers to the direction of cause-and-effect, it is not known whether the exposure has led to the outcome or the outcome has led to the exposure.

RR: Relative Risk

Narrative review: An article written to consider the critical points of current knowledge including substantive findings, as well as theoretical and methodological contributions to a particular topic

SD: Standard Deviation

SE: Standard Error

Surrogate outcome: A laboratory measurement or physical sign that is used in trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the exposure

Systematic reviews: Reviews of the evidence on a clearly formulated question that use systematic and explicit methods to identify, select and critically appraise relevant primary research, and to extract and analyse data from the studies that are included in the review

Type-I error: The incorrect rejection of a true null hypothesis

UFW: United Farm Workers

EFSA supporting publication 2013:EN-497

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EFSA 支援出版 2013:EN-497

ナラティブ・レビュー (Narrative review):実質的な知見や、特定のトピックに対する理論的・方 法論的な貢献を含めて、現在の知識の重要な点を調査するために書かれた論文

ばく露につながったのかはわからない。

Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

因子が知られていないため)。

いて、その人の病歴を調べる。

SD:標準偏差

RR・相対リスク

- SE:標準誤差
- 代替健康影響(surrogate outcome):患者がどのように感じているか、機能しているか、または生存 しているかを直接測定するものや、ばく露の影響を予測することが期待されるものである臨床 的に意味のあるエンドポイントの代わりに試験で使用される測定値または物理的徴候。

後ろ向き研究:参加した個人を何らかの影響あり(症例)と影響なし(対照)に分類する疫学研究

逆因果関係: 逆因果関係とは、因果関係の方向性のことで、ばく露が影響につながったのか、影響が

で、影響は特定の疾患である可能性があり、その影響と関連する可能性のある特定の因子につ

システマティックレビュー:関連する主要研究を特定、選択、批判的に評価し、レビューに含まれる 研究からデータを抽出、分析するために、体系的かつ明示的な方法を用いて、明確に定式化さ れた問題に関するエビデンスのレビュー。

第一種の誤り:真の帰無仮説の誤った棄却

UFW: 米国農場労働者 (United Farm Workers)

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<sup>159</sup> 

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## **Environment International**

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研究の質を評価するための提案:バイオモニタリング、環境疫学、短寿命化学物質 (BEES-C)の指標

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記事情報

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バイオモニタリング
至る所に存在する(ユビキタス)化学物質
短い生理的半減期
評価
指標
環境疫学

要約

ばく露評価の質は、環境疫学研究の全体的な質を決定する主要な要素である。生理的半減期の 短い至る所に存在する(ユビキタス)化学物質へのばく露を評価するためのツールとしてのバイ オモニタリングの使用は比較的最近になってから始まった。これらの化学物質には、分析室やサ ンプリング機器に含まれる化学物質の存在、横断研究における時間的順序の確立の難しさ、ばく 露とバイオマーカーの濃度の短期及び長期の変動、適切なばく露分類に必要な測定数に関する情 報不足など、いくつかの問題がある。現在までのところ、科学研究分野において、ばく露指標と してバイオモニタリングを使用する短寿命化学物質の研究を計画、実行、解釈するための体系的 なガイドラインを開発しておらず、また、この種の研究の質を評価するための WOE 評価、また は助成金や出版物のピアレビューのための体系的なガイドラインを開発していない。本研究では、 短寿命化学物質のバイオモニタリングデータを用いた疫学研究に影響を与える重要な問題点を説 明し、短寿命化学物質のバイオモニタリングデータを取り入れた研究提案や研究の質を評価する ための体系的な指標であるバイオモニタリング、環境疫学、短寿命化学物質(BEES·C)を提案 する。短寿命化学物質の生物学的測定を含む疫学研究の評価に不可欠と考えられる3つの分野の 品質基準について説明している。1)バイオマーカーの選択と測定、2)試験デザインと実行、3) 一般的な疫学研究デザインに関する考察。BEES-C のような評価ツールの開発は単純ではなく、 議論の余地のないものでもないことを認識している。

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## 1. 序文

疫学研究は、様々な化学的、物理的、生物学的、放射線学的、行動学的なばく露がヒトの健康 に及ぼす影響を評価する上で重要な役割を果たしている。しかし、ヒトにおける因果関係の仮説 を検証するために特別に設計され、厳密に実行された疫学研究であっても、しばしば矛盾した結 果が報告される。保健政策の提言を行う規制機関やコンセンサス・パネルは、一般的に疫学研究 結果を評価するために WOE (weight-of-evidence) アプローチに依存している。

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WOE 評価は、結論が利用可能な最も強力な証拠に基づいていることを確認するために研究の 質を評価しない場合、不完全であったり、誤解を招く可能性がある。さらに、助成金申請書や原 稿のピアレビューにおける研究の質を評価することで、ヒトのばく露や健康に関する研究の全体 的な質を高めるのに役立つ。

研究の質の決定には常にある程度専門的な判断が必要であるが、疫学的証拠の強さの評価は、 体系的に適用される合意された基準に依存すべきであるという考えが出てきている

(Vandenbroucke ら、2007 年)。これらの考えが、いくつかの研究の質評価ツールの開発と改善の動機となった。これらのツールのいくつか(例えば、STROBE(Vandenbroucke ら、2007 年);CONSORT(Moher ら、2001 年))は、分野を超えて適用される一般的な問題を扱っている。他のツールは、医学や生命科学の様々な分野に特化して開発された(例えば、遺伝学的研究のための STREGA(Little ら、2009 年)、治療効果の比較研究のための GRADE(Owens ら、2010 年)、診断精度の研究のための STARD(Bossuyt ら、2004 年))。

研究の質を取り入れた WOE 評価の標準化が進んでいる現在の傾向に鑑みると、環境疫学研究 を評価するための手段が、研究デザインの開発段階や原稿の審査段階でも、相対的に少なくなっ ていることは注目に値するが、説明が難しい。エビデンスの重み付け評価のための研究の質の評 価に焦点を当てた評価スキーム(Harmonization of Neurodevelopmental Environmental Epidemiology Studies)(Youngstrom ら、2011 年)では、診断精度研究の質の評価

(QUADAS)をコーディングツールの基礎として使用しているが(Whiting ら、2003 年)、その名が示すように、この評価指標は神経発達研究を中心としたものである。National Toxicology Program は最近、研究の質を評価するためのアプローチを開発し(NTP、2013 年)、これを用いて環境化学物質と糖尿病に関する文献を調査した(Kuo ら、2003 年)。この事業計画には、疫学及び毒性学の文献の評価を含み、非難分解性及び難分解性の化学物質は含まれていたが、短寿命化学物質のバイオモニタリング固有の問題は含まれていなかった。

環境疫学研究の最適な実行に関する体系的なガイダンスを提供するツールがないことは、集団 ベースの研究に依存する規制上の意思決定にとって重要な制約となっている。他の研究分野とは 異なり、ヒトに有害性影響を誘発することを目的とした介入研究は、倫理的に可能であるとして も、ほとんど行われていないため、環境疫学データに基づく WOE 評価は、独特のものである。 そのため、環境疫学研究はほとんど常に観察的な研究であり、様々な情報源から生じる避けられ ない不確実性の影響を受けている。環境疫学における不確実性の重要なソースの一つであると同 時に、急速に進展している分野でもあるのが、ばく露科学である。

ばく露評価は、生理学的半減期の短い化学物質を含む環境疫学研究(Hertz-Picciotto、1998 年)において、全体的なデータの質を決定する主要な要素である。短寿命化学物質とは、化学物 質分量の半分を体外に排出するのに必要な時間が、およそ数分から数時間あるいは数日である化 学物質のことである。短寿命化学物質のばく露評価の質は、ばく露の様々な側面を調べるための バイオモニタリングを用いた研究と同様に、健康影響との関連性を評価するためのデータの有用 性と密接に関連している。近年、ばく露科学の手法は、バイオモニタリングによる環境化学物質 の検出能力の向上により、特に恩恵を受けている。バイオモニタリングとは、血液、尿、呼気、 母乳、毛髪など、ヒトの様々なマトリックス中の化学物質を測定することである。バイオモニタ リングデータは、すべての経路(経口、吸入、経皮、経胎盤)からのばく露を統合したものであ り、以下のような場合に有用である。(1)集団参照範囲の設定、(2)亜集団における異常ばく 露の特定、(3)集団内の時間的変動と傾向の評価、(4)個人のばく露量を推定するためにデザ インされたアンケートの検証、(5)疫学研究における健康影響との関連性の検討。

難分解性有機汚染物質や金属のばく露量を測定するための基礎としてのバイオモニタリングを 用いた疫学研究は、何十年にもわたって実行されてきた。対照的に、至る所に存在する(ユビキ タス)生理的半減期の短い化学物質(ベンゼン、フタル酸エステル類、特定の農薬など)のバイ オモニタリングは、比較的最近始まったもので、ばく露のばらつきや、分析室やサンプリング機 器に含まれる多くの化学物質のユビキタスな性質によって複雑になるため、これらの化学物質に 関するデータの解釈にはいくつかの新たな問題がある。また、これらの化学物質は、研究に使用 するマトリックスを選択する際にも問題となる。現在までのところ、これらの化学物質のバイオ モニタリング研究を実行し、解釈するための体系的なガイドラインは開発されていない。同様に、 WOE 評価あるいは助成金や出版物の査読のため、この種の研究の質を評価するための公表され た方法はない。

この知識ギャップは、2013年の国際ワークショップ「Best Practices for Obtaining, Interpreting and Using Human Biomonitoring Data in Epidemiology and Risk Assessment: Chemicals with Short Biological Half-Lives (疫学とリスク評価におけるヒトのバイオモニタリ ングデータの取得、解釈、利用のための最適な実行:生物学的半減期の短い化学物質)」の明確 な目的となった。このワークショップには、分析化学、ばく露とリスクの評価、疫学、医学、生 理学的薬物動態 (PBPK) モデリング、臨床バイオマーカーを専門とする政府機関、学界、民間 機関の専門家が参加した。ワークショップの目的は以下の通りであった。(i) 生理的半減期の 短い化学物質の生物学的モニタリングデータを用いた疫学研究に影響を与える問題を説明するこ と。(ii) 短寿命化学物質のバイオモニタリングデータを取り入れた研究提案や研究の質を評価 するための体系的なスキームを構築する。

本論文では、短寿命化学物質の生物学的測定を含む疫学研究の評価の基礎となると考えられる 3 つの分野の品質基準について説明する。1) バイオマーカーの選択と測定、2) 試験デザインと 実行、3) 一般的な疫学研究デザインの考慮事項である。これらのトピック領域の主要な側面に ついて議論し、次に、階層化されたマトリックスとして構成された評価指標(バイオモニタリン グ(Biomonitoring)、環境疫学(Environmental Epidemiology)、短寿命化学物質(Short-Lived Chemicals (BEES-C))の指標)の提案に組み入れた(表1)。提案されている評価指標 のいくつかの側面には、難分解性化学物質と短寿命化学物質の両方の疫学研究に関連する研究デ ザインの要素が含まれている。実際、STROBE のような広く受け入れられている評価指標の側 面は、ここで提案する評価指標に意図的に織り込まれている(Gallo ら、2011 年; Little ら、 2009 年; Vandenbroucke ら、2007 年) (STROBE は、観察研究の報告を改善するための方法 や、これらの研究を批判的に評価するためのガイダンスを提供している。STROBE は査読者、 ジャーナル編集者、読者が利用できるようにデザインされている「(Vandenbroucke ら、 2007)」。)。STROBE の既定及び新規の側面は、ばく露評価のアプローチとして短寿命化学 物質のバイオモニタリングを用いた研究の質を評価する上で批判的な要素であるが、本コミュニ ケーションの第一の目的は、短寿命化学物質の研究の批判的な側面をカバーすることである。

ある研究を評価するために使用できる品質問題のリストは長い。すべてを網羅しているが扱い にくい手段を開発することと、最も重要な問題のみを含む(短寿命化学物質に特有の、あるいは 特に重要な研究の側面に焦点を当てている)より差別化された実用的な手段を開発することとの 間とは対立する状態にある。提案された BEES-C 指標を開発するにあたり、後者を選択した。

この指標は、ばく露と健康影響との関係を調べる研究だけでなく、ばく露の様々な側面(例え ば、時間的・空間的傾向)を調べるバイオモニタリングデータを用いた研究にも適用可能である。 ここで提起され、BEES-C指標によって対処された問題は、職業に関係のある研究と栄養疫学を 含めて短寿命化学物質の生物学的指標に関わる複数の分野に影響を及ぼす。

環境疫学研究における短命化学物質の特徴として、特に注意が必要なのは、対象となる健康影響に関連するばく露範囲を表すサンプルの数と時期、パーソナルケア製品、実験器具、塵埃、食品など、現在製造されている製品にはこれらの化学物質の多くが普遍的に存在しているため、サ

ンプルの汚染を避けるために特別な配慮が生じること、適切な生物学的マトリックスの選択により、1 つのサンプルで多数の化学物質を測定することができるため、完全な報告と多重比較に関する問題に注意を払う必要性が高まることである。

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表 1

バイオモニタリング、環境疫学、短寿命化学物質(BEES-C)の評価指標。生理的半減期の短い 化学物質のバイオモニタリングを含む疫学研究の質を評価するための評価基準。評価基準は、バ イオモニタリングをばく露指標とした環境疫学研究のいくつかの側面をカバーしている(表の下 部に頭字語が定義されている)。Justification 欄は、意思決定のプロセスの透明性を高めるため に使用される。

研究評価の	MIDD 1			т с	
構成要素	TIER 1	TIER 2	TIER 3	Justification	
バイオマーカーの選択と測定					
生物学的関連性 (親化合物/代 替の関係)					
ばく露バイオマ ーカー		クス内のバイオマー カーと外部ばく露、 内部ばく露、または 目標ばく露量との間	ックスに含まれる バイ オマーカー は、ばく露/用量に		
効果バイオマー カー	イベントのバイオイ ンジケーター。	健康影響との関連性 が示された効果のバ イオマーカーである が、その作用機序は 不明である。	バイオマーカーは 未確定の結果をも たらす(例えば、 バイオマーカーは 健康影響に特異的 ではない)。		
特異度	バイオマーカーは、1 つの親化合物へのば く露から得られる。	バイオマーカーは、 類似の有害性エンド ポイントを持つ複数 の親化合物に由来す る。	バイオマーカー は、有害性エンド ポイントの種類が 異なる複数の親化 合物に由来してい る。		
手法の感度 (検出限界)	検出限界は、研究課 題に取り組むのに十 分な割合のサンプル から化学物質を検出 するのに十分な低さ である。	NA	検出頻度が低すぎ て研究仮説に取り 組めない。		
バイオマーカー の安定性	既知の履歴と記録さ れた安定性データを 持つサンプル、また はリアルタイム測定 を使用しているサン プル。		プル、または目的 の分析物の安定性		
サンプルの汚染	サンプルは、収集時 から測定時まで無汚 染である(例えば、	これらの手順を使用 しない/記録しない ことを検討する。	汚染の問題が知ら れており、問題が 解決されたという		

r	A time of the time	I		
	分析物・無含有保証		記録はない。	
	の収集用品や標準物			
	質の使用、現場と実			
	験室の両方でのブラ			
	ンクの適切な使用な			
	ど)。研究には、研究			
	データの信頼性を保			
	証するために必要な			
	手順を記録したもの			
	が含まれる。			
玉汁の再供	<u>が占まれる。</u> バイオマーカーの明	高い信頼性と必要な	NIL-	
手法の要件		商い信頼住と必要な 感度でバイオマーカ		
	確な同定と定量を必		定量が可能なだけ	
	要な感度で提供する	ーの同定を可能にす	で、その手法が既	
	機器	る機器	知の干渉物質を持	
	(例:GC-HRMS、	(例:GC-MS、	っている機器	
	GC-MS/MS、LC-	$GC-ECD)_{\circ}$	(例:GC-FID、	
	MS/MS) 。		分光法)。	
マトリックス調	調整が必要な場合に	研究では、1 つの手	調整手法が確立さ	
整	は、調整済み濃度と	法(マトリックス調		
	非調整済み濃度の結	整済みか否か)を用	毛髪調整)。	
	果が含まれている。	いた結果のみを提供		
		している。		
研究デザインと実	行			
時系列性	ばく露と発現事象の	ばく露と発現事象の	ばく露と発現事象	
	間に確立された時間	間に時間的順序が確	の間に時間的順序	
	順序;ばく露と発現		が確立されていな	
	事象/再構成された	連するばく露範囲は	い研究。	
	ばく露の間の関連す	考慮されていない。		
	る間隔、及び関連す			
	るばく露範囲の適切			
	な考慮。			
ばく露変動と誤	なっ慮。    十分なサンプル数。	1 つ以上のサンプル	記主な老虐みポル	
分類	精度(例えば、感度			
	と特異度)と信頼性		奉ついたはく路。	
	(例えば、ICC)の	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	尺度を計算すること			
	によって考慮される			
	誤差。			
	1つのサンプルが使用			
	されている場合、1つ			
	の測定値からの誤差			
	が無視できる程度で			
	あるという証拠があ			
	る。			
一般的な疫学研究	『デザインの考察			
研究の論拠	先験的に定式化され	既存のサンプルやデ	あらかじめ指定さ	
	た仮説を評価するた	ータを用いて、先験	れた仮説を持たな	
	めに特別にデザイン	的に定式化された仮	いデータマイニン	
	された研究。	説を評価する研究。	グ研究;複数の同	
			時仮説検証。	
研究参加者	母集団ベースの不偏	集団ベースの不偏的	サンプル選択の方	
	的選択プロトコル;	選択プロトコル;応	法、応答率/脱落例	
	高い応答率または脱		率は報告されてい	
	同じ心合キよには肮	ロートで良い、みたは		

	落例が少ない。	脱落例が多い。	ない。	
データ分析	因果モデルと予測モ	外的因子を十分に考	外的因子の制御が	
	デルの明確な区別;	慮しているが、感度	不十分。	
	効果修飾の評価と交	分析は伴っていな		
	絡因子の調整を伴う	V ،		
	外的因子への十分な			
	配慮;感度分析。			
報告	研究は、その目的を	結論は保証されたよ		
	明確に述べ、読者が			
	検証された仮説の数	された仮説の数が不	された結果の方法	
	(結果が与えられた	明瞭である(明示さ	や選択に関して透	
	仮説の数だけではな	れていないか、判別	明性を欠いている	
	く)を評価できるよ	が困難である)、ま	研究。	
	うにする。複数の同	たは複数の検証を考		
	時仮説検証が行われ	慮していない。		
	る場合は、その影響			
	をなるべく PFP また			
	は FP:FN 比を推定			
	することで評価す			
	る。発現事象報告の			
	バイアスの証拠がな			
	く、結論は観察され			
	た結果を超えたもの			
	にはならない。			

AOP = 有害性転帰経路; FP = 偽陽性; FN = 偽陰性; GC-HRMS = ガスクロマトグラフィー/高分解能質量分析計; GC-MS = ガスクロマトグラフィー/質量分析; GC-ECD = ガスクロマトグラフィー電子捕足検出器; GC-FID = ガスクロマトグラフィー-炎イオン化検出器、ICC = クラス内相関係数; NA= 非該当; PFP = 偽陽性確率

以下 198ページ

これらについては、以下のセクションで詳しく説明し、それぞれの問題について例を示す。機器 のトピックのほとんどはばく露のバイオマーカーに関連するものであるが、効果のバイオマーカ ーについても関連性があれば記載している。

## 2. BEES-C 指標の使用

BEES-C 指標は、研究デザインの開発、助成金提案書のレビュー、論文原稿のピアレビュー、 WOE 評価の実行など、複数の目的に使用することができる。

#### 2.1. BEES-C の使用目的

BEES-C ツールの最終的な目標は、ヒトにおける短寿命化学物質の研究に関する文献全体の改善で研究者を支援することである。BEES-C は、以下のような目的で使用されることを意図したものではない: (i)研究者が仮説作成のための研究を行わないようにすること、(ii)低レベルの研究をWOE 評価に含めないようにすること。

どのようなタイプの検査機器でもそうであるように、評価プロセスには、階層化の観点からも、 また、検査機器のどの点が特定の研究に関連しているかを判断するためにも専門家の判断が必要 である。

以下の項では、BEES-C の主な特徴を事例を交えて説明する。ここでは、BEES-C を利用する ための推奨事項について述べる。BEES-C は、ばく露のバイオマーカーと何らかの効果指標 (例:効果のバイオマーカー、医師による診断疾患)との関連性に関する疫学研究の一部である ヒトのバイオモニタリング研究に適用されるが、他の目的(例:時間的または空間的傾向分析のためのばく露評価)のために計画されたヒトのバイオモニタリング研究には、BEES-C指標の一部のみが適用される。

#### 2.2. BEES-C の利用方法

表1は、研究デザインの側面(行)と評価段階(列)に沿って整理されている。審査対象の各 研究について、重要な側面が行ごとに評価され、該当するセルは色分けされている(図1)。こ れにより、研究者/査読者は研究の質の全体像を把握することができる。この指標の使用者は、 各決定に対して正当な根拠を示す必要がある(表1);これはプロセスの透明性を高めることに なる。BEES-C 指標は以下のように使用される: (i)提案された研究デザインを評価する研究 者が、研究の質が最大化されていることを確認するための手段として使用、(ii)研究の質を体 系的に評価し、質を向上させることができる分野を特定するために、論文や出版物の査読者が使 用、(iii)意思決定(例:規制基準の策定に使用するには十分に質の高い研究か?研究はメタ解 析に含まれるか?)を通知する手段として研究の質を評価するためにシステマティックレビュー を実行している者が使用、(iv) BEES-C を現在の既存のレビュースキームに組み入れたいと望 む者が使用。例えば、短寿命化学物質に特異的に適用可能な我々の提案するアプローチの問題点 の多くは、OHAT (Office of Health Assessment and Translation Approach)の草案(NTP、 2013 年)にはまだ含まれていないが、「環境化学物質、天然物質、または混合物(総称して 「物質」と呼ばれる)が有害性健康影響を引き起こすという証拠を評価するための文献ベースの 評価」を実行するためのアプローチに組み込まれる可能性がある。

本研究の品質評価指標には、以下の各項目について、原稿または企画書で明示的に報告するこ とが暗黙の了解となっている。言い換えれば、その研究が特定のレベルの基準を満たしているか どうかを評価するためには、その問題に関する情報が明確に記述されていなければならないとい うことである。以前に発表されたバイオモニタリングデータ(例:米国国民健康栄養調査 (NHANES))に依存している研究については、同じ報告要件を満たさなければならない。著 者は、研究の検出限界、相対標準偏差、関連する品質管理パラメータなどの関係がある項目を含 め、方法の記述を明確にすべきである。

このプロセスに数値スコアリングがないのは意図的なものである。ほとんどの構成要素につい ては質の高い研究であるにもかかわらず、研究結果の信憑性を大幅に低下させるような重要な問 題に対処していない場合があることは間違いない。全体的に高い「スコア」は、この問題を覆い 隠す。その代わりに、柔軟性を高める質的アプローチを提案する。

補足:評価のすべての側面でTier1に分類される研究は知られていない。すべての側面でTier 1 に分類される研究は確かに目標であり、強固なデータを提供することになるが、ほとんどの研 究はTier2 またはTier3と考えられる側面を含むことになる。研究データに対する利用者の意 図にもよるが、評価上の問題点によっては問題にならない場合もある。一方、Tier3の指定によ り、その研究の有用性が低いと判断される問題もある(例:サンプルに汚染がないことを証明で きない)。

#### 3. BEES-C の構成要素

最初に、短寿命バイオマーカーに関連した BEES-C の構成要素について説明する。これに続いて、より一般的な疫学研究デザインの問題に関連する BEES-C の側面について述べる。 3.1. バイオマーカーの選択と測定

バイオマーカー/生物学的マーカーは、「生物学的システムにおける変化や事象の指標」と定 義されている。「ばく露の生物学的マーカーとは、組織、細胞、または体内液などの生物学的媒 体から得られる細胞状、生化学的、分析的、または分子的な測定値を指し、薬剤へのばく露を示 すものである」(Zartarian ら、2005 年)。このように、バイオマーカーは、体内の化学物質や その代謝物の量を測定することで、化学物質へのばく露を評価するために使用することができる。 さらに、バイオマーカーは健康影響の指標として使用することができる。ばく露と効果のバイオ マーカーの多くは短寿命であり、環境化学物質へのばく露と環境化学物質による健康影響に関するヒトの研究では、両方のタイプのバイオマーカーが一般的に使用されている。この評価ツールは主にばく露のバイオマーカーに焦点を当てているが、ここで明らかにした原則の多くは効果のバイオマーカーにも適用される。

一般的なルールとして、ばく露と健康影響との関連を観察するように設計された研究は、適切 に確立されたバイオマーカーをばく露または健康のエンドポイントの代替として使用される場合 において、より正当性が高まる。バイオマーカーが高品質とみなされるために満たすべき基準に ついては、一般的なコンセンサスが得られている(NRC、2006 年; Zelenka ら、2011 年)。こ れらの基準の中には、バイオマーカーに固有の特性(例えば、化学物質ばく露との関連性や生物 学的関連性)に基づくものもある。その他の基準は、バイオマーカーの測定に関するもので、す なわちバイオマーカーを定量するために使用される方法の精度と正確さ、保存中のバイオマーカ ーの安定性、バイオマーカーの定量に誤差をもたらすサンプル汚染の可能性、測定誤差を引き起 こす可能性のある生物学的マトリックスの効果を調整する必要性などがある。バイオマーカーの 選択と測定の重要な側面については以下のサブセクションで説明し、BEES-C のために提案して いる階層化スキームを表1に示す。

#### 3.1.1. 関連性

化学物質の生成からヒトへの接触、目標の投与量、ならびにその後の分子、細胞、器官、個体、 集団での反応までの経路を示すために発生源から結果までの連続性が頻繁に使用される。

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	研究 1			仮想研究 2
研究評価の構成要素	TIER 1	TIER 2	TIER 3	研究評価の構成要素 TIER 1 TIER 2 TIER 3
バイオマーカーの選択と測	定	•	•	バイオマーカーの選択と測定
生物学的関連性				生物学的関連性
ばく露バイオマーカー				ばく露バイオマーカー
効果バイオマーカー				効果バイオマーカー
特異度				特異度
手法の感度				手法の感度
バイオマーカーの安定性				バイオマーカーの安定性
サンプル汚染				サンプル汚染
手法の要件				手法の要件
マトリックス調整				マトリックス調整
研究デザインと実行				研究デザインと実行
時系列性				時系列性
ばく露変動と誤分類				ばく露変動と誤分類
一般的な疫学研究デザイン	の考察	•		一般的な疫学研究デザインの考察
研究の論拠				研究の論拠
研究参加者				研究参加者
報告				報告
データ分析		2		

図 1. BEES-C 指標を用いてバイオモニターされた短寿命化学物質の 2 つの仮想研究についての品質比較の例。各 仮想研究のレビューでは、重要な側面が行ごとに評価され、適切なセルが色分けされているため、研究者/査読者 は研究の質の全体像を把握することができる。セル内のテキストは読みやすくするために削除されている。

またバイオマーカーは、ばく露、用量、及び生物学的反応を経験的に特徴付ける手段として使用 されることがある。本セクションでは、ばく露のバイオマーカー(すなわち、標的における親化 合物、代謝物、相互作用生成物(WHO、2001 年))と効果のバイオマーカー(すなわち、測定 可能な生化学的または生理学的反応で、健康影響に関連するもの(WHO、2001 年))の両方を、 ばく露と健康影響との関連性に関する疫学研究の重要な構成要素として検討する。

3.1.1.1. ばく露のバイオマーカー 疫学研究は、仮説駆動型の研究であってもよいし、より仮説

生成に向けた研究であってもよい。後者の場合、ばく露の最も適切なバイオマーカーは、外部ば く露または内部ばく露の正確で精密な代替となるものである。強固な生物学的根拠が存在し、生 物学的な「標的」が分かっている場合、最も適したバイオマーカーは、標的(分子、細胞、また は臓器レベル)で直接測定されるもの、または目標の用量を正確かつ精密に代替するものである。

理想的には、疫学研究で使用されるバイオマーカーについて、ばく露、用量、バイオマーカー のレベル間の定量的な関連性を明確に理解していることが必要である。標的組織のサンプリング の侵襲性を考慮すると、ほとんどのバイオマーカーベースの疫学研究では、血液、尿、毛髪、ま たはその他の容易にアクセス可能なマトリックスのサンプルを利用している。これらのマトリッ クスからのバイオマーカー測定値とばく露/用量のレベルとの間の定量的関係を解明するには、 化学物質の吸収、分布、代謝、排泄(ADME)の理解が必要である;これらのプロセスは、しば しば薬物動態(PK)モデルまたは生理学的薬物動態(PBPK)モデルを用いて記述される。疫 学研究でバイオマーカーを使用する前に、ADME に影響を与える可能性のある内的要因(例え ば、遺伝、ライフステージ、妊娠、性別)と外的要因(例えば、食事、薬、病状)と同様に、化 学物質の ADME についてもしっかりと理解しておく必要がある。さらに、短寿命のバイオマー カーについては、サンプル採取に関連して、特定のタイミングの詳細(例えば、時間帯、食事ば く露に関連する化学物質については最後の食事をしてからの時間、最後の尿を排泄してからの時 間)を知ることが重要である。理想的には、バイオマーカー濃度とばく露/用量のレベルとの関 係、及び疫学研究でバイオマーカーを使用する前に、これらの関係に対する内的、外的、タイミ ングの要因の影響を徹底的に評価する。(ばく露/用量に関する)バイオマーカーを適切に解釈 するために必要な重要情報は、その後、研究の一部として収集され、慎重に評価されるべきであ る。ばく露の各バイオマーカーの経費と利益は、疫学評価の一部として慎重に検討され、解釈さ れるべきである。

マトリックスの選択はばく露研究や疫学研究に不可欠な要素であり、測定能力、汚染の問題、 ばく露や健康影響と標的分析物との関連性など、複数の要因を考慮しなければならないことに注 意することが重要である。BEES-Cでは、これらの問題を個別に扱う。

3.1.1.1.1. 短寿命化学物質の例 ビスフェノール A (BPA) は、遊離形 (親化合物)、硫酸抱 合体またはグルクロン酸抱合体、または遊離形と抱合体の組み合わせ(総 BPA)として尿中に 存在する(Harthé ら、2012年; LaKind ら、2012年 a; Völkel ら、2008年; Ye ら、2005年)。 最近のいくつかの研究では、BPA ばく露に関連した内分泌関連の健康影響が調査されている。 最も生物学的に有用性の高いバイオマーカーは遊離(親化合物)BPA であるが、これは親化合 物 BPA のみがエストロゲン活性を有すると考えられているためである(EPA、2013年; WHO、 2011 年)。尿中の遊離 BPA の定量は、非抱合体の BPA がごく一部しか存在しないため、分析 が困難である(Ye ら、2005年)。この限界を考えると、抱合体または総 BPA の測定が遊離 BPAの有用な代替となる可能性がある。特に、個人内と個々人の間での遊離 BPAと抱合体 BPA の比率にばらつきが少ない場合(ばく露レベルのばらつきに対して)、抱合体 BPA または総 BPA は、遊離 BPA 及び一般的な BPA ばく露での正確で精密な代替となりうる。この事例は、 ばく露とバイオマーカー、異なるタイプのバイオマーカー(親化合物と代謝物のマトリックス)、 バイオマーカーと生物学的標的の関係を理解することの重要性を強調している。さらに、個々の ばく露のバイオマーカーを選択する際には、トレードオフの可能性があることを強調している (BPA の場合、生物学的関連性を最適化するために、化学物質の検出能力を犠牲にすることが ある)。

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3.1.1.1.2. 研究の評価(表 1) 標的マトリックス中のばく露の Tier 1 バイオマーカーは、目標用量(既知の標的がある仮説駆動型の研究の場合)または外部ばく露(既知の標的がない研究の場合)の正確で精密な代替である。Tier 2 のバイオマーカーについては、特定マトリックス中のバイオマーカーと外部ばく露、内部ばく露、または目標用量との間に関係があることを示す証拠が存在する。標的マトリックス中の Tier 3 バイオマーカーは、ばく露/用量の低い代替(精度と正確さが低い)である。

3.1.1.2. 効果のバイオマーカー 疫学研究では、短期的なバイオマーカー測定値と長期的な健康 影響の意味のある比較することが困難な場合がある。特に横断的な研究では、現在のバイオマー カーレベルが疾患発症に関連する時間帯の過去のばく露を再現していることが重要な前提となる。 効果のバイオマーカーのレベルは、疾患発症前の重要な時間帯に、対象となる集団におけるばく 露と反応の関係を評価する手段となる。調査結果は、ばく露のバイオマーカーとばく露効果、及 びばく露効果のバイオマーカーと有害性健康影響の関連性の強さに基づいて解釈される。

ばく露事象から健康への有害性影響への進行は、有害性転帰経路(AOP)を用いて定義するこ とができる(Ankley ら、2010年)。特定の健康影響の AOP は、体内の標的における分子的な 開始イベントから始まる。分子レベルでの効果は、ばく露イベントによって開始され、細胞レベ ル、体内液レベル、臓器レベルでの効果へと進行し、最終的には生体全体へと効果を及ぼすこと になる。"キーイベント"とは、AOP に沿った中間的なステップであり、AOP に沿った進行を評 価するために実験的にモニターすることができる。これらの重要な事象を、生体から採取可能な 媒体を用いて測定することを、バイオマーカーと呼ぶ。バイオマーカーは、特定の有害性発現事 象に関連する生物学的機能を反映しているため、理想的な効果をもつバイオマーカーと考えられ、 「個人または集団における潜在的有害性影響の予測で信頼性が高い」 (www.epa.gov/pesticides/science/biomarker.html)とされている。一方、「未確定の結果」に 分類される効果のバイオマーカーは、変化を特定疾患の発現事象への結びつける経路があまり確 実ではないことを反映している(www.epa.gov/pesticides/science/biomarker.html))。したがっ て、バイオ指標の代わりにこれらのバイオマーカーを使用した場合、個人または集団の発現事象 の予測は、確実性が低くなる。

3.1.1.2.1 研究の評価(表1) Tier1効果のバイオマーカーは、AOPにおける重要なイベントのバイオ指標である。Tier2の効果のバイオマーカーは、健康影響との関係があることが示されているが、作用機序は解明されていない。結果が不確定な効果のバイオマーカーは、Tier3と考えられる。

### 3.1.2. 特異度

単一のばく露のバイオマーカーが複数の親化合物に由来する場合があり、親化合物へのばく露 の評価は困難である(Barr and Needham、2002年; Barr ら、1999年、2006年)。ばく露評価 や疫学研究の解釈において、親化合物の毒性や作用機序が異なる場合に特に問題となる。さらに、 親化合物へのばく露評価を阻害する事例として、代謝物の一つが環境中にも存在する場合(外的 ソース)がある。

3.1.2.1. 短寿命化学物質の例 3-フェノキシ安息香酸(3PBA)は、研究の質を評価する際に特異度の評価の重要性を強調する短寿命化学物質の一例である。3PBAは、少なくとも18種類の合成ピレスロイドの代謝物であり(Barr ら、2010年; Leng ら、1997年)、3PBA環境分解物である3-フェノキシベンジルアルコールの潜在的な代謝物でもある。尿中3PBA測定は、神経毒性が知られていない環境分解物へのばく露に加えて、神経毒性の程度が異なる複数の殺虫剤へのばく露を表している(Barr ら、2010年)。尿中3PBA測定は、ピレスロイドへのばく露の保守的な推定値を提供することができるが、追加のばく露データがない場合、ピレスロイド系殺虫剤へのばく露に関連した神経毒性作用を正確に推定することはできない。神経毒性とばく露の関係を明らかにすることは、真のばく露量が不明であるため、より困難であると考えられる。

3.1.2.2. 研究の評価(表 1) Tier 1 研究には、1 つの親化合物へのばく露に由来するばく露のバイオマーカーが含まれる。Tier 2 研究には、同様の種類の有害性エンドポイントを持つ複数の親化合物に由来するバイオマーカーが含まれる。Tier 3 研究には、有害性エンドポイントの種類が異なる複数の親化合物に由来するバイオマーカーが含まれる。

### 3.1.3. 手法の感度

バイオマーカーは、分析対象のマトリックス中に評価できるほどに存在しなければならない

(Calafat and Needham, 2008 年)。ばく露に関係なく、マトリックス中で頻繁に検出されない バイオマーカーは、結果の有用性が限定的となる可能性があるため、環境疫学研究では望ましく ない。

3.1.3.1. 短寿命化学物質の例 4 つ以上の環を持ついくつかの多環芳香族炭化水素(PAH) は、 ヒトでの発がん性が疑われているか、または既知の発がん性物質である(例:benzo[a]pyrene)。 標準的な分析法(例えば、GC-MS [ガスクロマトグラフィー/質量分析]または LC-MS/MS [液体 クロマトグラフィー/タンデム質量分析]) は、採取可能な媒体(例えば、尿)中でのこれらの PAH の代謝物を定量するのに十分な感度を持っていないことが多く(Bouchard and Viau、 1997 年)、疫学調査の妨げとなっている。ナフタレン、フェナントレン及びピレンを含む低分 子量の PAH のバイオマーカーは、より高分子量の発がん性化学種の代替として評価されている (Bouchard ら、1998 年;Sobus ら、2009 年;Viau ら、1999 年;Withey ら、1991 年)。こ れらの代替物質は分析上の限界を克服する手段を提供するが、標的化学種のばく露を反映する能 力、PAH 間の共起を評価する能力、ばく露ソースの相関関係に関する情報を評価する能力につ いて十分に評価されなければならない。

3.1.3.2. 研究の評価(表 1) Tier 1 研究手法は、研究の問題に対処するために十分な割合のサンプルから化学物質を検出するのに十分な検出限界を持っている(例えば、研究仮説が母集団濃度の代表値と上端の両方の推定を必要とする場合、50~60%の検出値を有する) (Barr ら、2010年; Zota ら、2014年)。この要素はTier 2 にはない。Tier 3 の研究では、検出頻度が低すぎて研究仮説に対応できない。

#### 3.1.4. バイオマーカーの安定性

バイオマーカーは、保存及び使用の時間にわたって、所定のマトリックス中で安定でなければ ならない(Barr ら、2005 年 a)。サンプルの安定性は記録されるべきである。凍結/融解サイク ルを経たサンプルを用いた研究では、それらのサンプルの安定性を実証すべきである。試料の採 取から測定までの時間を記録すべきである。

3.1.4.1. 短寿命化学物質の例 難分解性有機汚染物質は、通常、-20℃以下で凍結した場合、血液 製剤中では永久に安定であるが、難分解性でない化学物質は血液中での安定性が低い場合がある。 例えば、現在使用されている殺虫剤は非常に反応性が高く、血液中の酵素で容易に分解される (Barr ら、1999 年)。EDTA を事前に添加した血液は、エステラーゼ活性を最小限に抑えるが、 測定は採取後数ヶ月以内に行う必要がある。解凍/再凍結の過程や熱湯での試料の解凍も分解の 原因となる。長期間保存された尿または血液サンプルを使用することで、背景的に収集されたサ ンプル(NHANES III サンプルなど)のデータが得られるが、多くの場合、解凍/再凍結の過程 が発生し、敏感な化学物質の劣化やサンプル自体の汚染が生じる可能性がある。背景的なサンプ ルの安定性を確認するために、1 つのサンプルを小量で複数に等分して保存する必要がある。バ イオマーカーの損失は、容器の壁との結合や揮発によっても発生する可能性がある。

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プラスチック容器はガラスに比べて安価で取り扱いや凍結が容易であるが、一部の化学物質の汚 染源になることがある。また、金属や有機化合物を吸収してしまうため、化学物質の濃度を過小 評価してしまうこともある。保存中や解凍・凍結の過程で損失がないことを検証するために、実 際のサンプルに含まれると予想されるレベルと一致するレベルのスパイクされたマトリックスを 使用した保存研究や、保存前のサンプルに安定同位体標識された化合物を添加することが必要で ある。

3.1.4.2. 研究の評価(表 1) Tier 1 研究には、既知の履歴と記録された安定性データを持つサンプルが含まれる。Tier 2 の研究では、保存中の損失は判明しているが、低量ばく露と高量ばく 露の違いを定性的に評価することができる(すなわち、研究の目的上、試験参加者を低量ばく露 か高量ばく露かのどちらかに分類することは十分に可能である)。Tier 3の研究では、目的とする分析物の履歴が不明なサンプル、または安定性データがないサンプルを使用している。

#### 3.1.5. 試料の汚染

この BEES・C 評価基準は、至る所に存在する(ユビキタス)短寿命化学物質を測定する研究を 評価する上で最も重要な基準の一つである。これは、汚染を避けるために多大な時間をかけてい るにもかかわらず、これらの化学物質の多くについて、採取時から測定時までの間に試料が汚染 される可能性があることが実証されているからである。生理的半減期の短い化学物質は、環境的 に至る所に存在するもの(ユビキタス)であるだけでなく、疫学研究で使用されるサンプリング や分析機器にも含まれている可能性がある。したがって、試料採取から試料測定までの研究のす べての段階において試料汚染を回避/防止するためには、細心の注意が必要である(Barr ら、 1999年; Calafat and Needham、2008年、2009年; Needham ら、2007年)。試料採取中に、 標的の化学物質を含む物や、採取材料やマトリックスを環境媒体(空気や水など)にさらすと、 測定された濃度が誤って上昇する可能性がある。予防措置を講じたとしても、研究では分析物の 汚染が問題であることが報告されており、研究結果の解釈に不確実性をもたらしている。

3.1.5.1. 短寿命化学物質の例 Ye ら(2013 年)は、最善の努力をしたにもかかわらず、米国疾 病予防管理センター(Centers for Disease Control Prevention)の研究室のサンプルがトリクロ サンで汚染されていたことを指摘している。同様に、いくつかの研究グループは、このような汚 染を避けるために細心の注意を払っているにもかかわらず、(溶剤や試薬を含む)汚染が原因で、 血液サンプル中の BPA を測定することが困難であったことを指摘している(Calafat ら、2013 年、Markham ら、2010年、Teeguarden ら、2011年、Ye ら、2013年)。

3.1.5.2. 研究の評価(表 1) Tier 1 研究では、サンプルが収集時から測定時まで汚染されてい ないことを保証する(例えば、分析物を含まないと保証された収集用品や標準物質を使用したり、 現場と実験室の両方でブランクを適切に使用したりする)。研究では、研究データが信頼性が高 く正確であることを保証するために必要な手順を記録したものを含む。これらの手順を使用して いない、または記録していない研究は Tier 2 に分類される。Tier 3 の研究では、既知の汚染問題 があり、その問題に対処したという記録がない。

#### 3.1.6. 手法の要件

ばく露を評価するためのバイオマーカーの品質は、測定に使用される手法の質に大きく依存す る。これは、バイオマーカー測定の評価が難しい側面となる。例えば、習熟度テスト練習への研 究室参加と成果は、Tier 1 研究のための合理的なテストのように見えるかもしれないが、多くの 習熟度テスト研究では、200%変動する可能性のある許容範囲がある(すなわち、「許容できる」 分析物濃度値は、真の値の±200%になる)。一般的に、研究手法は適切な機器を備え、付随す る手順(例えば、QC、手法の強固性、確認イオンの存在、同位体希釈の使用)を記述しておく 必要がある。

3.1.6.1. 研究の評価(表 1) Tier 1 研究では、バイオマーカーの明確な同定と定量を必要な感度で提供する機器が含まれている(例:GC-HRMS [ガスクロマトグラフィー/高分解能質量分析]、GC-MS/MS、LC-MS/MS)。Tier 2 研究では、高度な信頼性と必要な感度でバイオマーカーの同定を可能にする機器を使用する(例:GC-MS、GC-ECD [ガスクロマトグラフィー・電子捕足検出器])。Tier 3 の研究では、バイオマーカーの定量化のみを可能にする機器を使用するが、その手法には既知の干渉物質が含まれている(例えば、GC-FID [ガスクロマトグラフィー・炎イオン化検出器]、分光器)。

#### 3.1.7. マトリックスの調整

バイオマーカーは濃度の単位で最も一般的に測定され、報告される。尿のバイオマーカー濃度 は、尿量変動(食事、運動、水分補給、年齢、病状などによって引き起こされる)によって強く 影響を受け、血液中のバイオマーカー濃度は、血液量と脂肪含量によって強く影響を受ける。尿 のバイオマーカー濃度は、クレアチニン濃度(筋肉内でのクレアチン・リン酸分解に由来する)、 比重、尿量、及びその他の手法を用いて、被験者間及び被験者内で尿希釈の変動を補正するため に標準化されている。しかし、補助情報のないスポット試料の未補正の尿中濃度は、一般に報告 され、ばく露や健康影響との関係の評価に利用される(Barr ら、2005 年 b; LaKind and Naiman、2008 年、2011 年; Lorber ら、2011 年; Meeker ら、2005 年)。尿希釈の変動に対す る尿のバイオマーカー測定値を「補正」するための最良の手法については、現在のところコンセ ンサスはない。最低限、研究間での適切な比較を可能にするために、容量ベースの濃度と補正さ れた(クレアチニンまたは他の手法)濃度の両方が提供されるべきである。また、全排尿量と排 尿間隔時間を取得することも有益である。

血液ベースのバイオマーカーレベルは、全血、血清、血漿、及び脂質調整値として報告されて いる。脂質調整を決定するために使用した手法、または血液中の脂質の異なる成分を分離するた めに使用した手法を提供し、入手可能な場合には、すべての濃度を報告すべきである(例えば、 全体量及び脂質調整値)。同様に、親油性化学物質の測定における絶食サンプル及び血清脂質調 整に関連する問題も考慮しなければならない(Schisterman ら、2005 年)。脂質やその他の組 織成分の調整の妥当性は、現在使用されている農薬のような特定の短寿命化学物質については確 立されていない。このような場合には、調整の妥当性が確立されていないことを明記して、全量 濃度と調整濃度を報告すべきである。さらに、血漿量は妊娠中に増加し(また、いくつかの既往 症や健康状態でも増加する可能性がある)、妊娠前や集団間で血漿濃度を比較する際にも考慮す る必要があるかもしれない(Hytten、1985 年)。

サンプル収集要件とマトリックス処理に関する情報は、研究間のデータを比較したり、基準範囲と比較したりする際に重要である。異なる政府機関(例えば、欧州連合、特定の欧州諸国、米国NHANES、カナダ健康測定調査、欧州規模でヒト・バイオモニタリングを実行する共同体、州ベースのHANES)及び他の大規模なバイオモニタリングデータ収集による研究では、マトリックス及び報告されたバイオマーカー濃度を変化させる可能性のあるサンプルの収集及び処理のための異なるプロトコルを持っている可能性がある。例えば、サンプル採取前の絶食についての参加者への指示は、血液中の脂質含有量を最小化することができるため、サンプル中の親油性バイオマーカー濃度を最小化することができる(Barr ら、2005年 a)が、これらの指示は国によって必ずしも同じではない(LaKind ら、2012年 a)。同様に、朝の起床直後の尿では、単純なスポットサンプルよりもマトリックス成分の濃度が高い可能性があり、分析対象物を検出または識別する能力が変化する可能性がある(Kissel ら、2005年; Scher ら、2007年)。

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さらに、朝の起床直後の尿収集は、以前のばく露とサンプルの収集・測定の間の関係によってデ ータにバイアス(システマティックエラー)をもたらす。抗凝固剤として EDTA とヘパリンを 用いて採取した血漿は、マトリックスの特性を変化させる可能性がある(Barr ら、2005 年 a)。 採取要件及びサンプル処理の違い(及びバイオマーカー濃度に影響を与える可能性のある腎臓病 などの研究参加者の健康状態)は、研究間で結果を比較する際に報告され、考慮され、それに応 じて重み付けされる必要がある。

3.1.7.1. 研究の評価(表 1) マトリックス調整のための最良の手法は、調査する仮説と目的の 特定化学物質が密接に関連していることであるが、この分野でのコンセンサスはまだ得られてい ないことを認識している。ただし、調整は研究結果に大きな影響を与える可能性がある。したが って、Tier 1 研究では、調整濃度と非調整濃度(調整が必要な場合)の結果を提供し、それによ ってマトリックス調整の影響について読者が独自の結論に達することができるようにすることを 提案する。Tier 2 研究は、1 つの手法(マトリックス調整の有無にかかわらず)を用いた結果の みを提示する研究である。Tier 3 の研究には、有効な調整手法がまだ確立されていないマトリッ クス中の化学物質の測定が含まれている。 3.2. 研究デザインと実行

研究デザイン、ばく露のばらつきと誤分類の両方を考慮することは、短寿命化学物質では特に 重要である。

3.2.1. 疫学研究のデザイン

短寿命化学物質に関するバイオモニタリングデータと疾患との関連性を探る研究は、バイオマ ーカーの血中または尿中のレベルが、通常数時間前または数日前に発生した最近のばく露を再現 しており、バイオマーカーのサンプル採取に関連したばく露のタイミングは通常知られていない ため、独特の問題を抱えている。しかし、目的の健康影響の多くは、発症に数年から数十年を要 する慢性疾患(例:肥満、高血圧、生殖機能の測定値)である。このため、短寿命化学物質を測 定する研究における因果関係仮説の評価は複雑であり、状況によっては実行不可能な場合もある。 因果関係の決定的で異論のない特性は時系列性であり、発現事象に先行する因果ばく露の観察に よって裏付けられなければならないということである(Potischman and Weed、1999 年; Rothman and Greenland、2005 年; Weed、1997 年; Weed and Gorelic、1996 年)。

時系列性を確立することができるのは、発症時の新たな疾患症例や、ベースラインと比較した 健康関連指標の変化など、健康関連のイベントを特定する「発生率」研究のみである(Pearce、 2012 年)。発生率研究には、介入研究(例:臨床試験)と観察研究(コホート研究や症例を確 認する症例対照研究)がある。しかし、どのようなデザインであっても、発生率研究の主な特徴 は、疾病の発症時期(または少なくとも診断時期)を特定できることであり、それによってばく 露と発現事象の順序を評価できる可能性がある。ばく露レベルが時間の経過とともに急速に変化 する場合、有用なアプローチは、ばく露の反復測定値と健康バイオマーカーの反復測定値との関 係を評価する縦断研究である。

因果関係を評価するためには時間的関係を確立する能力が重要であるが、環境疫学研究ではば く露と研究の発現事象との間の間隔が別の研究デザインの問題となっている。ヒトのバイオモニ タリングデータを病因研究に利用するためには、ばく露は疾患発症に関連する時期に測定されな ければならない。これは簡単な作業ではないが、関連する時間帯に難分解性化学物質のばく露を 調査し、それらのばく露と特定の有害性発現事象の発生とを相関させたバイオモニタリング研究 の成功例がある。

例えば、鉛の血中濃度は、過去 5~6 週間のばく露を反映しており、適切に実行された疫学研 究では、子供の血中濃度と認知能力への有害性影響を関連付けることができた(Lanphear ら、 2000 年)。しかし、半減期の短い化学物質の場合、関連するばく露と疾患発症の間の間隔を評 価することは困難である。研究デザインは、後述するばく露の誤分類とともに、短寿命化学物質 のバイオモニタリング研究において最も重要であり、まだ十分に調査されていない側面である。

「有病率」研究では、「罹患」研究に比べて時系列性の立証が困難であり、因果関係についての 結論を出すことが困難である。典型的な有病率調査は、ばく露と疾病情報を同時に把握する横断 的デザインに依存している(Rothman and Greenland、1998年)。短寿命化学物質に焦点を当 てた研究の場合、多くの症例対照研究は、たとえ症例を用いていたとしても、バイオマーカーレ ベルが病気の発症に先行するのではなく、最近のばく露を反映しているため、解釈は困難である。 特筆すべき例外は、コホート内症例対照研究やケース・コホート研究で行われているように、将 来の使用のために採取したサンプルを使用する研究である(Gordis、2008年)。

3.2.1.1. 短寿命化学物質の例 フタル酸代謝物と肥満、糖尿病、心血管疾患との関連性に関する 最近の疫学文献のレビュー(Goodman ら、2014 年)において、研究の大部分は横断的デザイン であった。研究結果は発現事象の間で一貫性がなく、フタル酸代謝物への事前ばく露とそれに伴 う健康影響との関係を識別する能力における重要な制限要因として、時系列性の欠如が考えられ た。

3.2.1.2. 研究の評価(表 1) Tier 1 研究は、追跡期間または反復測定値の縦断分析を含む発生 率研究であり、ばく露と発現事象の間の時間順序と関連する間隔の両方が確立される(表 1)。 Tier 2 研究には、ばく露が発現事象に先行する発生率研究が含まれるが、ばく露の特定の関連範 囲は考慮されない。最も情報量の少ない(Tier 3)研究は、現在のばく露(例:化学物質の血中 レベル)と、急性ばく露ではなく慢性ばく露に関連している可能性が高くて頻繁に測定される発 現事象(例:BMI)との関連を検討する研究である(この評価基準は、集団内または集団間の時 間的または空間的な関係を調査する研究といったばく露のみに焦点を当てた研究には適用されな いことに注意する。)。

## 3.2.2. ばく露のばらつきと誤分類

多くの短寿命化学物質については、個人内での時間的変動が大きい場合があり、そのような化 学物質と疾患との間の関連を特定しようとすることは支持できない。個人の食生活、健康状態、 製品の使用、活動または場所の変化による短寿命化学物質のバイオモニターされたレベルの違い が予想される(Pleil and Sobus、2013 年)。Meeker ら(2013 年)が指摘しているように「ば く露測定基準における時間的変動を特徴づけることは、特に非難分解性化合物のバイオマーカー において、ばく露測定誤差の可能性に関連する疫学研究をデザインし解釈する上で重要なステッ プである。」

慢性ばく露または平均的ばく露を推定しようとする短寿命化学物質の多くの発表された研究は、 尿または血液の一回限りのサンプルを使用したばく露の一つの尺度に依存しているため、誤差が 生じる可能性がある(Goodman ら、2014 年; LaKind ら、2012 年 b、2014 年; Preau ら、2010 年; Wielgomas、2013 年)。バイオマーカーレベルの時間的変動を平均化するために、同じ個人 から異なる時間に複数のサンプルを採取することで、ばく露を推定する能力を向上させることが できる(NRC、2006 年)。信頼性は通常、クラス内相関係数(ICC)を計算することによって 測定される。ICC は、数時間、数日または数週間に渡って反復収集されたサンプル中の化学物質 を測定し、個々人の間の分散を総分散で割った値を計算することで推定できる。

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ICC の値は 0 から 1 の範囲であり、1 に等しいまたは 1 に近い ICC 値は、単一のサンプルから 集団の長期ばく露を推定する際の信頼性が高いことを示唆している。Symanski ら(1996 年) は、職業ばく露における安定しない行動を考慮するために混合効果モデルを使用し、ばく露にお ける系統的な変化が適切にモデル化されていない場合、分散成分(ICC の計算に使用される)の 推定値が大幅に偏る可能性があることを発見した。「もし ICC が数週間または数ヶ月間の反復サ ンプルを採取して作成された場合、その値は目的の多くの慢性疾患の発症の時間枠である数年間 のばく露に関連するか?」という疑問について提起されなければならない。この問題については、 標的の短寿命化学物質の多くについて現在のところ研究が進んでいない。

短寿命化学物質の単一の測定値を使用する場合のもう一つの問題は、ばく露の誤分類を引き起 こす可能性のあるエラーである。ばく露の誤分類は、割り当てられたばく露が実際のばく露レベ ルやカテゴリーを正しく再現していない場合に起こる。ばく露の誤分類は、方向性と大きさの両 方の観点から予測することが困難であることが示されている(Cantor ら、1992 年; Copeland ら、 1977 年; Copeland ら、1977 年; Dosemeci ら、1990 年; Sorahan and Gilthorpe、1994 年; Wacholder ら、1995 年)。ばく露誤差とばく露誤分類の用量反応関係への効果は問題である (Rhomberg ら、2011 年)。ばく露の誤分類は、重大なばく露が発生する際に関連するサンプ ル収集のタイミングを含め、測定誤差の多くのソースから発生する。例えば、多くの揮発性有機 化合物は半減期が数分のオーダーである。すなわちばく露は毎日発生しても、短い時間間隔で発 生する。このように、ばく露のバイオマーカーの濃度は、ばく露が発生した際に関連してサンプ ルが採取された時期に大きく依存しており、体内の長期的なレベルを適切に反映しない場合があ る。

複数のサンプルの使用または長時間(例:24 時間)のサンプル収集は、時間的変動、研究の 亜集団の特性、及びサンプルに関連する問題の効果を軽減することにより、誤差を減少させるの に役立つかもしれない(Scherら、2007年)。誤差が回避できない場合(例:利用可能なすべて のサンプルが絶食後に得られた場合)は、感度と特異度を計算することにより、ばく露特性の精 度を評価することが重要である(Jurek ら、2006 年)。感度とは、もしも個人が本当に高いば く露カテゴリーに属しているならば、高レベルのばく露を受けた個人と正しく分類できる確率で ある。特異度とは、本当に低レベルのばく露を受けた参加者を正しく低レベルばく露と割り当て る確率である。感度と特異度の推定値は、多くの測定値に対する真の感度と特異度は不明である ので、被験者ごとに複数のサンプルを絶対的基準として使用して1つの尿サンプルについて計算 される。これは、研究期間中に収集された各個人の反復サンプルの中から無作為に1つのサンプ ルを選択することによって実行される(2008 年に Adibi らによってフタル酸エステル類につい て実証されたように)。

3.2.2.1. 短寿命化学物質の例 Goodman ら (2014 年) は、フタル酸エステル類と肥満、糖尿病、 心血管疾患との関連性に関する疫学文献の最近のシステマティックレビューにおいて、利用可能 な 26 件の研究のうち、3 件を除くすべての研究がフタル酸エステル類の単一の測定値に依存し ていることを明らかにした。同様に、BPA と肥満、糖尿病、心血管疾患に関するシステマティ ックレビューでは、LaKind ら (2014 年) は、利用可能な 45 件の研究のうち、4 件を除くすべ ての研究が BPA の単一測定値に依存していたことを明らかにした。しかし、BPA の個人内変動 は大きく (ICC は 0.10~0.35 の範囲) (Lassen ら、2013 年; Teitelbaum ら、2008 年)、1 人 の長期ばく露を記述するには複数のばく露尺度が必要である。フタル酸エステル類の ICC は BPA よりも高いことが報告されているが (例えば、フタル酸モノエチルは 0.18~0.61、フタル 酸モノイソブチルは 0.21~0.51、フタル酸モノ (2-エチルへキシル) は 0.08~0.27 (Goodman ら、2014 年でレビュー)、個人間のばらつきは依然として大きい。最近、Attfield ら (2014 年) は、小児における尿中農薬測定値の変動性に関する研究において、各研究参加者からのサンプル 数が少ない研究では、「…不正確な四分位分類によるばく露の誤分類の可能性が高く、ばく露を 正確に特定のカテゴリーに分類するための保証はほとんどない」と述べた。

3.2.2.2. 研究の評価(表 1) 上記の考察により、利用可能な文献を以下の段階に分けることができる(表 1)。Tier 1 には、ばく露評価が、適切な期間にわたるばく露量を推定するために、 一人当たりのサンプル数が十分な数であるか、または適切な長期サンプリング(例えば、複数の 24 時間尿採取)を使用している研究が含まれている。Tier 1 に含まれるためには、研究は、精度(例:感度及び特異度)及び信頼性(例:ICC)の測定値を計算することによって誤差を評価 すべきである。化学物質によっては、ばく露を完全に特徴づけるには 1 つのサンプルで十分であ る可能性がある。このような場合、Tier 1 研究では、単一の測定の誤差が十分に小さいと考えら れるという証拠を提供する必要がある。これは常に実現可能なことではないが、研究者がバリデ ーション研究を実行する必要があると考える状況があることは理解している(Teeguarden ら、 2011年)。Tier 2 には、2 つ以上のサンプルを使用する研究が含まれるが、測定数の選択に根拠 を示さず、誤差の明示的な評価も含まれていない。Tier 3 は、誤差を考慮せずに単一のサンプル に基づいてばく露評価を行う研究のためにある。

### 3.3. 一般的な疫学研究デザインの考察

このセクションでは、必ずしも短寿命化学物質に特化したものではないが、研究全体の質を評価する上で重要な研究デザインの側面について議論する。これらの問題のいくつかは、ばく露と 健康影響との関連性を検討する研究に適用できるものもあれば、ばく露のみに焦点を当てた研究 に適用できるものもある。

#### 3.3.1. 研究の論拠

このセクションでは、バイオモニタリングデータと健康影響データの間の関連性を検証する仮 説検証研究を述べる。臨床観察または基礎科学実験から生じる十分に練られた仮説は、研究の種 類にかかわらず、疫学調査の基礎となるものである(Boet ら、2012 年; Fisher and Wood、2007 年; Moher and Tricco、2008 年)。現在、様々な分野で推奨されているのは、目的の集団、調査 対象のばく露(またはそれに対応するマーカー)及び懸念される結果に関する情報を伝えるよう に構成された調査課題を提起することの重要性を強調している(Sampson ら、2009 年; Walker ら、2012 年)。

バイオモニタリング研究、特に1つのサンプルが多数の化学物質に関するデータを提供できる

ような短寿命化学物質を対象とした研究では、多くの場合、複数の変数を含むデータを生成し、 複数の仮説検証を同時に行う機会を得ることができる。バイオモニタリング研究のこの特徴は、 いくつかの関連する発現事象に対して有意な関連性が観察された場合(Lord ら、2004 年)のよ うな状況では、強みとみなすことができる。例えば、仮説を立てられた肥満因子が体重指数、ウ エスト周囲長、または体脂肪率に同様の影響を及ぼす場合である。一方で、複数のばく露-発現 事象の関連を評価する能力は、特に過去に収集されたデータを扱う場合、結果の解釈を複雑にす る(Clarke ら、2003 年; Lee and Huang、2005 年; Marco and Larkin、2000 年)。過去に収集 されたデータを使用した研究の中で、優先的に定式化された仮説に導かれた研究と、強い生物学 的根拠を持たずに実行された研究を区別することが重要であるが、後者のカテゴリーは新しい仮 説の策定に役立つことが証明されている(Liekens ら、2011 年; Oquendo ら、2012 年)。仮説 が十分に定式化されている研究は、その研究がこれまでの知識の上に築かれていることを示して おり、これは WOE 評価にとって重要な考慮事項である。既存の知識ベースに追加するために特 別にデザインされた研究は、より容易に WOE に組み込むことができる。

3.3.1.1 研究の評価(表1) 優先的に定式化された仮説を、この仮説に対処するために特別に 設計されたバイオモニタリング戦略で評価する研究は、最高品質(Tier 1)と考えるべきである。 Tier 2 の研究とは、優先的に定式化された仮説を評価するために既存のサンプルやデータを使用 した研究であり、この目的のために特別にデザインされたバイオモニタリング戦略ではない。

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Tier 3 の研究では、事前に定められた仮説を持たずに既存のサンプルやデータに依存する研究または複数の同時仮説検証を含む。現在のところ、短寿命化学物質を対象としたほとんどのバイオモニタリング研究の研究理論拠は、Tier 3 研究として記述されることになると認識している。

## 3.3.2. 研究参加者

研究参加者の選択に関する評価方法は、難分解性化学物質と短寿命化学物質の両方の研究に適用される。疫学研究における参加者選択の目的は、サンプルから得られる情報と対象となる集団について求められる情報との間を「仲立」することである(Kalsbeek and Heiss、2000 年)。 偏りのない集団サンプルを選択する実際のプロセスは、症例対照研究、縦断(コホート)研究、 横断研究において継続的な問題である(Vandenbroucke ら、2007 年)。

参加者選択の問題は、短寿命化学物質の疫学研究に特有のものではない。しかし、バイオモニ タリング研究はこの問題に十分な注意を払っていない。これまでのバイオモニタリング研究のレ ビューでは、選択バイアスが内的妥当性に対する重要な脅威となる可能性があるという証拠が示 されている(Bull ら、2006 年; Faust ら、2004 年)。同じ問題は、フタル酸エステル類のよう な短寿命化学物質のバイオモニタリング研究にも当てはまる(Durmaz ら、2010 年; Wang ら、 2013 年; Wirth ら、2008 年)。

3.3.2.1. 研究の評価(表 1) Tier 1 研究には、偏りのない選択またはフォローアッププロトコルが含まれており、横断研究または症例対照研究では高い(例:80%以上)応答率、コホート研究では低い(例:20%未満)脱落例が認められる。Tier 2 の研究は、偏りのない選択/フォローアッププロトコルを有し、横断研究または症例対照研究では低い(例:50%~80%)、応答率、コホート研究では高い(例:20%~50%)脱落例が認められる。Tier 3 の研究とは、望ましい参加者の数が 50%未満であるか、サンプルの選択方法、無応答率または脱落例率を報告していない研究である。この情報が報告されていない研究は、Tier 3 研究とみなされるべきである。

低い応答率や高頻度の脱落例を選択バイアスと同一視すべきではないことを念頭に置いておく ことが重要である。選択バイアスは、最終のデータセットに含まれる個人の割合(別名:選択確 率)がばく露と発現事象の両方で異なる場合に起こる(例えば、ばく露群の発症例、非ばく露群 の発症例、ばく露群の非発症例、非ばく露群の非発症例の間で)。実際の選択確率は通常不明で あるが、望ましい参加者の10%しか脱落していない研究では、バイアスの大きさは、被験者の 50%以上が脱落している研究でのバイアスの大きさよりもはるかに小さいと予想できる。 3.3.3. データ分析

疫学研究におけるデータ分析に必須な側面は他で検討されており、生理学的半減期が短い化学 物質には特化したものではない。しかし、提案されている段階的評価システムの完全性のために、 これらの考慮事項を簡単に説明する。観察研究における全体的な分析戦略は、研究の主な目的に よって決まる。一般的に、統計モデルは予測分析と説明分析の2つのカテゴリーに分類される (Shmueli、2010年)。予測分析では、モデルへの変数の選択はデータに基づいて行われ、デ ータセットごとに異なる場合がある。このアプローチの目標は、モデル適合を最大化することで あり、目的の特定の共変量を保持するかどうかの決定は、目的の特定のばく露なしで、統計的検 定と適合良好に基づいて行われる(Bellazzi and Zupan、2008年)。説明的(仮説検証)分析 では、発現事象とリスク因子の関係が交絡している場合に、潜在的に重要な変数を誤って排除し たり、交絡因子として作用しない変数を間違って保持したりする可能性があるので、このアプロ ーチは不適切であるかもしれない(Kleinbaum and Klein、2002年)。

事前に定義されたばく露と発現事象の関係に焦点を当てた説明モデルでより重要なことは、コントロール変数(交絡因子、媒介因子、または効果修飾因子)の包含と排除は、少なくとも部分的には、先験的な理由に基づいて行われるべきである(Beran and Violato、2010年; Concato ら、1993年; Hernan ら、2002年)。

観察研究の結果は必然的に不確実性の影響を受けることを念頭に置くことが重要である。この 不確実性は、原因不明のバイアスの様々なソースや、様々なデータ処理の決定や仮定に起因して いる可能性がある。不確実性の大きさは、定量的な感度分析によって正式に評価できる。感度分 析を通じた残留バイアスへの対処方法は、基礎的な理論(Greenland、1996 年)と実践的な応 用の両面で十分に発達している(Goodman ら、2007 年; Lash and Fink、2003 年; Maldonado ら、2003 年)。代替的な決定や仮定の感度分析に関しては、経済学、ばく露評価、定量的リス ク分析の過去の経験から多くを学ぶことができる(Koornneef ら、2010 年; Leamer、1985 年; Spiegelman、2010 年)。

3.3.3.1. 研究の評価(表 1) Tier 1 の研究には、因果関係モデルと予測モデルを明確に区別し、 効果修飾と交絡因子の調整を評価して外的要因を十分に考慮していることを論証することが含ま れる。Tier 1 の資格を得るためには、研究は正式な感度分析も行うべきである。外部要因の考慮 が十分であり、モデル選択が適切であると考えられる場合でも、感度分析のない研究は不完全と みなされる可能性がある。このような研究は Tier 2 に分類される。Tier 3 の研究は、共変量の選 択方法が不適切であったり、重要な交絡因子を考慮していなかったり、効果修飾を考慮に入れて いなかったりするために、外部要因を適切にコントロールできなかった研究である。

「外部要因」という用語は、対照となるばく露や発現事象以外の参加者の特性を示しており、 それらは交絡因子または効果修飾因子あるいはその両方として作用するので、研究のデザインま たは解析の段階で考慮する必要がある(Kleinbaum ら、2007年)。

3.3.4. 結果報告

報告の3つの側面(透明性、複数のテスト、報告の偏り)を考慮する。

*3.3.4.1. 報告の透明性* STROBE 声明で述べられているように、結果の報告は「観察研究において何が計画され、何が行われ、何が発見されたかを明確に提示」(Vandenbroucke ら、2007 年) すべきである。これらの考慮事項はすべての研究に当てはまるが、短寿命化学物質のバイオモニタリング研究には特に関連性のある研究報告の側面がある。

生物学的サンプル分析は、1回の分析で複数の分析物を迅速に分析できるように、ますます最 適化されてきている。このような技術の発展により、ばく露(及び該当する場合は発現事象)バ イオマーカーの完全なリストを含むデータの完全な報告の重要性が増してきており、代表値や分 散などの要約統計量の提示も重要である。その他の重要な情報要素には、欠落データのパターン とその取り扱い、及び LOD 以下の測定値の説明が含まれるべきであり、これらはすべて研究結 果の解釈に影響を及ぼす(Albert ら、2010 年; Barnes ら、2008 年; LaKind ら、2012 年 b)。 さらに、研究参加者の数を決定する際に使用された検出力計算や、有意な関連性を特定する能力 に影響を与えるばく露勾配に関する情報も提供されるべきである。これらの情報の一部は、スペ ースの制約のために論文には含まれないかもしれないが、補足資料に組み入れたり、要望に応じ て提供したりすることが可能となる。

3.3.4.2. 多重検定の問題点 複数の仮説検定を行う場合の主な懸念は、偽陽性(FP)の結果となる可能性が高まることである(Boffettaら、2008年; Ioannidis、2014年; Jager and Leek、2014年; Rothman、1990年; Sabatti、2007年)。その他、FPの結果の問題は、対応する偽陰性(FN)の問題よりも重要ではないとの主張がある(Blair ら、2009年)。どのタイプのエラー(FPか、FNか)がより大きな懸念をもたらすかの決定は、化学的特性及び発現事象特性であり、ケース・バイ・ケースで行われるべきである。

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遺伝疫学及び分子疫学における最近の進歩は、FN 結果のリスクを増加させることなく FP (PFP) の確率を減少させることを目的とした新しいアプローチの開発された(Datta and Datta、2005 年; Wacholder ら、2004 年)。さらに最近になって、これらのアプローチは、さらに FP:FN 比を計算できるまでに及んだ(Ioannidis ら、2011 年)。

3.3.4.3. バイアス報告 メタ分析や WOE 評価のために一連の研究を評価する際には、利用可能 なデータの分析と統合の両方に影響を及ぼす可能性のある 2 つの特定のバイアスのソース、すな わち、出版バイアスと論文報告バイアスを考慮しなければならない。出版バイアスとは、「研究 者や編集者が研究結果の方向性や強さに基づいて研究結果を発表しない傾向」と定義されている (Dickersin and Min、1993 年)。これと密接に関連する概念として、選択的研究内報告(別 名:結果報告バイアス)があり、これは「論文に含めるために記録された元の変数の一部の結果 に基づいて選択すること」と定義されている(Dwan ら、2008 年)。

出版バイアスは短寿命化学物質を含む研究には特有のものではない。しかし、上記の理由から、 短寿命化学物質の研究では、発現事象報告バイアスがより問題になる可能性がある。特に、高性 能分析設備が利用しやすくなることで、より多くのサンプル数でより多くの分析物を測定するこ とが可能になっている。

3.3.4.4. 研究の評価 Tier1 研究では、その目的が明確に述べられており、読者は検証された仮説の数(結果が与えられた仮説の数だけではない)を評価することができる。複数の同時仮説検証が行われている場合は、その影響が評価し、可能なら PFP または FP: FN 比を推定することで評価を行う。結果報告バイアスの証拠はなく、結論は観察された結果を超えたものではない。 Tier 2 の研究では、結論は正当であると思われるが、検証された仮説の数が不明確(明示されていないか、識別が困難である)であり、及び/または多重牲の検討がなされていない。データ要約を選択的に報告し、提示された結果の方法や選択に関して透明性を欠いている研究は Tier 3 に含まれる。

## 4. 考察/結論

環境疫学研究の質を評価するための体系的なアプローチの必要性は明らかである。評価スキー ムを開発するための2つの先行研究は、環境化学物質ばく露と神経発達に関する疫学研究に焦点 を当てたものであった(Amler ら、2006年; Youngstrom ら、2011年)。これらの提案された スキームで提示された概念の多くは、生理学的半減期の短い化学物質のバイオモニタリングを検 討する際に、研究の質を評価し、研究結果を伝える上で貴重なものである。例えば、Amler ら、

(2006 年)が提示した基本的な最適な実行/基準は以下とおりである。明確に定義された生物学的に妥当な仮説、前向きで縦断的なコホートデザインの使用、研究間での研究デザインプロトコルの一貫性、研究結果がどの程度決定的で一般化可能であるかについての率直で規律正しく知的に正直な扱い、実際の研究課題、検証手順及び研究での発見についての報告の制限、否定的な結果も報告する倫理的義務の研究者の認識ならびに結果を最小化したり誇張したり

## しないことの重要性。

生理的半減期が短い化学物質は、分析室やサンプリング機器に存在すること、横断研究における時間的順序の確立が困難であること、ばく露とバイオマーカー濃度の短期及び長期の変動性、 正確なばく露分類に必要な測定数に関する情報不足など、いくつかの重要な問題を抱えている。 BEES-Cは、研究や提案の中でこれらの問題を評価するためにデザインされている。

BEES-C のような評価ツールの開発は、単純でもなく、議論の余地のないものでもないことを 認識しており、CONSORT やその他の既存方法の一部、あるいは臨床データの質評価であるデ ータ品質スキームと同様に、これが反復的なプロセスになることを期待している。この種の評価 スキームは探索的研究には有用ではなく、むしろ、半減期の短い化学物質へのばく露と有害性健 康影響の関連性の理解を深めるために最も有用な研究をデザインし、特定することに焦点が当て られていることにも留意したい。本ワークショップで開発された評価指標が、このテーマについ てのさらなる議論のきっかけとなることを期待する。

#### 利益相反

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#### 免責事項

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## 参考文献

- Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, et al. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ Health Perspect 2008;116:467–73.
- Albert PS, Harel O, Perkins N, Browne R. Use of multiple assays subject to detection limits with regression modeling in assessing the relationship between exposure and outcome. Epidemiology 2010;21(Suppl. 4):S35-43.
- Amler RW, Barone Jr S, Belger A, Berlin Jr CM, Cox C, Frank H, et al. Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical exposure.

Neurotoxicology 2006;27:861-74.

- Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 2010;29:730-41.
- Attfield KR, Hughes MD, Spengler JD, Lu C. Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. Environ Health Perspect 2014;122:201-6.
- Barnes SA, Mallinckrodt CH, Lindborg SR, Carter MK. The impact of missing data and how it is handled on the rate of false-positive results in drug development. Pharm Stat 2008;7:215-25.
- Barr DB, Needham LL. Analytical methods for biological monitoring of exposure to pesticides: a review. J Chromatogr B Analyt Technol Biomed Life Sci 2002;778:5–29.

以下 206 ページ

- Barr DB, Barr JR, Driskell WJ, Hill Jr RH, Ashley DL, Needham LL, et al. Strategies for biolog- ical monitoring of exposure for contemporary-use pesticides. Toxicol Ind Health 1999;15:168–79.
- Barr DB, Wang RY, Needham LL. Biologic monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's Study. Environ Health Perspect 2005a;113:1083–91.
- Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005b;113:192–200.
- Barr DB, Landsittel D, Nishioka M, Thomas K, Curwin B, Raymer J, et al. A survey of laboratory and statistical issues related to farmworker exposure studies. Environ Health Perspect 2006;114:961–8.
- Barr DB, Olsson AO, Wong LY, Udunka S, Baker SE, Whitehead RD, et al. Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999–2002. Environ Health Perspect 2010;118:742–8.
- Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. Int J Med Inform 2008;77:81–97.
- Beran TN, Violato C. Structural equation modeling in medical research: a primer. BMC Res Notes 2010;3:267.
- Blair A, Saracci R, Vineis P, Cocco P, Forastiere F, Grandjean P, et al. Epidemiology, public health, and the rhetoric of false positives. Environ Health Perspect 2009;117: 1809–13.
- Boet S, Sharma S, Goldman J, Reeves S. Review article: medical education research: an overview of methods. Can J Anaesth 2012;59:159–70.
- Boffetta P, McLaughlin JK, La Vecchia C, Tarone RE, Lipworth L, Blot WJ. False-positive results in cancer epidemiology: a plea for epistemological modesty. J Natl Cancer Inst 2008;100:988–95.
- Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Fam Pract 2004;21:4–10.
- Bouchard M, Viau C. Urinary excretion of benzo[a]pyrene metabolites following intrave- nous, oral, and cutaneous benzo[a]pyrene administration. Can J Physiol Pharmacol 1997;75:185–92.
- Bouchard M, Krishnan K, Viau C. Kinetics of tissue distribution and elimination of pyrene and 1-hydroxypyrene following intravenous administration of [<sup>14</sup>C]pyrene in rats. Toxicol Sci 1998;46:11–20.
- Bull S, Fletcher K, Boobis AR, Battershill JM. Evidence for genotoxicity of pesticides in pesticide applicators: a review. Mutagenesis 2006;21:93–103.
- Calafat AM, Needham LL. Factors affecting the evaluation of biomonitoring data for human exposure assessment. Int J Androl 2008;31:139–43.

- Calafat AM, Needham LL. What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data? Environ Health Perspect 2009;117:1481–5.
- Calafat AM, Koch HM, Swan SH, Hauser R, Goldman LR, Lanphear BP, et al. Misuse of blood serum to assess exposure to bisphenol A and phthalates. Breast Cancer Res 2013;15: 403.
- Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, et al. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 1992;52:2447–55.
- Clarke P, Sproston K, Thomas R. An investigation into expectation-led interviewer effects in health surveys. Soc Sci Med 2003;56:2221–8.
- Concato J, Feinstein AR, Holford TR. The risk of determining risk with multivariable models. Ann Intern Med 1993;118:201–10.
- Copeland KT, Checkoway H, McMichael AJ, Holbrook RH. Bias due to misclassification in the estimation of relative risk. Am J Epidemiol 1977;105:488–95.
- Datta S, Datta S. Empirical Bayes screening of many p-values with applications to microarray studies. Bioinformatics 2005;21:1987–94.
- Dickersin K, Min YI. Publication bias: the problem that won't go away. Ann N Y Acad Sci 1993;703:135–46. [discussion 146–138].
- Dosemeci M, Wacholder S, Lubin JH. Does nondifferential misclassification of exposure always bias a true effect toward the null value? Am J Epidemiol 1990;132:746–8.
- Durmaz E, Ozmert EN, Erkekoglu P, Giray B, Derman O, Hincal F, et al. Plasma phthalate levels in pubertal gynecomastia. Pediatrics 2010;125:e122-9.
- Dwan K, Altman DG, Arnaiz JA, Bloom J, Chan AW, Cronin E, et al. Systematic review of the empirical evidence of study publication bias and outcome reporting bias. PLoS One 2008;3:e3081.
- EPA (US Environmental Protection Agency). America's children and the environment3rd ed. ; 2013 [Available: http://www.epa.gov/ace/ Accessed November 25, 2013].
- Faust F, Kassie F, Knasmuller S, Boedecker RH, Mann M, Mersch-Sundermann V. The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. Mutat Res 2004;566:209–29.
- Fisher CG, Wood KB. Introduction to and techniques of evidence-based medicine. Spine (Phila Pa 1976) 2007;32:S66–72.
- Gallo V, Egger M, McCormack V, Farmer PB, Ioannidis JPA, Kirsch-Volders M, et al. STrengthening the Reporting of OBservational studies in Epidemiology Molecular Epidemiology STROBE-ME: an extension of the STROBE statement. J Clin Epidemiol 2011;64:1350-63.
- Goodman M, Barraj LM, Mink PJ, Britton NL, Yager JW, Flanders WD, et al. Estimating uncertainty in observational studies of associations between continuous variables: ex- ample of methylmercury and neuropsychological testing in children. Epidemiol Perspect Innov 2007;4:9.
- Goodman M, LaKind JS, Mattison DR. Do phthalates act as obesogens in humans? A systematic review of the epidemiology literature. Crit Rev Toxicol 2014;44(2): 151–75.
- Gordis L. Epidemiology. Philadelphia, PA: Saunders Elsevier; 2008.
- Greenland S. Basic methods for sensitivity analysis of biases. Int J Epidemiol 1996;25: 1107–16.
- Harthé C, Rinaldi S, Achaintre D, de Ravel MR, Mappus E, Pugeat M, et al. Bisphenol A-glucuronide measurement in urine samples. Talanta 2012;100:410–3.
- Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol 2002;155:176–84.
- Hertz-Picciotto I. Environmental epidemiology. In: Rothman KJ, Greenland S, editors.Modern epidemiology. Lippincott Williams and Wilkins; 1998.
- Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985;14:601-12.
- Ioannidis JP. Discussion: why "an estimate of the science-wise false discovery rate and application to the top medical literature" is false. Biostatistics 2014;15:28–36. [dis- cussion

39-45].

- Ioannidis JP, Tarone R, McLaughlin JK. The false-positive to false-negative ratio in epidemiologic studies. Epidemiology 2011;22:450–6.
- Jager LR, Leek JT. An estimate of the science-wise false discovery rate and application to the top medical literature. Biostatistics 2014;15:1–12.
- Jurek AM, Maldonado G, Greenland S, Church TR. Exposure-measurement error is frequently ignored when interpreting epidemiologic study results. Eur J Epidemiol 2006;21:871-6.
- Kalsbeek W, Heiss G. Building bridges between populations and samples in epidemiological studies. Annu Rev Public Health 2000;21:147–69.
- Kissel JC, Curl CL, Kedan G, Lu C, Griffith W, Barr DB, et al. Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington State. J Expo Anal Environ Epidemiol 2005; 15:164–71.
- Kleinbaum DG, Klein M. Logistic regression: a self-learning text. NY: Springer-Verlag New York; 2002.
- Kleinbaum DG, Sullivan KM, Barker ND. A pocket guide to epidemiology. New York: Springer Science + Business Media; 2007. p. 228–9.
- Koornneef J, Spruijt M, Molag M, Ramirez A, Turkenburg W, Faaij A. Quantitative risk assessment of CO<sub>2</sub> transport by pipelines — a review of uncertainties and their impacts. J Hazard Mater 2010;177:12–27.
- Kuo CC, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr Diab Rep 2013;13:831– 49.
- LaKind JS, Naiman DQ. Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003–2004 NHANES urinary BPA data. J Expo Sci Environ Epidemiol 2008; 18:608– 15.
- LaKind JS, Naiman DQ. Daily intake of bisphenol A (BPA) and potential sources of exposure 2005–2006 NHANES. J Expo Sci Environ Epidemiol 2011;21:272–9.
- LaKind JS, Levesque J, Dumas P, Bryan S, Clarke J, Naiman DQ. Comparing United States and Canadian population exposures from national biomonitoring surveys: bisphenol A intake as a case study. J Expo Sci Environ Epidemiol 2012a;22:219–26.
- LaKind JS, Goodman M, Naiman DQ. Use of NHANES data to link chemical exposures to chronic diseases: a cautionary tale. PLoS One 2012b;7(12):e51086. http://dx.doi. org/10.1371/journal.pone.0051086.
- LaKind JS, Goodman M, Mattison DR. Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: a systematic review of epidemiologic research. Crit Rev Toxicol 2014;44(2):121-50.
- Lanphear BP, Dietrich K, Auinger P, Cox C. Cognitive deficits associated with blood lead concentrations b 10 µg/dL in US children and adolescents. Public Health Rep 2000; 115:521–9.
- Lash TL, Fink AK. Semi-automated sensitivity analysis to assess systematic errors in observational data. Epidemiology 2003;14:451–8.
- Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Main KM, Skakkebæk NE, et al. Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples. Environ Res 2013;126:164–70.
- Leamer EE. Sensitivity analyses would help. Am Econ Rev 1985;75:308-13.
- Lee WC, Huang HY. Data-dredging gene-dose analyses in association studies: biases and their corrections. Cancer Epidemiol Biomarkers Prev 2005;14:3004–6.
- Leng G, Kuhn KH, Idel H. Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. Sci Total Environ 1997;199:173–81.
- Liekens AM, De Knijf J, Daelemans W, Goethals B, De Rijk P, Del-Favero J. Biograph: unsupervised biomedical knowledge discovery via automated hypothesis generation. Ge- nome Biol 2011;12:R57.
- Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. Strengthening the reporting of genetic association studies (STREGA): an extension of the strengthening the

reporting of observational studies in epidemiology (STROBE) statement. J Clin Epidemiol 2009;62(597–608):e594.

- Lorber M, Koch HM, Angerer J. A critical evaluation of the creatinine correction approach: can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthal- ate. J Expo Sci Environ Epidemiol 2011;21:576–86.
- Lord SJ, Gebski VJ, Keech AC. Multiple analyses in clinical trials: sound science or data dredging? Med J Aust 2004;181:452–4.
- Maldonado G, Delzell E, Tyl RW, Sever LE. Occupational exposure to glycol ethers and human congenital malformations. Int Arch Occup Environ Health 2003;76:405–23.
- Marco CA, Larkin GL. Research ethics: ethical issues of data reporting and the quest for authenticity. Acad Emerg Med 2000;7:691–4.
- Markham DA, Waechter Jr JM, Wimber M, Rao N, Connolly P, Chuang JC, et al. Development of a method for the determination of bisphenol A at trace concentrations in human blood and urine and elucidation of factors influencing method accuracy and sensitivity. J Anal Toxicol 2010;34:293–303.
- Meeker JD, Barr DB, Ryan L, Herrick RF, Bennett DH, Bravo R, et al. Temporal variability of urinary levels of nonpersistent insecticides in adult men. J Expo Anal Environ

Epidemiol 2005;15:271–81.

以下 207 ページ

- Meeker JD, Cantonwine DE, Rivera-González LO, Ferguson KK, Mukherjee B, Calafat AM, et al. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. Environ Sci Technol 2013; 47:3439–47.
- Moher D, Tricco AC. Issues related to the conduct of systematic reviews: a focus on the nutrition field. Am J Clin Nutr 2008;88:1191–9.
- Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001; 357:1191-4.
- National Research Council (NRC). Human biomonitoring for environmental chemicals.Washington, DC: The National Academies Press; 2006.
- National Toxicology Program (NTP). Draft OHAT approach for systematic review and evidence integration for literature-based health assessments — February. Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health; 2013 [Available: http://ntp.niehs.nih.gov/?objectid= 960B6F03-A712-90CB-8856221E90EDA46E [accessed 25 October 2013]].
- Needham LL, Calafat AM, Barr DB. Uses and issues of biomonitoring. Int J Hyg Environ Health 2007;210:229–38.
- Oquendo MA, Baca-Garcia E, Artes-Rodriguez A, Perez-Cruz F, Galfalvy HC, Blasco-Fontecilla H, et al. Machine learning and data mining: strategies for hypothesis generation. Mol Psychiatry 2012;17:956–9.
- Owens DK, Lohr KN, Atkins D, Treadwell JR, Reston JT, Bass EB, et al. AHRQ series paper 5: grading the strength of a body of evidence when comparing medical interventions Agency for Healthcare Research and Quality and the effective health-care program. J Clin Epidemiol 2010;63:513–23.
- Pearce N. Classification of epidemiological study designs. Int J Epidemiol 2012;41:393-7.
- Pleil JD, Sobus JR. Estimating lifetime risk from spot biomarker data and intraclass correlation coefficients (ICC). J Toxicol Environ Health Part A 2013;76:747–66.
- Potischman N, Weed DL. Causal criteria in nutritional epidemiology. Am J Clin Nutr 1999; 69:1309S-14S.
- Preau Jr JL, Wong LY, Silva MJ, Needham LL, Calafat AM. Variability over 1 week in the uri- nary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. Environ Health Perspect 2010;118: 1748–54.
- Rhomberg LR, Chandalia JK, Long CM, Goodman JE. Measurement error in environmental

epidemiology and the shape of exposure-response curves. Crit Rev Toxicol 2011;41: 651–71.

- Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1: 43– 6.
- Rothman KJ, Greenland S. Modern epidemiology. Philadelphia, PA: Lippincott Williams and Wilkins; 1998.
- Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health 2005;95(Suppl. 1):S144-50.
- Sabatti C. Avoiding false discoveries in association studies. Methods Mol Biol 2007;376: 195–211.
- Sampson M, McGowan J, Cogo E, Grimshaw J, Moher D, Lefebvre C. An evidence-based practice guideline for the peer review of electronic search strategies. J Clin Epidemiol 2009;62:944–52.
- Scher DP, Alexander BH, Adgate JL, Eberly LE, Mandel JS, Acquavella JF, et al. Agreement of pesticide biomarkers between morning void and 24-h urine samples from farmers and their children. J Expo Sci Environ Epidemiol 2007;17:350–7.
- Schisterman EF, Whitcomb BW, Buck Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. Environ Health Perspect 2005;113:853–7.
- Shmueli G. To explain or to predict? Stat Sci 2010;25:289–310.
- Sobus JR, McClean MD, Herrick RF, Waidyanatha S, Nylander-French LA, Kupper LL, et al. Comparing urinary biomarkers of airborne and dermal exposure to polycyclic aro- matic compounds in asphalt-exposed workers. Ann Occup Hyg 2009;53:561–71.
- Sorahan T, Gilthorpe MS. Non-differential misclassification of exposure always leads to an underestimate of risk: an incorrect conclusion. Occup Environ Med 1994;51:839–40.
- Spiegelman D. Approaches to uncertainty in exposure assessment in environmental epidemiology. Annu Rev Public Health 2010;31:149–63.
- Symanski E, Kupper LL, Kromhout H, Rappaport SM. An investigation of systematic changes in occupational exposure. 1996;57:724–35.
- Teeguarden JG, Calafat AM, Ye X, Doerge DR, Churchwell MI, Gunawan R, et al. Twentyfour hour human urine and serum profiles of bisphenol a during high-dietary exposure. Toxicol Sci 2011;123:48–57.
- Teitelbaum SL, Britton JA, Calafat AM, Ye X, Silva MJ, Reidy JA, et al. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. Environ Res 2008;106:257–69.
- Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the reporting of observational studies in epidemiology (strobe): explanation and elaboration. Epidemiology 2007;18:805–35.
- Viau C, Bouchard M, Carrier G, Brunet R, Krishnan K. The toxicokinetics of pyrene and its metabolites in rats. Toxicol Lett 1999;108:201–7.
- Völkel W, Kiranoglu M, Fromme H. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicol Lett 2008; 179:155–62.
- Wacholder S, Hartge P, Lubin JH, Dosemeci M. Non-differential misclassification and bias towards the null: a clarification. Occup Environ Med 1995;52:557–8.
- Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004;96:434–42.
- Walker DG, Wilson RF, Sharma R, Bridges J, Niessen L, Bass EB, et al. Best practices for conducting economic evaluations in health care: a systematic review of quality assessment tools. AHRQ Methods for Effective Health Care. Rockville (MD): Agency for Healthcare Research and Quality; 2012.
- Wang H, Zhou Y, Tang C, He Y, Wu J, Chen Y, et al. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One 2013;8:e56800.

Weed DL. On the use of causal criteria. Int J Epidemiol 1997;26:1137–41.

- Weed DL, Gorelic LS. The practice of causal inference in cancer epidemiology. Cancer Epidemiol Biomarkers Prev 1996;5:303-11.
- Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in system- atic reviews. BMC Med Res Methodol 2003;3:25.
- World Health Organization. Biomarkers & Human Biomonitoring. Children's Health and the Environment WHO Training Package for the Health Sector; 2011. October. www.who.int/ceh/capacity/biomarkers.pdf.
- WHO (World Health Organization). Toxicological and health aspects of bisphenol A. Re- port of Joint FAO/WHO Expert Meeting. 2–5 November 2010 and Report of Stake- holder Meeting on Bisphenol A; 2011. [Available: whqlibdoc.who.int/publications/ 2011/97892141564274\_eng.pdf [accessed 25 November 2013]].
- Wielgomas B. Variability of urinary excretion of pyrethroid metabolites in seven persons over seven consecutive days implications for observational studies. Toxicol Lett 2013;221:15–22.
- Wirth JJ, Rossano MG, Potter R, Puscheck E, Daly DC, Paneth N, et al. A pilot study associating urinary concentrations of phthalate metabolites and semen quality. Syst Biol Reprod Med 2008;54:143–54.
- Withey JR, Law FC, Endrenyi L. Pharmacokinetics and bioavailability of pyrene in the rat. J Toxicol Environ Health 1991;32:429–47.
- Ye X, Kuklenyik Z, Needham LL, Calafat AM. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 2005;383:638-44.
- Ye X, Zhou X, Hennings R, Kramer J, Calafat AM. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomoni- toring analysis: an elusive laboratory challenge. Environ Health Perspect 2013;121: 283–6.
- Youngstrom E, Kenworthy L, Lipkin PH, Goodman M, Squibb K, Mattison DR, et al. A proposal to facilitate weight-of-evidence assessments: Harmonization of Neurodevelopmental Environmental Epidemiology Studies (HONEES). Neurotoxicol Teratol 2011;33:354–9.
- Zartarian V, Bahadori T, McKone T. Adoption of an official ISEA glossary. J Expo Anal Environ Epidemiol 2005;15:1–5.
- Zelenka MP, Barr DB, Nicolich MJ, Lewis RJ, Bird MG, Letinski DJ, et al. A weight of evidence approach for selecting exposure biomarkers for biomonitoring. Biomarkers 2011;16:65–73.
- Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001–2010. Environ Health Perspect 2014;122:235–41.

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization, the International Labour Organization, or the United Nations Environment Programme.

## Harmonization Project Document No. 4

# PART 1: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS AND CASE-STUDIES

# PART 2: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS

This project was conducted within the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.

Published under the joint sponsorship of the World Health Organization, the International Labour Organization, and the United Nations Environment Programme, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.



The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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### FOREWORD

Harmonization Project Documents are a family of publications by the World Health Organization (WHO) under the umbrella of the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP). Harmonization Project Documents complement the Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as authoritative documents on methods for the risk assessment of chemicals.

The main impetus for the current coordinated international, regional, and national efforts on the assessment and management of hazardous chemicals arose from the 1992 United Nations Conference on Environment and Development (UNCED). UNCED Agenda 21, Chapter 19, provides the "blueprint" for the environmentally sound management of toxic chemicals. This commitment by governments was reconfirmed at the 2002 World Summit on Sustainable Development and in 2006 in the Strategic Approach to International Chemicals Management (SAICM). The IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter 19, and contributes to the implementation of SAICM. In particular, the project addresses the SAICM objective on Risk Reduction and the SAICM Global Plan of Action activity to "Develop and use new and harmonized methods for risk assessment".

The IPCS Harmonization Project goal is to improve chemical risk assessment globally, through the pursuit of common principles and approaches, and, hence, strengthen national and international management practices that deliver better protection of human health and the environment within the framework of sustainability. The Harmonization Project aims to harmonize global approaches to chemical risk assessment, including by developing international guidance documents on specific issues. The guidance is intended for adoption and use in countries and by international bodies in the performance of chemical risk assessments. The guidance is developed by engaging experts worldwide. The project has been implemented using a stepwise approach, first sharing information and increasing understanding of methods and practices used by various countries, identifying areas where convergence of different approaches would be beneficial, and then developing guidance that enables implementation of harmonized approaches. The project uses a building block approach, focusing at any one time on the aspects of risk assessment that are particularly important for harmonization.

The project enables risk assessments (or components thereof) to be performed using internationally accepted methods, and these assessments can then be shared to avoid duplication and optimize use of valuable resources for risk management. It also promotes sound science as a basis for risk management decisions, promotes transparency in risk assessment, and reduces unnecessary testing of chemicals. Advances in scientific knowledge can be translated into new harmonized methods.

This ongoing project is overseen by a geographically representative Harmonization Project Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.

### PART 1

### IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS AND CASE-STUDIES

### PREFACE

Following publication of the International Programme on Chemical Safety (IPCS) Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (in animals),<sup>1</sup> an IPCS Cancer Working Group convened on 3–5 March 2004 in Arlington, Virginia, USA. The working group agreed that the issue of human relevance of animal tumours should be further explored with the goal of developing a unified IPCS Human Relevance Framework for use of mode of action information in risk assessment for regulatory and other purposes, and it provided initial guidance for this task. The members of this working group, including secretariat support and a representative of the Organisation for Economic Co-operation and Development, were as follows:

- Professor Hermann Bolt, Institut für Arbeitsphysiologie, Germany
- Professor Alan R. Boobis, Department of Health Toxicology Unit, Imperial College London, United Kingdom
- Dr John Bucher, National Institute of Environmental Health Sciences, USA
- Dr Vincent Cogliano, Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, France
- Dr Samuel M. Cohen, Pathology and Microbiology, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center, USA
- Dr William Farland, Office of Research and Development, Environmental Protection Agency, USA
- Dr Jun Kanno, Division of Cellular & Molecular Toxicology, National Institute of Health Sciences, Japan
- Dr Lois D. Lehman-McKeeman, Bristol-Myers Squibb, USA
- Ms Bette Meek, Environmental Health Centre, Health Canada, Canada
- Ms Laurence Musset, Environment, Health and Safety Division, Organisation for Economic Co-operation and Development, France
- Dr Jerry Rice, Consultant, USA
- Ms Cindy Sonich-Mullin, International Programme on Chemical Safety, World Health Organization, USA
- Ms Carolyn Vickers, International Programme on Chemical Safety, World Health Organization, Switzerland
- Ms Deborah Willcocks, Existing Chemicals, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australia

Extending the Mode of Action Framework to include consideration of human relevance, taking into account guidance from the Arlington meeting, was the subject of an IPCS international workshop convened in Bradford, United Kingdom, from 21 to 23 April 2005. This workshop prepared draft text for an IPCS Human Relevance Framework, including updating the 2001 Mode of Action Framework. The workshop participants, including

<sup>&</sup>lt;sup>1</sup> Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

secretariat support and representatives of the European Food Safety Authority and European Chemicals Bureau, were as follows:

- Dr Peter Abbott, Scientific Risk Assessment and Evaluation Branch, Food Standards Australia New Zealand, Australia
- Dr Antero Aitio, International Programme on Chemical Safety, World Health Organization, Switzerland
- Dr Diana Anderson, Department of Biomedical Sciences, University of Bradford, United Kingdom
- Professor Sir Colin Berry, United Kingdom
- Professor Hermann Bolt, Institut für Arbeitsphysiologie, Germany
- Professor Alan R. Boobis, Department of Health Toxicology Unit, Imperial College London, United Kingdom
- Dr Susy Brescia, Health and Safety Executive, United Kingdom
- Dr John Bucher, National Institute of Environmental Health Sciences, USA
- Dr Vincent Cogliano, Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, France
- Dr Samuel M. Cohen, Pathology and Microbiology, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center, USA
- Dr Vicki Dellarco, Office of Pesticide Programs, Environmental Protection Agency, USA
- Ms Christine Dove, School of Life Sciences, University of Bradford, United Kingdom
- Dr Jun Kanno, Division of Cellular and Molecular Toxicology, National Institute of Health Sciences, Japan
- Dr Janet Kielhorn, Department of Chemical Risk Assessment, Fraunhofer Institute for Toxicology and Experimental Medicine, Germany
- Mrs Sandra Kunz, International Programme on Chemical Safety, World Health Organization, Switzerland
- Dr Christian Laurent, Scientific Expert Services, European Food Safety Authority, Italy
- Dr Douglas McGregor, Toxicity Evaluation Consultants, United Kingdom
- Ms Bette Meek, Environmental Health Centre, Health Canada, Canada
- Ms Sharon Munn, Toxicology and Chemical Substances, European Chemicals Bureau, Italy
- Dr R. Julian Preston, National Health and Environmental Effects Research Laboratory, Environmental Carcinogenesis Division, Environmental Protection Agency, USA
- Dr Jerry Rice, Consultant, USA
- Dr Hans-Bernhard Richter-Reichhelm, Federal Institute for Risk Assessment (BfR), Germany
- Ms Carolyn Vickers, International Programme on Chemical Safety, World Health Organization, Switzerland
- Ms Deborah Willcocks, Existing Chemicals, National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australia
- Dr William P. Wood, Risk Assessment Forum, Environmental Protection Agency, USA
- Dr Zheng Yuxin, Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, and WHO Collaborating Centre of Occupational Health, People's Republic of China

The draft was published on the Internet for public comment and sent to a number of WHO Collaborating Centres and IPCS Participating Institutions for peer review. An expert meeting that convened in London in December 2005 considered the comments received and finalized the framework. The expert meeting participants were as follows:

- Professor Alan R. Boobis, Department of Health Toxicology Unit, Imperial College London, United Kingdom (*Rapporteur*)
- Dr Samuel M. Cohen, Pathology and Microbiology, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center, USA
- Dr Vicki Dellarco, Office of Pesticide Programs, Environmental Protection Agency, USA
- Dr William Farland, Office of Research and Development, Environmental Protection Agency, USA (Chair)

Dr Douglas McGregor, Toxicity Evaluation Consultants, United Kingdom

- Ms Carolyn Vickers, International Programme on Chemical Safety, World Health Organization, Switzerland
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### LIST OF ACRONYMS AND ABBREVIATIONS

ADH	alaahal dahudraganaga
ANOVA	alcohol dehydrogenase analysis of variance
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bw CAR	body weight
-	constitutively active receptor
cDNA	complementary deoxyribonucleic acid
CoA	coenzyme A
CpG	cytosine and guanine separated by a phosphate
CYP	cytochrome P-450
dA	deoxyadenosine
dG	deoxyguanosine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPX	DNA-protein cross-links
FAO	Food and Agriculture Organization of the United Nations
HRF	Human Relevance Framework
IARC	International Agency for Research on Cancer
ILO	International Labour Organization
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
IU	International Units
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
$K_{\mathrm{M}}$	Michaelis-Menten constant
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NAT	N-acetyltransferase
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program (USA)
OAT	O-acetyltransferase
PCNA	proliferating cell nuclear antigen
PPX	protein–protein cross-linkage
RNA	ribonucleic acid
RSI	Risk Science Institute (ILSI)
rT3	reverse triiodothyronine
S9	$9000 \times g$ supernatant from rat liver
SCE	sister chromatid exchange
SHE	Syrian hamster embryo
Т3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TGF	tumour growth factor
TSH	thyroid stimulating hormone
UDP	uridine diphosphate
UDS	unscheduled DNA synthesis
UGT	uridine diphosphate glucuronosyltransferase

ULLI	unit length labelling index
UNEP	United Nations Environment Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

### IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Alan R. Boobis, Samuel M. Cohen, Vicki Dellarco, Douglas McGregor, M.E. (Bette) Meek, Carolyn Vickers, Deborah Willcocks, & William Farland

The use of structured frameworks can be invaluable in promoting harmonization in the assessment of chemical risk. The International Programme on Chemical Safety (IPCS) has therefore updated and extended its Mode of Action (MOA) Framework for cancer to address the issue of human relevance of a carcinogenic response observed in an experimental study. The first stage is to determine whether it is possible to establish an MOA. This comprises a series of key events along the causal pathway to cancer, identified using a weight-of-evidence approach based on the Bradford Hill criteria. The key events are then compared first qualitatively and then quantitatively between the experimental animals and humans. Finally, a clear statement of confidence, analysis, and implications is produced. The IPCS Human Relevance Framework for cancer provides an analytical tool to enable the transparent evaluation of the data, identification of key data gaps, and structured presentation of information that would be of value in the further risk assessment of the compound, even if relevancy cannot be excluded. This might include data on the shape of the dose–response curve, identification of any thresholds, and recognition of potentially susceptible subgroups, for example, the basis of genetic or life stage differences.

Fundamental to the evolution of cancer risk assessment over the last three decades has been our increasing understanding of the biology of cancer and the identification of key events in carcinogenesis. Through the mid-1980s, national and international assessments of human cancer hazard and risk depended primarily on lifetime assays in rodents of potentially carcinogenic agents. For few agents was there sufficient human evidence on which to base retrospective cancer assessments, and fewer still would be expected to be detected prospectively, given modern controls on general exposures in the workplace and in the environment generally. Inherent in rodent-based assessments was the assumption that the observation of tumours in laboratory animals could be meaningfully extrapolated to identify potential human carcinogens and, by the use of mathematical models, to provide upper-bound estimates of risk at human doses of regulatory significance. During the same period, the potential significance of mutagenesis in carcinogenesis was becoming accepted by the scientific community. Subsequently, it has become increasingly apparent that an appreciable number of chemicals cause cancer in laboratory animals by processes that do not involve direct interaction with DNA. These developments in our understanding of the biological basis of carcinogenesis in both laboratory animals and humans have benefited risk assessment processes by providing more data on the pharmacokinetics and pharmacodynamics of suspect carcinogenic agents. Consideration of the biological processes involved in the carcinogenesis of specific compounds has led to the concept of mode of action (MOA).

<sup>&</sup>lt;sup>1</sup> This article, to which WHO owns copyright, was originally published in 2006 in *Critical Reviews in Toxicology*, Volume 36, pages 781–792. It has been edited for this WHO publication and includes corrigenda.

A postulated MOA for carcinogenesis is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed carcinogenicity—in a logical framework. MOA contrasts with mechanism of action, which generally involves a sufficient understanding of the molecular basis for an effect and its detailed description so that causation can be established in molecular terms.

In 2001, as part of its efforts to harmonize risk assessment practices, the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP) published a framework for assessment of MOA for carcinogenesis in laboratory animals (animal MOA), based on Bradford Hill criteria for causality. The IPCS Human Relevance Framework (HRF) presented in this document updates this MOA Framework and extends it to consider human relevance. It is an analytical tool to provide a means of evaluating systematically the data available on a specific carcinogenic response to a chemical in a transparent manner. While it is envisaged that the framework will be of value to risk assessors both within and outside of regulatory agencies, it will also be a valuable tool to the research community. Among reasons for using the framework are:

- to provide a generic approach to the analysis of data to contribute to harmonization;
- to encourage transparency of the consideration and use of available data and reasons for the conclusions drawn;
- to provide guidance in the presentation of data;
- to identify critical data deficiencies and needs;
- to inform the quantitative assessment of carcinogenic risk to humans.

These and other topics will be discussed in more detail below.

## THE ROLE OF IPCS IN DEVELOPING THE FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MOA FOR HUMANS

IPCS has been leading an effort to harmonize approaches to cancer risk assessment as part of its larger project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. The first phase of this work resulted in the publication of the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis in experimental animals (Sonich-Mullin et al., 2001). As described in that publication, a major impediment to harmonization identified in the consideration of weight of evidence was the evaluation of MOA in animals. Sonich-Mullin et al. (2001) provided a framework for evaluating MOA of chemical carcinogenesis in animals and recognized the importance of moving on to the next step in the overall characterization of cancer hazard and risk in humans: the assessment of relevance of the MOA of animal carcinogenesis to humans. Adoption of the MOA Framework concept is proceeding through its incorporation in the revised United States Environmental Protection Agency (USEPA) Guidelines for Carcinogen Risk Assessment (USEPA, 1999, 2005), and the framework is now commonly used by other regulatory agencies and international organizations. In the United Kingdom, the framework is being used for the assessment of pesticides and industrial chemicals. The United Kingdom

Committee on Carcinogenicity (2004) has noted the framework's value with regard to both harmonization between agencies and internal consistency in its latest guidelines. The framework has also been adopted and is being used by agencies in Australia and in Canada, in the evaluation of Existing Chemicals under the Canadian Environmental Protection Act. The European Union has incorporated the framework into its technical guidance documents on evaluating new and existing industrial chemicals and biocides, including carcinogenicity. With regard to international organizations, of particular note is the use of the framework by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), for example, in its evaluation of pyrethrin extract and its incorporation into the resulting monograph.

The step to extend the MOA Framework to include consideration of human relevance has been undertaken by IPCS in cooperation with international partners. It was the subject of an IPCS international workshop convened in Bradford, United Kingdom, from 21 to 23 April 2005. This workshop prepared draft text for an IPCS HRF, including updating the 2001 MOA Framework. The draft was published on the Internet for public comment and sent to a number of WHO Collaborating Centres and IPCS Participating Institutions for peer review. An expert meeting convened in London in December 2005 considered the comments received and finalized the framework. The framework text and the steps leading to its development are discussed in detail in the following sections.

### THE 2001 IPCS CONCEPTUAL MOA FRAMEWORK FOR EVALUATING ANIMAL CARCINOGENESIS

### **Purpose of the framework**

The IPCS MOA Framework for evaluating carcinogenesis in animals (Sonich-Mullin et al., 2001) remains a fundamental basis for the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans. The animal MOA Framework provides a generic approach to the principles commonly used when evaluating a postulated MOA for tumour induction in animals by a chemical carcinogen. Thus, the framework is a tool that provides a structured approach to the assessment of the overall weight of the evidence for the postulated MOA. In this context, a supported MOA would have evidence provided by robust experimental observations and mechanistic data to establish a biologically plausible explanation.

The framework is designed to bring transparency to the analysis of a postulated MOA and thereby promote confidence in the conclusions reached through the use of a defined procedure that encourages clear and consistent documentation supporting the analysis and reasoning and that highlights inconsistencies and uncertainties in the available data. The purpose of the framework is to provide a systematic means of considering the weight of the evidence for an MOA in a given situation; it is not designed to give an absolute answer on sufficiency of the information, as this will vary depending on the circumstance. It is not a checklist of criteria, but rather an analytical approach. However, the process can be greatly aided by the presentation of tabular summaries of comparative data on incidence of key events and tumours.

The animal MOA Framework analysis is an important step in the hazard characterization. It is envisaged that the animal MOA Framework will contribute to risk assessments of chemical

carcinogens across all sectors (drugs, industrial chemicals, pesticides, food additives, etc.). In the resulting risk assessment documentation, the framework analysis would be appropriately positioned within the hazard characterization section. In the absence of adequate epidemiological data, it may be regarded as an essential component in any discussion of human relevance, dose–response relationships, and risk characterization. It is also envisaged that the framework will be useful to both regulators and researchers in identifying research needs based on clear delineation of data gaps and inconsistencies.

MOA analysis can be used to establish either that a compound has an MOA that has been described previously or that it has a novel MOA. Thus, the output of an MOA analysis may serve to support the evaluation of a specific compound or contribute to the generation of a novel MOA. In the former, chemical-specific data play a vital role in the concordance analysis for human relevance. In the latter, it will be important to identify which events are key to the biological processes that represent the MOA.

Thus, an MOA comprising the same set of key events may apply to many different compounds. The evidence necessary to establish that a specific MOA is responsible for a given carcinogenic response will be substantial the first time such an MOA is proposed. As subsequent compounds are found to share this MOA, the "barrier" to acceptance will be lower, although it will always be necessary to establish rigorously that the key events comprising the MOA occur and that they fulfil the criteria described below. It will also be important to exclude other possible MOAs.

Scientific peer participation is a prerequisite for the development and acceptance of a novel postulated MOA. Peer participation includes both peer involvement in the development of an MOA and peer review by scientists who are independent of the process of development of the MOA. Publication in the scientific literature and presentation and discussion at scientific meetings and workshops constitute peer involvement that contributes to acceptance of an MOA by the scientific community.

While acceptance does not necessarily mean unanimity, most of the scientists reviewing an MOA analysis should agree that the relevant scientific information has been identified and appropriately analysed, that "key events" have been identified and are supported by the information presented, that their relationship to carcinogenesis has been clearly established in the hypothesized MOA, and that alternative MOAs have been considered and rejected.

As knowledge advances, the characterization of an MOA will change. Additional key events may be identified, and others may be refined or even dropped. Nevertheless, significant changes to the key events also need some general acceptance, through peer review, such as described above.

### **Update of framework guidelines**

In development of the IPCS HRF, the 2001 animal MOA Framework text has been updated, and this revised version is presented here.

#### Introduction to framework analysis

This section describes the cancer end-point or end-points that have been observed and identifies which of these are addressed in the analysis. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals. The nature of the framework is such that only one MOA is analysed at a time; hence, for example, different tumour types associated with chemical treatment, even if recorded in the same animals, will require separate framework analyses to discern each tumour's MOA. However, in considering the pathogenesis of a single type of tumour, it should be recognized that it is possible that a chemical could induce that tumour type by more than one MOA. Hence, it might be necessary to undertake an analysis of more than one MOA for the same tumour type for a single chemical. Consistent with species- and tissue-specific variation in metabolic activation and detoxication, there is often only poor site concordance for genotoxic carcinogens. This will need to be kept in mind when comparing animal and human data. In contrast, consistent with the observation that most carcinogens acting by a non-genotoxic MOA perturb physiological processes that tend to be site specific, site concordance is reasonably assumed, at least as an initial premise in the HRF.

#### 1. Postulated mode of action (theory of the case)

This section comprises a brief description of the sequence of events on the path to cancer for the postulated MOA of the test substance. This explanation of the sequence of events leads into the next section, which identifies the events considered "key" (i.e. necessary and measurable), given the database available for the analysis.

### 2. Key events

This section briefly identifies and describes the "key events"—measurable events that are critical to the induction of tumours as hypothesized in the postulated MOA. To support an association, a body of experiments needs to define and measure an event consistently. Pertinent observations include, for example, tumour response and key events in the same cell type, sites of action logically related to event(s), increased cell growth, specific biochemical events, changes in organ weight and/or histology, proliferation, perturbations in hormones or other signalling systems, receptor–ligand interactions, effects on DNA or chromosomes, and impact on cell cycle. For example, key events for tumours hypothesized to be associated with prolonged regenerative proliferation might be cytotoxicity as measured histopathologically and an increase in labelling index. As another example, key events for induction of urinary bladder tumours hypothesized to be due to formation of urinary solids composed primarily of calcium phosphate might include elevated urinary free calcium, phosphate, and pH and formation of urinary solids, followed by irritation and regenerative hyperplasia of the uro-thelium.

### 3. Concordance of dose–response relationships

This section should characterize the dose–effect/response relationships for each of the key events and for the tumour response and discuss their interrelationships, in the context of the Bradford Hill criteria. Ideally, one should be able to correlate the dose dependency of the increases in incidence of a key event with increases in incidence or severity (e.g. lesion progression) of other key events occurring later in the process, and with the ultimate tumour incidence. Comparative tabular presentation of incidence of key events and tumours is often helpful in examining dose–response. In the case of complex data sets, this is almost essential.

It is important to consider whether there are fundamental differences in the biological response (i.e. dose transitions) at different parts of the dose–response curve for tumour formation (Slikker et al., 2004). If so, key events relevant to the different parts of the dose–response curve will need to be defined and used in the framework analysis.

### 4. Temporal association

This section should characterize the temporal relationships for each of the key events and for the tumour response. The temporal sequence of key events leading to the tumour response should be determined. Key events should be apparent before tumour appearance and should be consistent temporally with each other; this is essential in deciding whether the data support the postulated MOA. Observations of key events at the same time as the tumours (e.g. at the end of a bioassay) do not contribute to considerations of temporal association, but can contribute to analysis in the next section. Most often, complete data sets to address the criterion of temporality are not available.

### 5. Strength, consistency, and specificity of association of tumour response with key events

This section should discuss the weight of evidence linking the key events, precursor lesions, and the tumour response. Stop/recovery studies showing absence or reduction of subsequent events or tumour when a key event is blocked or diminished are particularly important tests of the association. Consistent observations in a number of such studies with differing experimental designs increase that support, since different designs may reduce unknown biases or confounding. Consistency, which addresses repeatability of key events in the postulated MOA for cancer in different studies, is distinguished from coherence, however, which addresses the relationship of the postulated MOA with observations in the broader database (see point 6). Pertinent observations include tumour response and key events in the same cell type, sites of action logically related to event(s), and results from multistage studies and from stop/recovery studies.

#### 6. Biological plausibility and coherence

One should consider whether the MOA is consistent with what is known about carcinogenesis in general (biological plausibility) and also in relation to what is known for the substance specifically (coherence). For the postulated MOA and the events that are part of it to be biologically plausible, they need to be consistent with current understanding of the biology of cancer. However, the extent to which biological plausibility can be used as a criterion against which weight of evidence is assessed may be limited due to gaps in our knowledge. Coherence, which addresses the relationship of the postulated MOA with observations in the broader database-for example, association of MOA for tumours with that for other end-points-needs to be distinguished from consistency (addressed in point 5), which addresses repeatability of key events in the postulated MOA for cancer in different studies. For coherence, likeness of the case to that for structural analogues may be informative (i.e. structure-activity analysis). Information from other compounds that share the postulated MOA may be of value, such as sex, species, and strain differences in sensitivity and their relationship to key events. Additionally, this section should consider whether the database on the agent is internally consistent in supporting the purported MOA, including that for relevant non-cancer toxicities. Some MOAs can be anticipated to evoke effects other than cancer, such as reproductive effects of certain hormonal disturbances that are carcinogenic.

### 7. Other modes of action

This section discusses alternative MOAs that logically present themselves in the case. If alternative MOAs are supported, they need their own framework analysis. These should be distinguished from additional components of a single MOA that likely contribute to the observed effect, since these would be addressed in the analysis of the principal MOA.

### 8. Uncertainties, inconsistencies, and data gaps

Uncertainties should include those related to both the biology of tumour development and those for the database on the compound of interest. Inconsistencies should be flagged and data gaps identified. For the identified data gaps, there should be some indication of whether they are critical as support for the postulated MOA.

### 9. Assessment of postulated mode of action

This section should include a clear statement of the outcome with an indication of the level of confidence in the postulated MOA—for example, high, moderate, or low. If a novel MOA is being proposed, this should be clearly indicated. However, if the MOA is the same as that proposed for other compounds, the extent to which the key events fit this MOA needs to be stated explicitly. Any major differences should be noted, and their implications for the MOA should be discussed.

### ADDRESSING THE ISSUE OF HUMAN RELEVANCE

In 2000, an IPCS Harmonization Project Cancer Planning Work Group convened in Carshalton, United Kingdom (IPCS, 2000). (This initial IPCS working group differed in membership from the subsequent IPCS working group convened to work on the human relevance project.) Among the recommendations of that meeting was the suggestion that IPCS and the International Life Sciences Institute (ILSI) move forward together and in parallel on the development of the extension of the IPCS MOA Framework towards addressing human relevance. It was recognized that ILSI could provide much help in technical workshops. In June 2001, the ILSI Risk Science Institute (RSI) with support from the USEPA and Health Canada formed a working group to examine key issues in the use of MOA information to determine the relevance of animal tumours. These efforts have resulted in several published reports that are described below. An IPCS Cancer Working Group, convened on 3–5 March 2004 in Arlington, Virginia, USA, agreed that these reports should form the starting point for further exploration of the issue of human relevance of animal tumours by IPCS with the goal of developing a unified IPCS HRF for use of MOA information in risk assessment for regulatory and other purposes (IPCS, 2004).

To address the issue of the human relevance of the MOAs determined in animals, ILSI/RSI charged its working group with expanding the IPCS MOA Framework to include evaluation of the human relevance of a cancer MOA determined in animals. The details of the process, the case-studies, and the framework were published as a series of papers in the November 2003 issue of *Critical Reviews in Toxicology* (Cohen et al., 2003; Meek et al., 2003). These articles describe the ILSI/RSI HRF and provide guidance for its application. In addition, references to specific examples on which the framework is based are included. Several iterations of case-studies of chemicals with generally well known MOAs were used to

develop the integrated framework. The intent was to provide guidance for a disciplined, transparent process evaluating the MOA in animals and each key event with respect to human relevance.

The ILSI/RSI HRF is based on three fundamental questions:

- 1. Is the weight of evidence sufficient to establish the mode of action (MOA) in animals?
- 2. Are key events in the animal MOA plausible in humans?
- 3. Taking into account kinetic and dynamic factors, are key events in the animal MOA plausible in humans?

Questions 2 and 3 involve qualitative and quantitative considerations, respectively, in a concordance analysis of human information in relation to the animal MOA and its key events.

These are followed by an explicit description of confidence in the evaluation, identification of specific data gaps, and the implications for risk assessment. It was emphasized by ILSI/RSI that use of this framework would form part of the hazard characterization step of the overall risk assessment process.

## DEVELOPMENT OF AN IPCS HRF GUIDANCE DOCUMENT BASED ON THE IPCS MOA FRAMEWORK AND THE ILSI/RSI HRF

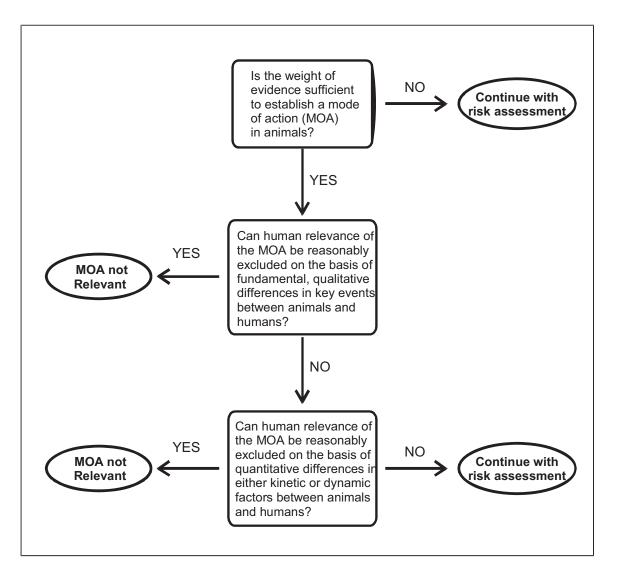
The 2004 IPCS Cancer Working Group discussed the type of document that would be produced as a result of its task to extend the IPCS MOA Framework to address human relevance. It was recognized that one integrated guidance document that worked as a whole would be needed to facilitate uptake and use by regulatory and other risk assessment bodies. The guidance could be supplemented by publication of the other materials generated through the process (e.g. issue papers and case-studies).

There was general agreement among working group members that the questions identified as the critical components of the ILSI/RSI HRF were important and in general appropriate for addressing the human relevance of an MOA determined in animals. However, several issues were identified that could benefit from additional clarification, development, or expansion.

These refinements of the ILSI/RSI HRF were developed through discussions of the IPCS Cancer Working Group and at a workshop convened for this purpose in Bradford, United Kingdom, on 21–23 April 2005 (IPCS, 2005). The resulting IPCS HRF is presented as an approach to answering a series of three questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumours. The application of the guidance results in a narrative with four sections that may be incorporated into the hazard characterization of a risk assessment. The sections are as follows (see Figure 1):

- 1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?
- 2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

- 3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?
- 4. Conclusion: Statement of confidence, analysis, and implications.



**Figure 1. IPCS general scheme illustrating the main steps in evaluating the human relevance of an animal MOA for tumour formation.** The questions have been designed to enable an unequivocal answer *yes* or *no*, but recognizing the need for judgement regarding sufficiency of weight of evidence. Answers leading to the left side of the diagram indicate that the weight of evidence is such that the MOA is not considered relevant to humans. Answers leading to the right side of the diagram indicate either that the weight of evidence is such that the MOA is likely to be relevant to humans or that it is not possible to reach a conclusion regarding likely relevance to humans, owing to uncertainties in the available information. In these cases, the assessment would proceed to risk characterization. It should be noted that only at this stage would human exposure be included in the evaluation.

In applying this framework for a given chemical, tumours of each animal target organ observed are evaluated independently, with the assumption that different MOAs are possible in different organs, although based on this analysis, MOAs in different tissues may be similar. Similarly, an evaluation of the likelihood of congruence between target organ(s) in different species and in humans needs to be made, based on the MOA analysis.

### Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?

Answering this first question in the IPCS HRF requires application of the (updated) IPCS MOA Framework described previously in this document. The steps in the MOA Framework, which are based on the Bradford Hill criteria for causality, are:

- 1. postulated MOA;
- 2. key events; associated critical parameters;
- 3. dose-response relationships;
- 4. temporal association;
- 5. strength, consistency, and specificity of association of key events and tumour response;
- 6. biological plausibility and coherence;
- 7. possible alternative MOAs;
- 8. uncertainties, inconsistencies, and data gaps;
- 9. conclusion about the MOA.

This process incorporates an evaluation of the weight of evidence for possible alternative MOAs at a given site and an evaluation of the overall strength of evidence supporting the MOA under consideration. Ultimately, a decision concerning the weight of evidence supporting the MOA and the level of confidence in that decision must be made. The process also identifies critically important data gaps that, when filled, would increase confidence in the proposed MOA. It is also necessary to establish whether the postulated MOA has already been described for other chemicals, in which case human relevance will already have been evaluated, or whether the proposed MOA is novel, in which case human relevance needs to be assessed de novo.

For a given chemical, the primary sources of information for evaluating an MOA are likely to be data generated for that specific chemical in the animal model in which tumours were produced. Obviously, data from other sources can and should also be used, as appropriate, along with data on chemicals with similar chemical structures, the same or similar MOAs, or both. If the MOA for a chemical is novel, considerably more data will be required to support the conclusion that it is related to the carcinogenic process of the tumours induced by that chemical than for subsequent examples of chemicals acting by the same MOA. The ILSI/RSI working group and the IPCS Bradford workshop did not address the issue of how many data are sufficient to support a specific MOA for a given chemical per se, except by way of example within the case-studies and recognition that acceptance of a novel MOA requires scientific consensus (described above). Consideration at this stage of the MOA analysis of potential variations between animals and humans also facilitates addressing subsequent steps in the framework.

# Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS workshop on the implications of a *yes* or a *no* answer to the original question. In answering the original question, only an unequivocal *no* would be sufficient to

permit the conclusion that the animal MOA was not relevant to humans. Also, it was recognized that translation of the word "plausible" into other languages could be problematic. The question was therefore reworded to enable a *yes/no* answer, but qualified by the descriptor "reasonably", based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve scientific judgement based on transparent analysis of the available data.

This step represents a qualitative assessment of the relevance of the MOA to human cancer potential. Listing the critical specific key events that occur in the animal MOA and directly evaluating whether each of the key events might or might not occur in humans facilitate consideration and transparent presentation of the relevant information. Presentation in tabular form, referred to as a concordance table, can be helpful in delineating the relevant information (for an example, see Meek et al., 2003, case-study 6: kidney and liver tumours associated with chloroform exposure, Table 7; McGregor et al., current document, case-study on formaldehyde, Table 3). The key events (and possibly some of the critical associated processes) are listed with the information regarding these events for the animals in which the tumour was observed. It is intended that the information in these tables be brief, since a narrative explanation is expected to accompany the table. In the right-hand column, the effect on humans for each of the key events is evaluated. An additional column for the results in a different strain, species, sex, or route of administration that does not result in tumours can be useful if information is available for comparison with the model that leads to tumours. In addition, factors may be identified that, while not key themselves, can modulate key events and so contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology. Any such factors identified should be noted in a footnote to the concordance table.

The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical specific—for example, the enzyme induction response by phenobarbital in rodent liver, or the formation of a cytotoxic metabolite from chloroform by specific cytochrome P-450 enzymes. Later events are more generic to the MOA—for example, pleiotropic stimulation of hepatic proliferation or regenerative hyperplasia. Information that can be utilized to evaluate the key events in humans can come from in vitro and in vivo studies on the substance itself, but also can involve basic information regarding anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans. Information concerning an evaluation of the key event in humans exposed directly to the specific chemical is frequently unavailable.

As knowledge concerning the development of cancer evolves, it may become possible to combine some MOAs on the basis of the basic biology of the processes involved, thus relying less on chemical-specific information to reach a conclusion on the human relevance of a given MOA.

In evaluating the concordance of the information in humans to that in animals, a narrative describing the weight of evidence and an evaluation of the level of confidence for the human information need to be provided. Some specific types of information that are useful include the following:

- 1. cancer incidences at the anatomical site and cell type of interest, including age, sex, ethnic differences, and risk factors, including chemicals and other environmental agents;
- 2. knowledge of the nature and function of the target site, including development, structure (gross and microscopic), and control mechanisms at the physiological, cellular, and biochemical levels;
- 3. human and animal disease states that provide insight concerning target organ regulation and responsiveness;
- 4. human and animal responses to the chemical under review or analogues following short-, intermediate-, or long-term exposure, including target organs and effects.

Obviously, a substantial amount of information is required to conclude that the given MOA is not relevant to humans. If such a conclusion is strongly supported by the data, then chemicals producing animal tumours only by that MOA would not pose a cancer hazard to humans, and no additional risk characterization for this end-point is required. Since there is no cancer hazard, there is no cancer risk for the tumour under consideration.

The question of relevance considers all groups and life stages. It is possible that the conditions under which an MOA operates occur primarily in a susceptible subpopulation or life stage—for example, in those with a pre-existing viral infection, hormonal imbalance, or disease state. Special attention is paid to whether tumours could arise from early-life exposure, considering various kinetic and dynamic aspects of development during these life stages. Any information suggesting quantitative differences in susceptibility is identified for use in risk characterization.

# Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS workshop on the implications of a *yes* or a *no* answer to the original question. In answering the original question, only an unequivocal *no* would be sufficient to permit the conclusion that the animal MOA was not relevant to humans. The question was therefore reworded to enable a *yes/no* answer, but qualified by the descriptor "reasonably", based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve judgement based on transparent analysis of the available data.

For purposes of human relevance analysis, if the experimental animal MOA is judged to be qualitatively relevant to humans, a more quantitative assessment is required that takes into account any kinetic and dynamic information that is available from both the experimental animals and humans. Such data will of necessity be both chemical and MOA specific and will include the biologically effective doses required to produce the relevant dynamic responses from which neoplasia can arise. Kinetic considerations include the nature and time course of chemical uptake, distribution, metabolism, and excretion, while dynamic considerations include the consequences of the interaction of the chemical with cells, tissues, and organs. On occasion, the biologically effective dose that would be required to create these conditions would not be possible in humans. It may also be that quantitative differences in a biological process involved in a key event-for example, the clearance of a hormone-are so great that the animal MOA is not relevant to humans. However, the IPCS workshop recognized that only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. As with the qualitative assessment, a tabular comparison of quantitative data from the experimental animals and humans can facilitate the evaluation (for example, see Meek et al., 2003, case-study 5, thyroid tumours associated with exposure to phenobarbital, Table 6; Dellarco et al., current document, case-study on thiazopyr, Table 4). Useful comparisons can also be made with key events identified from studies of other compounds believed to induce effects by a similar MOA. For example, in the case of thiazopyr, information on the effects of phenobarbital in humans was particularly informative in evaluating the relevance of the MOA. As molecular and kinetic approaches continue to evolve, understanding of the similarities and differences of responses in animals and humans will be improved. It may become apparent that qualitative differences in a key event between an animal model and humans will be identified as being due to a specific quantitative difference, thus changing the answer to the second question (described above) to no.

As with question 2, if the conclusion to this question is *yes*, then chemicals producing animal tumours only by that MOA would not pose a cancer hazard to humans, and no additional risk characterization for this end-point is required.

### Statement of confidence, analysis, and implications

Following the overall assessment of each of the three questions, a statement of confidence is necessary that addresses the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database, and the nature and extent of the concordance analysis. An evaluation of alternative MOAs, using comparable analyses and rigour, is also essential. A critically important outcome of adequate consideration of the weight of the evidence for an overall MOA and the qualitative and quantitative concordance is the identification of specific data gaps that can be addressed experimentally in future investigations to increase confidence.

Infrequently, there may be conclusive epidemiological data on the cancer risk from a chemical that shares the MOA of the compound under consideration—that is, the compound does or does not cause cancer in humans. Obviously, such data would lend considerable weight to the conclusion of the human relevance evaluation. However, there may be occasions when, despite it being possible to establish an MOA in animals, there is insufficient information on the key events in humans to reach a clear conclusion on human relevance. In such circumstances, it might be possible to bridge this data gap by using epidemiological data. For example, the database on key events in humans for compounds that act like phenobarbital via activation of the constitutively active receptor (CAR) to induce hepatic tumours is incomplete. However, there are robust epidemiological data showing that exposure to phenobarbital for prolonged periods at relatively high doses does not cause cancer in humans. One possibility, therefore, is to "read across" from these findings with phenobarbital to any other compound that shares its MOA in animals in inducing rodent liver tumours and to conclude that the tumours caused by such a compound are not relevant to the risk assessment of the compound in humans (Holsapple et al., 2006). Such a conclusion would be critically dependent on the reliability of the epidemiological data and the similarity between the MOA for the chemical under test to that of the compound for which there are epidemiological data available.

In applying the framework to case-studies, it is apparent that much current research does not address key questions that would facilitate an analysis of an animal MOA or its relevance to humans. Often this has been because of lack of transparent delineation of key data gaps based on consideration of the data in analytical frameworks such as that presented here. Thus, use of the HRF can be very informative to researchers from the outset in the design of their studies.

The output of formal human relevance analysis provides information that is useful for more than just determining whether or not an end-point in animals is relevant to humans. Rather, consideration of the relevant information in a transparent, analytical framework provides much additional information that is critically important in subsequent steps in the risk characterization for relevant effects. Based on a human relevance analysis for a proposed MOA for relevant effects, it may be possible to predict, for example, site concordance or not of observed tumours in animals to humans. Application of the HRF also often provides information on relevant modulating factors that are likely to affect risk, such as hepatitis B and aflatoxin B<sub>1</sub> (see Cohen et al., current document, case-study on 4-aminobiphenyl). Analysis often also provides an indication of those components of a proposed MOA that may operate only over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in an MOA, then the relevance to human risk becomes a matter of exposure. Thus, the exposure assessment step of the subsequent risk characterization is critical to the proper evaluation of human cancer potential. In addition, information identified during the framework analysis can prove invaluable in hazard quantification based on the key events for the MOA.

Importantly, the human relevance analysis also contributes to identification of any special subpopulations (e.g. those with genetic predisposition) who are at increased risk and often provides information relevant to consideration of relative risk at various life stages. In some cases, this may be based not on chemical-specific information but rather on inference, based on knowledge of the MOA, as to whether or not specific age groups may be at increased or decreased risk.

The data and their analysis using the framework should be reported in a transparent manner, enabling others to determine the basis of the conclusions reached with respect to the key events, the exclusion of other MOAs, and the analysis of human relevance. As the specific form of presentation will vary with the type of data available, it is not helpful to be prescriptive on how the information should be reported. However, presentation should include sufficient details on the context and thought processes to ensure transparency of the conclusions reached. The use of appropriate tables can be helpful in presenting certain data, such as comparative analysis of key events in experimental animals and humans.

#### **Dissemination of the framework**

To assist in the dissemination and application of the IPCS HRF, a database of generally accepted MOAs and informative cases should be constructed and maintained. This would comprise a series of MOAs and their associated key events, for reference by those developing framework analyses for compounds that may act by similar MOAs. The case-studies would comprise worked examples that have been analysed using the framework, to provide an indication of the relevant level of detail of the analyses and nature of the weight of evidence required to support acceptance of a proposed MOA in causing the carcinogenic response. Such cases would be particularly valuable early in the development of a new MOA.

#### Application of the IPCS HRF to DNA-reactive carcinogens

Because of similarities in the carcinogenic process between rodents and humans and the comparable initial interactions with DNA by DNA-reactive carcinogens, it would be expected that, in general, DNA-reactive carcinogens would be assessed as progressing to the step of "yes, the key events in the animal MOA could occur in humans" in the ILSI/RSI HRF, as was the case for ethylene oxide (Meek et al., 2003), and "no" to the equivalent step in the IPCS HRF that asks the question, "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?", as was the case for 4-aminobiphenyl (Cohen et al., current document). In a recent paper, Preston & Williams (2005) presented a set of key events for tumour development that provided a guide for the use of the ILSI/RSI HRF with DNA-reactive carcinogens. This guide supported the view that for most DNA-reactive chemicals, the animal MOA would be predicted to be relevant to humans. However, it was also argued that there could be exceptions and that the ILSI/RSI HRF would be a valuable tool for identifying these. Use of the ILSI/RSI HRF and the IPCS HRF can also assist in quantifying differences in key events between rodents and humans that may be of value in extrapolating risk to humans. Not all rodent DNA-reactive carcinogens have been established to be human carcinogens, as judged by the International Agency for Research on Cancer (IARC) review process. For some of these exceptions, this human-rodent difference in tumour response is attributable to lower exposure of humans to the agent or to the relative insensitivity of epidemiological studies to detect tumour responses at low exposure levels. However, there are other reasons for such differences that are based on biological considerations. For example, if a DNA-reactive carcinogen induces tumours *only* in a species-specific organ, it is possible that the animal MOA based on key events might not be relevant to humans, although available data on MOA would need to be considered to permit such a conclusion. Similarly, the generally more proficient DNA repair processes that occur in humans compared with rodents (Cortopassi & Wang, 1996; Hanawalt, 2001) or a unique pathway of bioactivation in rodents could result in there being yes answers to the steps in the IPCS HRF that address the queries "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?" and/or "Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?" Alternatively, the IPCS HRF could provide quantitative information on these processes for use later in the risk characterization step.

The need in applying the IPCS HRF for DNA-reactive carcinogens is to develop a set of key events that would clearly describe the cancer process and use these as the guide for establishing the human relevance of a rodent tumour MOA for any particular DNA-reactive carcinogen under consideration.

### The IPCS HRF and risk assessment

Among the strengths of the framework are its flexibility, general applicability to carcinogens acting by any MOA, and the ability to explore the impact of each key event on the carcinogenic response. This includes determination of the nature of the dose–response curve, the identification and location of thresholds for individual key events, and their consequences for the overall tumour response curve. In addition, by considering the kinetic and dynamic factors involved in each key event, it may be possible to reach conclusions regarding the relevance or not of the carcinogenic response to specific subpopulations—for example, in early life, in those with particular diseases, or in those with specific polymorphisms. Alternatively, application of the framework can provide quantitative information on the differences between such groups. Application of the framework can also more generally inform the risk characterization of the chemical, even when it is concluded that the carcinogenic response per se is not relevant to humans.

As stated at the outset, MOA analysis and its human relevance counterpart are aspects of the hazard identification and characterization phases of risk assessment (National Research Council, 1983; Meek et al., 2003). Consistent with this paradigm, the human relevance case-studies referred to in the present report contribute to, but do not complete, a risk assessment for the chemicals under study. This is because a complete risk characterization requires not only evaluation of doses in the range of observations from experimental or occupational hygiene studies but also extrapolation to human exposure levels of interest in daily and lifetime activities.

Hazard characterization—and related MOA analysis—deals with the potential for harm in general terms, while the complete risk assessment puts this potential hazard into context with respect to exposure for decision-makers. Risk characterization seeks to describe the relationship between these effects and the doses to which humans are exposed in order to understand and estimate the nature and likelihood of effects in humans who are generally exposed at lower dose levels.

Understanding dose–response can have a profound effect on hazard characterization and therefore is an important component of the MOA analysis, particularly when non-linear processes or dose transitions are inherent in the relevant biology. Similarly, quantifying hazard in the context of dose informs the process of risk assessment by suggesting extrapolation models that are consistent with our understanding of the biology.

Estimating these generally lower human exposure levels is the task of the exposure analysis component of the risk assessment process. This usually involves extensive analysis of data collected from environmental media and plant and animal tissues, as well as those derived from pharmacokinetic models. This process also depends on analyses of human activity patterns and life stage and lifestyle factors that may bring about exposure. Ideally, based on

this information, a range of exposure scenarios is developed for different groups (men, women, children, infants, special groups, based, for example, on ethnicity or occupation) for use in identifying populations of concern. While hazard characterization, which is largely included in the framework analysis, involves quantification (dose–response analysis), estimating external exposures and contextualizing the hazard with respect to these estimates comprise subsequent steps in the risk assessment process. For example, in the case of melamine (Meek et al., 2003, case-study 7), it was concluded that the animal MOA was potentially relevant to humans. However, recognition that bladder carcinoma formation occurred only at very high doses carried forward to the subsequent stages of the risk assessment, exposure assessment, and risk characterization. The full risk assessment established that human exposures would not achieve levels necessary to produce bladder carcinomas, by a substantial margin.

### CONCLUSIONS

This IPCS HRF has been developed based on experience gained from the original 2001 IPCS MOA Framework and consideration of the 2003 ILSI/RSI human cancer relevance framework. Many aspects of these frameworks have been adopted, but a number of changes have been made to improve clarity and to introduce some elements not previously considered (e.g. sensitive subpopulations). The utility and role of the framework as an analytical tool within hazard characterization and within the overall risk assessment/characterization paradigm—that is, informing human relevance and dose–response extrapolation—have been emphasized. A number of general points and conclusions follow from the development of this framework:

- 1. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals.
- 2. Peer involvement and independent review are essential prerequisites for the general acceptance and scientific defensibility of a new MOA.
- 3. The framework is applicable to all MOAs for carcinogens, including DNA reactivity.
- 4. Although human relevance is likely to be assumed for most DNA-reactive carcinogens, the human relevance analysis is a valuable approach to enhance understanding, improve characterization of the hazard and risk, and identify exceptions.
- 5. When dealing with a chemical that may operate through a novel MOA, the analysis is focused on the chemical and entails a detailed evaluation via the HRF. However, when a specific chemical produces a tumour response consistent with an already established and peer-reviewed MOA through which other chemicals have been shown to operate, the analysis is then focused on the established MOA and a determination of whether the chemical produces its carcinogenic effect via the same key events established for the pathway.
- 6. When evaluating the human relevance of a tumour response found in experimental animals, the concordance analysis of key events is for the MOA and is not necessarily a chemical-specific evaluation. Chemical-specific and generic information relevant to the carcinogenic process can be valuable in the analysis. As knowledge advances, MOAs will become less chemical specific and will be based even more on the key biological

processes involved, allowing greater generalization of human relevance from one compound to another.

- 7. The biological understanding and significance of the key events can inform the approach to dose–response extrapolation for cancer risk, and thus understanding of the MOA can have a profound effect on the hazard and risk characterization, particularly when non-linear processes or dose transitions are inherent in the relevant biology.
- 8. It is recommended that a database of generally accepted MOAs and informative casestudies be established and maintained. It should provide examples that add to the existing case-studies developed by ILSI/RSI and IPCS and that are instructive in the application of the framework analysis. This database is particularly important as experience continues to evolve in the development of MOAs of carcinogens.
- 9. It is important to consider potentially susceptible subgroups and different life stages in the analysis.

In conclusion, the IPCS HRF provides a rigorous and transparent approach for judging whether data support a postulated mode of carcinogenic action for a chemical and for evaluating its relevance for humans. The scientific community is encouraged to use this approach as a means to increase the use of mechanistic information in cancer risk assessment and is encouraged to provide feedback, which may lead to additional refinements in the future. The framework is of value to both the risk assessment and research communities in furthering our understanding of carcinogenic processes, in identifying critical data gaps, and in informing the design of studies related to MOAs. When a carcinogenic response is considered potentially relevant to humans, information obtained on the key events during the analysis can prove invaluable in subsequent hazard quantification of the compound. It should be possible to extend the framework to non-cancer end-points, and further work on this is recommended. Thus, application of the IPCS HRF would be an invaluable tool for harmonization across end-points.

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#### REFERENCES

Cohen M, Meek ME, Klaunig JE, Patton DE, Fenner-Crisp PA (2003) The human relevance of information on carcinogenic modes of action: An overview. *Critical Reviews in Toxicology*, **33**:581–589.

Committee on Carcinogenicity (2004) *Guidance on a strategy for the risk assessment of chemical carcinogens*. London, Department of Health.

Cortopassi GA, Wang E (1996) There is substantial agreement among interspecies estimates of DNA repair activity. *Mechanisms of Ageing and Development*, **91**:211–218.

Hanawalt PC (2001) Revisiting the rodent repairadox. *Environmental and Molecular Mutagenesis*, **38**:89–96.

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP (2006) Mode of action in relevance of rodent livers to human cancer risk. *Toxicological Sciences*, **89**:51–56.

IPCS (2000) Scoping meeting to address the human relevance of animal modes of action in assessing cancer risk, Carshalton, United Kingdom, 8–10 November 2000. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2004) *Report of the first meeting of the Cancer Working Group, Arlington, Virginia, USA, 3–5 March 2004.* Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2005) *Record of the Cancer Framework Workshop, Bradford, United Kingdom, 21–23 April 2005.* Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

National Research Council (1983) *Risk assessment in the federal government. Managing the process.* Washington, DC, National Academy Press.

Preston JR, Williams GM (2005) DNA-reactive carcinogens: Mode of action and human cancer hazard. *Critical Reviews in Toxicology*, **35**:673–683.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity: Case studies. *Toxicology and Applied Pharmacology*, **20**:226–294.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

USEPA (1999) *Guidelines for carcinogen risk assessment (review draft)*. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (NCEA-F-0644).

USEPA (2005) *Guidelines for carcinogen risk assessment*. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (EPA/639/P-03/001F).

### THIAZOPYR AND THYROID DISRUPTION: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

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Thiazopyr increases the incidence of male rat thyroid follicular cell tumours; however, it is not carcinogenic in mice. Thiazopyr is not genotoxic. Thiazopyr exerts its carcinogenic effect on the rat thyroid gland secondary to enhanced metabolism of thyroxine leading to hormone imbalance. The relevance of these rat tumours to human health was assessed by using the 2006 International Programme on Chemical Safety Human Relevance Framework. The postulated rodent tumour mode of action (MOA) was tested against the Bradford Hill criteria and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well established MOA for thyroid follicular cell tumours. Although the postulated MOA could theoretically operate in humans, marked quantitative differences in the inherent susceptibility for neoplasia to thyroid hormone imbalance in rats allows for the conclusion that thiazopyr does not pose a carcinogenic hazard to humans.

A number of chemical substances have been shown to induce thyroid follicular cell tumours in rats through a mode of action (MOA) that involves perturbation of thyroid hormone homeostasis via reduction of circulating thyroid hormones (Hurley et al., 1998; Capen et al., 1999; IARC, 2001). Homeostatic responses to low thyroid hormone concentrations result in a compensatory increase in the release of thyroid stimulating hormone (TSH) from the pituitary gland, which in turn stimulates the thyroid gland to increase thyroid hormone synthesis and release. Persistent elevation of TSH levels leads to thyroid follicular cell hypertrophy and hyperplasia, which, if maintained (as a result of continuous exposure to the compound), can eventually lead to neoplasia. This neoplastic MOA in rats is well accepted by the scientific community, and both the International Agency for Research on Cancer (Capen et al., 1999; IARC, 2001) and the United States Environmental Protection Agency (USEPA, 1998) have established specific guidance or policies for evaluating the human relevance of rodent thyroid follicular cell tumours.

Thiazopyr, a herbicide that induces rat thyroid follicular cell tumours by its effect on thyroid homeostasis, was the case-study used to illustrate the original 2001 International Programme on Chemical Safety (IPCS) framework for mode of carcinogenic action analysis (Sonich-Mullin et al., 2001). Thiazopyr's MOA is revisited as a case-study here to illustrate the additional guidance provided in the 2006 IPCS Human Relevance Framework (HRF) for evaluation of a neoplastic MOA for humans. This updated case-study highlights how accumulating experience with a particular MOA can make subsequent analyses less difficult. Because this case-study is based on an established MOA in which the key events have been well defined, this analysis will focus on whether thiazopyr produces the biological effects

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expected of this pathway. This case-study also emphasizes the importance of understanding the basic physiological processes underlying a toxicity pathway in animals and humans. For some compounds, chemical-specific data might be critical in evaluating the key events in humans. For others, the underlying biology is sufficient to allow interpretation of the human relevance of the carcinogenic MOA, both qualitatively and quantitatively. Thiazopyr is an example of the latter. Another MOA case-study of thyroid hormone disruption and the human relevance of rat thyroid follicular cell tumours is available for phenobarbital (Lehman-McKeeman & Hill, in Meek et al., 2003).

The present MOA analysis begins with a brief summary of the available information on the carcinogenicity of thiazopyr, followed by a discussion of the experimental biochemical and histopathological data considered for this thyroid disruption MOA. It is not intended to be a comprehensive assessment of the chemical per se.

### CARCINOGENICITY DATA

Human epidemiological data on the carcinogenicity of thiazopyr are not available. Thiazopyr produces effects on liver and thyroid in various laboratory species, including mice, rats, and dogs. Thiazopyr was found to induce thyroid tumours in male rats only and appears to do so by increasing the hepatic metabolism and excretion of thyroid hormones.

Chronic dietary administration of thiazopyr to mice and rats resulted primarily in thyroid follicular cell tumours in male rats but not in female rats (Naylor & McDonald, 1992; Naylor & Raju, 1992). There were no significant increases in the incidences of any tumours in either sex in the chronic study of mice treated with thiazopyr at up to 800 mg/kg in the diet (128.4 mg/kg body weight [bw] per day in males and 215.9 mg/kg bw per day in females) (Naylor & Raju, 1992). In the rat carcinogenicity study, thiazopyr (technical, 94.8% pure) was administered to male and female Sprague-Dawley (SD) rats (60 per sex per group) at dietary concentrations of 0, 1, 10, 100, 1000, or 3000 mg/kg, providing dose levels of 0, 0.04, 0.4, 4.4, 44.2, or 136.4 mg/kg bw per day for males and 0, 0.06, 0.6, 5.6, 56.3, or 177.1 mg/kg bw per day for females (Naylor & McDonald, 1992). The incidences of thyroid follicular cell adenomas and carcinomas were increased in male rats of the 1000 mg/kg (44.2 mg/kg bw per day) and 3000 mg/kg (136.4 mg/kg bw per day) groups (Table 1). It should be noted that the increase in tumour incidence in male rats is primarily accounted for by benign tumours.

### POSTULATED MOA FOR THE INDUCTION OF THYROID FOLLICULAR CELL TUMOURS IN RATS

The postulated MOA for thiazopyr-induced thyroid follicular cell tumours involves the perturbation of homeostasis of the pituitary–thyroid axis by an extrathyroidal mechanism. Specifically, thiazopyr induces hepatic thyroxine (T4)-uridine diphosphate (UDP) glucurono-syltransferase (UGT) activity, leading to enhanced metabolism of T4 by conjugation and increased biliary excretion of the conjugated hormone. The result of this enhanced liver metabolism is a decrease in serum T4 (and sometimes triiodothyronine, or T3) half-life. The pituitary gland responds to a decrease in circulating serum levels of T4 by enhancing the output and serum level of TSH. Prolonged elevation of circulating TSH levels stimulates the

thyroid gland to deplete its stores of thyroid hormone and continues to induce hormone production. Thus, the thyroid follicular cells enlarge (hypertrophy) and are induced to proliferate at an increased rate and to increase in number (hyperplasia). With chronic exposure, thyroid hyperplasia eventually progresses to neoplasia.

### Table 1. Thyroid follicular cell tumour incidence in Sprague-Dawley male rats(2-year chronic study).

		Dose (mg/kg bw per day) <sup>a</sup>					
	0	0.04	0.4	4.4	44.2	136.4 <sup>b</sup>	
Adenomas	1/50	2/47	0/49	2/47	8/49	12/48	
Carcinomas	1/50	1/47	0/49	0/47	1/49	4/48	
Combined	2/50	3/47	0/49	2/47	9/49	14/48	
%	(2)	(6)	(0)	(4)	(18)	(29)	
Ρ	0.000 <sup>c</sup>	0.470	0.253	0.668	0.024*	0.001**	

Note: Tumour incidences were extracted from data submitted to the USEPA Office of Pesticide Programs (Naylor & McDonald, 1992). Significance: \* P < 0.05; \*\* P < 0.01 (statistical analyses based on Fisher's exact test).

<sup>a</sup> Doses in mg/kg bw per day were estimated.

<sup>b</sup> Two animals in the 136.4 mg/kg bw per day or 3000 mg/kg diet dose group had both benign and malignant tumours.

<sup>c</sup> For trend with dose.

### **KEY EVENTS IN EXPERIMENTAL ANIMALS**

The sequence of key events in thiazopyr's mode of carcinogenic action includes:

- induction of hepatic UGT activity;
- increase in hepatic metabolism and biliary excretion of T4;
- decrease in serum T4 half-life and concentration;
- increase in circulating TSH concentration;
- cellular thyroid hypertrophy and follicular cell hyperplasia.

An evaluation follows to determine whether thiazopyr works via disruption of thyroid– pituitary status by increasing hepatic clearance of circulating thyroid hormone. Thus, based on the key events listed above, biological indicators of thiazopyr's MOA should include changes in liver metabolism, alterations in hormone levels, increases in thyroid growth, and lesion progression in the thyroid. These effects have been observed and measured in male rats in short-term and subchronic studies, and at interim and terminal sacrifices in a chronic study (Hotz et al., 1997). The dose–response and temporal analyses of the key events and tumour response are presented below.

### DOSE-RESPONSE RELATIONSHIP AND CONCORDANCE

A summary of the no-observed-adverse-effect levels (NOAELs) and lowest-observedadverse-effect levels (LOAELs) for the key effects in thiazopyr's MOA are provided in Table 2. In the 56-day study by Hotz et al. (1997), male SD rats (20 per dose) were fed diets containing thiazopyr at 0, 10, 30, 100, 300, 1000, or 3000 mg/kg (doses not measured, but estimated to be 0, 0.5, 1.5, 5, 15, 50, and 150 mg/kg bw per day) for 56 days and evaluated for the effects on liver (weights, T4-hepatic UGT activity, T4 biliary elimination), thyroid (weights, hypertrophy/hyperplasia), and hormones (serum levels of T4, T3, reverse T3, or rT3, and TSH). In this study, the effects on liver, thiazopyr's primary site of action, appear to be the most sensitive indicator of pituitary-thyroid homeostasis perturbation. Statistically significant increases in hepatic T4-UGT activity in the 50 and 150 mg/kg bw per day groups (approximately 3- and 6-fold increases in activity over controls when adjusted for liver weight, respectively) were found at the end of the 56-day treatment period. Consistent with the increase in T4-UGT activity, clearance of T4 from the blood and elimination in bile (40% increase in excretion of <sup>125</sup>I-labelled T4) were increased after 150 mg/kg bw per day of thiazopyr (only dose evaluated). Statistically significant increases in liver weight were found at 15, 50, and 150 mg/kg bw per day of thiazopyr in the 56-day study in male rats by Hotz et al. (1997). In the 2-year rat study (Naylor & McDonald, 1992), absolute liver weights were increased by 122% at 44.2 mg/kg bw per day and by 178% at 136.4 mg/kg bw per day relative to controls. There were also statistically significant increases in the incidence of liver hypertrophy at 44.2 and 136.4 mg/kg bw per day (47/61 and 52/60 versus 0/60 in controls, respectively) in the 2-year rat study.

Effect	NOAEL/LOAEL			
Liver				
Induction of UGT	15/50 mg/kg bw per day (56-day study)			
Increase in T4 biliary elimination	<150/150 mg/kg bw per day (only dose tested in 56-day study)			
Increase in liver weight	5/15 mg/kg bw per day (56-day study)			
	44.2/136.4 mg/kg bw per day (2-year study)			
Hepatocellular hypertrophy	4.4/44.2 mg/kg bw per day (2-year study)			
Hormones				
Decrease in serum T4	50/150 mg/kg bw per day (56-day study)			
Increase in serum TSH	50/150 mg/kg bw per day (56-day study			
Thyroid				
Increase in thyroid weight	15/50 mg/kg bw per day (56-day study)			
	44.2/136.4 mg/kg bw per day (2-year study)			
Increase in thyroid hyperplasia	44.2/136.4 mg/kg bw per day (2-year study)			
Increase in thyroid tumours	4.4/44.2 mg/kg bw per day (2-year study)			

Table 2. Summary of effects on liver, hormones, and thyroid from a 56-day study (Hotz
et al., 1997) and the 2-year chronic study (Naylor & McDonald, 1992) in male rats.

Consistent with the enhanced hepatic clearance of T4 described above, when Hotz et al. (1997) treated male SD rats with doses of thiazopyr, statistically significant ( $P \le 0.05$ ) decreases in serum T4 levels (by 30%) and increases in TSH (by 60%) were found after 56 days of treatment at the highest dose tested (Table 3). T3 serum levels were non-significantly lower at 1.5 mg/kg bw per day and statistically significantly higher at 150 mg/kg bw per day after 56 days of treatment. In general, hepatic microsomal enzyme inducers appear to affect T3 less than T4; thus, T4 and TSH tend to be more reliable indicators of altered pituitary–

thyroid homeostasis (Liu et al., 1995; Hurley et al., 1998; Hood et al., 1999). In the case of thiazopyr, there appears to be a poor correlation between the doses causing the T4 and TSH effects and those causing an increased incidence of thyroid follicular cell tumours. The lowest dose of thiazopyr producing a statistically significant (P < 0.05) increase in thyroid follicular cell tumours in male SD rats was 44.2 mg/kg bw per day in the 2-year study, whereas the NOAEL for effects on T4 and TSH was 50 mg/kg bw per day in the 56-day study (Table 2). Generally, effects on liver enzymes/weight and pituitary-thyroid hormone concentrations would be anticipated to occur at doses at least as low as those that produce thyroid weight changes and increases in thyroid tumour incidence, given that this thyroid disruption MOA is a threshold phenomenon. This apparent discrepancy is probably not real, because neither of the doses quoted is accurate. In the 2-year study, the milligrams per kilogram body weight doses were averaged estimates for the entire study, whereas the relevant doses for comparison with the 56-day mechanistic study are those for rats of 12-20 weeks of age. These doses would have been at least 2-fold higher than those that were readily available (so the real LOAEL for neoplasia would have been about 90 mg/kg bw per day). They would also have been more relevant for neoplasia, because the critical period for hormonal perturbations (e.g. prolonged elevation of TSH) to initiate pathological changes would be early, not late, in the 2-year study. The doses calculated for the 56-day study are also likely to be inaccurate, because food intake information was not available in the publication; the doses are estimates based on assumed intakes. Having acknowledged this uncertainty, it is observed that thyroid weights were increased significantly at 50 mg/kg bw per day and liver weights were increased at 15 mg/kg bw per day, which is consistent with the liver being the initial target in thiazopyr's MOA.

	Dose (mg/kg bw per day) <sup>a</sup>							
	0	0.5	1.5	5	15	50	150	
T4 (µg/dl)	4.1 ± 0.2	4.3 ± 0.3	3.9 ± 0.2	4.1 ± 0.2	4.0 ± 0.2	4.0 ± 0.2	2.9 ± 0.1 <sup>a</sup>	
T3 (ng/dl)	84 ± 3	82 ± 4	68 ± 2	84 ± 3	82 ± 3	91 ± 4	110 ± 6 <sup>a</sup>	
TSH (ng/ml)	2.7 ± 0.2	$3.5 \pm 0.4$	2.7 ± 0.1	3.1 ± 0.4	2.9 ± 0.3	3.1 ± 0.2	$4.3 \pm 0.4^{a}$	

Table 3. Fifty-six-day study in male rats: Hormonal effects (Hotz et al., 1997).

Note: The mg/kg bw per day doses were estimated. Values are mean ± standard error of the mean; 19 or 20 animals per group.

Significantly different from control with Dunnett's test after analysis of variance (ANOVA) ( $P \le 0.05$ ).

As stated above, prolonged TSH stimulation leads to both hypertrophy and hyperplasia of the thyroid. In the 2-year rat study, there was a poor dose correlation between thyroid hyperplasia alone and tumour incidence. While tumour incidence was increased at 44.2 mg/kg bw per day, a statistically significant increase in the incidence of hyperplasia (8/58 versus 1/60 in controls) was found only at 136.4 mg/kg bw per day. Furthermore, in the 56-day rat study, where thyroid histology was reported as follicular cell hypertrophy and hyperplasia combined, there was a significant increase in the incidence of this diagnosis at 150 mg/kg bw per day but not at lower doses (Hotz et al., 1997). There was, however, a good dose correlation between increases in thyroid weights in the 56-day study and tumour incidence in the 2-year study. Statistically significant increases in thyroid weights of 46% were found at 150 mg/kg bw per day and 25% at 50 mg/kg bw per day (Hotz et al., 1997).

### **TEMPORAL RELATIONSHIP**

If an event (or events) is an essential element of tumorigenesis, it must precede tumour appearance. Multiple exposure time data at 7, 14, 28, 56, and 90 days are available in which male SD rats were offered diets containing thiazopyr at 3000 mg/kg (150 mg/kg bw per day) (Hotz et al., 1997). Liver weights and hepatic T4-UGT activity were increased at all observation times from the earliest time of assessment on day 7. Biliary excretion of conjugated T4 was not measured in this experiment; however, serum T4 was reduced at all observation times. Increases in circulating TSH were observed at all sampling times, although the increase was not significant at 14 days after treatment began. Increases in thyroid weight were also observed at all sampling times. Histologically, there was a time-related increase in hypertrophy/hyperplasia beginning at 14 days. In the 2-year rat study, the first thyroid adenoma was observed at week 69 at a dose of 136.4 mg/kg bw per day. Thus, there is a logical temporal response for the key events in thiazopyr-induced thyroid follicular cell tumour formation in which all key events precede tumour formation.

### STRENGTH, CONSISTENCY, AND SPECIFICITY OF ASSOCIATION OF THE TUMOUR RESPONSE WITH KEY EVENTS

Strength, consistency, and specificity of the association can be established from the studies described above. The quantifiable precursor events, fundamental to the proposed MOA, are relatively consistent with the emergence of thyroid follicular cell tumours. Observation of liver weight increase and induction of hepatic T4-UGT in rats receiving the thiazopyr in the diet would be consistent with perturbation of homeostasis of the pituitary-thyroid axis by an extrathyroidal mechanism. An increase in hepatic T4-UGT activity is a step occurring before the other key biochemical changes and before thyroid follicular cell hypertrophy and hyperplasia. Thiazopyr treatment clearly results in a decrease in circulating T4 and an increase in TSH following enhanced liver metabolism of T4. Furthermore, in subchronic studies, the increases in thyroid weight and the development of hypertrophy/hyperplasia were shown to appear to a statistically significant degree under the same conditions of dose and time as the appearance and reversal of changes in thyroid hormone levels and thyroid hormone metabolism. Stop/recovery studies (Hotz et al., 1997) showed that cessation of thiazopyr dosing was followed by a return of hormone levels to control values, as well as a reduction in liver and thyroid weights and reversal of hyperplasia of thyroid follicular cells. Early dosing withdrawal would be expected to result in a reversal of hypothyroidism and of lesion progression for this non-genotoxic MOA. The only sign that was slow to reverse was the increase in thyroid weight after the longest dosing period.

### **BIOLOGICAL PLAUSIBILITY AND COHERENCE**

There are considerable data from studies in laboratory rodents demonstrating the relationship between sustained perturbation of the hypothalamic–pituitary–thyroid axis, prolonged stimulation of the thyroid gland by TSH, and the progression of thyroid follicular cells to hypertrophy, hyperplasia, and eventually neoplasia (McClain, 1995; Hard, 1998; Hurley et al., 1998; Capen et al., 1999; IARC, 2001). Increased secretion of TSH may result via several mechanisms, including increased hepatic clearance of T4, as is the case with thiazopyr.

Circulating levels of T4 are monitored by the thyrotropic cells of the pituitary gland that are responsible for the synthesis of TSH. In the pituitary gland, T4 is metabolized by 5'-deiodinase type II to T3, which then binds to specific receptors in the cell nucleus. A decrease in T3 receptor occupancy results in stimulation of TSH synthesis and secretion. Studies in vivo have shown that injection of rats with TSH leads to reductions in thyroid follicular cell nuclear statin, a non-proliferation-specific nuclear antigen, indicating that these cells were leaving the non-dividing state to resume the cell cycle (Bayer et al., 1992). This study showed that low, repeated doses of TSH (0.25 IU per rat twice daily) produced a cumulative response in nuclear statin levels over 10 days, which returned to normal resting levels within 5 days of cessation of TSH injections. Reduction in nuclear statin is also an early event that parallels the earliest known pinocytotic response to TSH. These data are consistent with increased TSH concentrations alone causing thyroid follicular cells of rats to enter a state of preproliferation. Therefore, the suggestion that thiazopyr causes thyroid follicular cell neoplasms in rats by initially inducing hepatic T4-UGT is coherent with the known physiology of the hypothalamus-pituitary-thyroid dynamic control system, at least to the stage of hypertrophy and hyperplasia.

Lastly, the tumour response elicited by thiazopyr is typical of a rodent thyroid carcinogen, in that thyroid follicular cell tumours are found in male rats but not in female rats or mice. Rats tend to be more sensitive to thyroid carcinogenesis than mice, and male rats are frequently found to be more sensitive than female rats with respect to the proportion of chemicals that induce thyroid tumours (Hurley et al., 1998). In keeping with this, TSH levels are typically higher in male rats than in females (Hill et al., 1989). In addition, male rats are sometimes more prone to hepatic enzyme induction than females of the same strain, but this depends on the enzyme in question, the dose of the inducing compound, and the age of the animals (Sundseth & Waxman, 1992; Agrawal & Shapiro, 1996; Oropeza-Hernandez et al., 2003).

# OTHER MODES OF ACTION

Mutagenesis is always one possible MOA to consider, but no genetic toxicity has been demonstrated for thiazopyr in the following tests:

- mutation in four strains of *Salmonella typhimurium* (Bakke, 1989a);
- mutation at the *hgpt* locus of Chinese hamster ovary cells (Li & Myers, 1989);
- micronucleus induction in bone marrow cells of mice treated in vivo (Flowers, 1990);
- unscheduled DNA synthesis induction in hepatocytes of rats treated in vivo (Bakke, 1989b).

Therefore, the available evidence indicates that mutagenesis is not an alternative MOA for thiazopyr.

Additional effects on the hypothalamic–pituitary–thyroid axis and disruption of other pathways of thyroid hormone metabolism are other possibilities for altering thyroid homeostasis. These variations would not differ in any fundamental way from the one that has been proposed for thiazopyr, in that all would lead to prolonged TSH stimulation with continuous exposure.

# UNCERTAINTIES, INCONSISTENCIES, AND DATA GAPS

There appears to be a lack of dose concordance for thyroid tumours and hormone changes, but this is likely to be due to inaccuracies in the milligrams per kilogram body weight doses compared—which either were estimated (versus calculated on the basis of food consumption and body weight data) and cover an early period in the life of rats or were averages for the whole duration of the experiment—as well as experimental variability.

# ASSESSMENT OF POSTULATED MODE OF ACTION

The data presented are judged, with a moderately high degree of confidence, to be adequate to explain the development of thyroid follicular cell tumours in male rats following chronic dietary exposure to thiazopyr. Thiazopyr clearly increased liver weights (i.e. the initial target organ) at doses lower than those causing tumours and enhanced thyroid growth (i.e. increased thyroid weights) at the lowest tumorigenic dose.

#### Human applicability of the proposed MOA

The IPCS HRF, which was developed from the Risk Science Institute/International Life Sciences Institute "Human Relevance Framework" (Meek et al., 2003) and modified based on discussions by the IPCS Cancer Working Group (Boobis et al., current document), presents a four-part approach to addressing a series of three questions and leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumours.

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals? As described in detail above, there is clear evidence that thiazopyr alters thyroid homeostasis by UGT induction, by reducing serum T4 levels and consequently elevating serum TSH.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? The current understanding of the regulation of thyroid hormone homeostasis in humans and of the role of increased TSH levels (as a result of altered thyroid homeostasis) as a risk factor for thyroid cancer was considered in order to assess the human relevance of the key events in thiazopyr's animal mode of carcinogenic action. Although there are substantial quantitative dynamic differences (discussed below), the fundamental mechanisms involved in the function and regulation of the hypothalamic-pituitary-thyroid axis in rats are qualitatively similar to those in humans (Bianco et al., 2002). Therefore, an agent that decreases T4 levels in rats could likewise reduce T4 in humans; this, in turn, could potentially lead to an increase in TSH levels. There are data showing that rodents and humans respond in a similar fashion to perturbations of pituitary-thyroid function. For example, it is well known that iodine deficiency, which readily leads to decreased thyroid hormone levels, stimulates thyroid cell proliferation in humans, leading to goitre. If left untreated, iodine deficiency may lead to tumour formation, albeit rarely (Thomas & Williams, 1999). Although there is no evidence of increased susceptibility to thyroid cancer, a number of pharmaceuticals (e.g. propylthiouracil, lithium, amiodarone, iopanoic acid) that disrupt thyroid homeostasis by acting directly on the thyroid gland (e.g. by inhibiting hormone synthesis or release or by blocking the conversion

of T4 to T3) are known to lead to hypothyroidism and increases in TSH in humans (Ron et al., 1987).

In contrast to rats, no increases in TSH levels have been found in humans following exposure to agents that induce hepatic microsomal enzymes and reduce circulating T4 levels (discussed in Lehman-McKeeman & Hill, in Meek et al., 2003). For example, the pharmaceutical compounds phenytoin, rifampin, and carbamazepine induce hepatic microsomal enzymes, including UGT, and reduce circulating T4 levels, but TSH levels are unchanged (Curran & DeGroot, 1991); agents that produce thyroid tumours in rats by increasing glucuronidation and biliary excretion of T4 at high experimental doses (e.g. omeprazole, lansoprazole, and pantoprazole) produce no changes in thyroid hormones at clinical doses in humans (Masubuchi et al., 1997). Thus, there appears to be a substantial difference in the dose–response relationship for altered homeostasis of the pituitary–thyroid axis in rats compared with humans. As discussed below, this observation is due to quantitative dynamic differences between rats and humans in the basic physiological processes underlying pituitary–thyroid function.

3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans? Thiazopyr does not target the thyroid directly. Rather, its primary effect is on hepatic metabolizing enzymes, and the increase in metabolic activity indirectly increases the systemic clearance of T4, leading to the hypothyroid state and the compensatory increase in TSH found in rats. Although there are no chemical-specific data on the potential for thiazopyr to disrupt thyroid hormone homeostasis in humans, a number of other microsomal enzyme inducers have been extensively studied, such as phenobarbital (Lehman-McKeeman & Hill, in Meek et al., 2003). As discussed above, agents that produce hypothyroidism by altering hepatic clearance of T4 do not appear to result in elevated TSH levels in humans. Presumably, TSH is not increased because a critical reduction of T4 is not reached.

There are several important physiological and biochemical differences between rats and humans related to thyroid function. Rats have a smaller reserve capacity of thyroid hormones when compared with humans. The rat has a much shorter thyroid hormone half-life than humans. The half-life of T4 is about 12 h in rats compared with 5–9 days in humans (Dohler et al., 1979). The shorter half-life in rats is likely related to the absence of a high affinity binding globulin for T4 that is present in humans (Hill et al., 1989). In rats, the increased clearance contributes to the need for a higher rate of production of T4 (per unit of body weight) to maintain normal levels of T4. In contrast, in humans, the binding of thyroid hormone to this globulin accounts for a slower metabolic degradation and clearance, which in turn result in the thyroid gland being less active than in rats. The constitutive TSH levels are approximately 25 times higher in rats to agents that reduce T4 and lead to elevated TSH. There is no increased risk of thyroid tumour development if TSH is not elevated.

Another difference of rats compared with humans is the histological appearance of the thyroid. This histological difference is related to the higher rate of production of T4 to

maintain a consistent serum concentration, thus making the rat thyroid more "functionally active" than that of primates, including humans (McClain, 1995). More of the follicular epithelium in the rat is stimulated to synthesize thyroglobulin, and therefore more of the follicular cells are tall cuboidal and appear to be active in synthesis. In contrast, more of the follicular cells in humans tend to be short cuboidal or almost squamous in appearance, suggesting they are quiescent. Because rat follicular cells are already generally active, under stimulation from TSH, they will respond with hyperplasia more readily than human follicular cells. Because of the greater storage capability of the human thyroid and the greater numbers of cells in a quiescent state, human thyroid follicular cells will be roused from their quiescent state to synthesize and secrete additional thyroid hormone without the need for a hyperplastic response to re-establish homeostasis. Therefore, the primary response in the human thyroid gland would be thyroglobulin reabsorption and cellular hypertrophy rather than hyperplasia. In short, there is much greater buffering capacity in the biochemistry of the human than the rat thyroid.

Even though certain agents can cause a reduction in thyroid hormone levels in humans, there is no clear evidence that these agents increase susceptibility to thyroid cancer (Ron et al., 1987). For example, epidemiological studies with phenobarbital do not show any increased risk of thyroid cancer (Olsen et al., 1993). Studies of individuals with conditions that would lead to elevated TSH (patients with Graves disease or goitre) indicate that the occurrence of thyroid cancer is rare in these circumstances (e.g. Mazzaferri, 2000; Gabriele et al., 2003). A study of environmental and heritable causes of cancer among 9.6 million individuals, using the nationwide Swedish Family-Cancer Database, found that the environment did not appear to play a principal causative role in thyroid cancer (Lichtenstein & Hemminki, 2002). The only known human thyroid carcinogen is radiation, a mutagenic exposure.

As summarized in Table 4, there is sufficient evidence in the general literature on the biochemical and physiological differences in thyroid function to indicate differences in tumour susceptibility between rats and humans. In contrast to humans, rats are very susceptible to thyroid neoplasia secondary to hypothyroidism. In particular, modest changes in thyroid hormone homeostasis will promote tumour formation in rats. Thus, thyroid tumours induced by thiazopyr involving increased hepatic clearance of hormone and altered homeostasis of the pituitary–thyroid axis in rodents are considered not relevant to humans, based on quantitative dynamic differences.

4. Conclusion: statement of confidence, analysis, and implications. There is sufficient experimental evidence to establish a thyroid disruption MOA for thiazopyr-induced thyroid follicular cell tumours in rats. Although thiazopyr may potentially result in hypothyroidism in humans, there is sufficient quantitative evidence on the basic physiological processes in the general literature to conclude that thyroid tumours induced by a process involving increased hepatic clearance of thyroid hormone and altered homeostasis of the pituitary–thyroid axis in rodents is not likely to lead to an increase in susceptibility to tumour development in humans. Although there are no human data on thiazopyr, clinical data on other hepatic microsomal enzyme inducers were critical to this human relevance analysis. The general literature provided sufficient evidence to show that unlike in the rat, decreased T4 levels typically show no evidence of compensatory increases in TSH levels in humans. There is also cellular and biochemical evidence that the rat pituitary-thyroid axis is much more sensitive than that in humans to such perturbations. This sensitivity is likely the result of the rapid turnover of T4 in rats coupled with the higher demand for TSH to maintain thyroid activity.

Key event	Evidence in rats	Evidence in humans
Increased hepatic clearance of T4	In short-term and chronic rat studies, the liver is found to be the most sensitive target, and evidence of increased T4 hepatic clearance is provided by studies on T4-hepatic UGT activity, T4 half-life, T4 biliary elimination, liver weights, and hypertrophy.	No data available for thiazopyr, but microsomal enzyme induction is plausible.
Decreased serum T4	Direct experimental evidence.	No data available for thiazopyr, but plausible given that other microsomal enzyme inducers have been shown to reduce T4 in humans.
Increased TSH levels	Direct experimental evidence.	No data available for thiazopyr, but other microsomal enzyme inducers have not been shown to increase TSH levels even when T4 is decreased.
Increased TSH increases thyroid cell proliferation and tumour formation	Direct experimental evidence.	Induction of thyroid follicular cell tumours secondary to hypothyroidism is remote in humans, given the quantitative differ- ences in thyroid function/homeostasis. Occurrence of thyroid cancer is rare even in severely hypothyroid individuals.

#### Table 4. A comparison of key events in rats and humans.

# **IMPLICATIONS OF THE IPCS HRF**

The thiazopyr example is an illustration of an induced tumour response consistent with an MOA that has been previously defined and established. Thus, addressing the first question in the framework analysis, "Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?", became a determination of whether the data set on the chemical conforms to the same key events defined for the pathway of interest. This example further demonstrates how data on the basic understanding of the biological processes involved in the MOA provide an important means to compare the rodent and human key events. Thus, this generic human information was essential to evaluating the qualitative and quantitative differences between experimental animals and humans in addressing the plausibility of the cancer MOA for humans (i.e. questions 2 and 3 in the HRF).

### REFERENCES

Agrawal AK, Shapiro BH (1996) Phenobarbital induction of hepatic CYP2B1 and CYP2B2: Pretranscriptional and post-transcriptional effects of gender, adult age, and phenobarbital dose. *Molecular Pharmacology*, **49**(3):523–531.

Bakke JP (1989a) *Ames/*Salmonella *mutagenicity assay with MON 13200: Study No. ML-88-191/EHL No. 88124.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275535).

Bakke JP (1989b) *Evaluation of MON 13200 to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay in the male F-344 rat: Study No. SR-88-204/SRI No. LSC 6327.* Testing facility: SRI International, Menlo Park, CA. Submitted by Monsanto, St. Louis, MO (MRID No. 42275538).

Bayer I, Mitmaker B, Gordon PH, Wang E (1992) Modulation of nuclear statin expression in rat thyroid follicle cell following administration of thyroid stimulating hormone. *Journal of Cellular Physiology*, **150**:276–282.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, **23**(1):38–89.

Capen CC, Dybing E, Rice JM, Wilbourn JD, eds (1999) *Species differences in thyroid, kidney and urinary bladder carcinogenesis.* Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 147).

Curran PG, DeGroot LJ (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Reviews*, **12**:135–150.

Dohler KD, Wong CC, Von Zur Muhlen A (1979) The rat as a model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacology and Therapeutics*, **5**:305–318.

Flowers LJ (1990) *Micronucleus assay with MON 13200: ML-88-390/EHL Study No. 88230.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275537).

Gabriele R, Letizia C, Borghese M, De Toma G, Celia M, Izzo L, Cavalla A (2003) Thyroid cancer in patients with hyperthyroidism. *Hormone Research*, **60**(2):79–83.

Hard GC (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. *Environmental Health Perspectives*, **106**(8):1–21.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF (1989) Thyroid follicular cell carcinogenesis. *Fundamental and Applied Toxicology*, **12**(4):629–697.

Hood A, Liu YP, Gattone VH 2nd, Klaassen CD (1999) Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicological Sciences*, **49**:263–271.

Hotz KJ, Wilson AG, Thake DC, Roloff MV, Capen CC, Kronenberg JM, Brewster DW (1997) Mechanism of thiazopyr-induced effects on thyroid hormone homeostasis in male Sprague-Dawley rats. *Toxicology and Applied Pharmacology*, **142**:133–142.

Hurley PM, Hill RN, Whiting RJ (1998) Mode of carcinogenic action of pesticides inducing thyroid follicular-cell tumors in rodents. *Environmental Health Perspectives*, **106**(8):437–445.

IARC (2001) *Some thyrotropic agents*. Lyon, International Agency for Research on Cancer, 763 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 79).

Li AP, Myers CA (1989) *CHO/HGPRST gene mutation assay with MON 13200: Study No. ML-88-382/EHL No. 88071.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275536).

Lichtenstein CK, Hemminki K (2002) Environmental and heritable cause of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. *International Journal of Cancer*, **1099**(2):260–266.

Liu J, Liu Y, Barter RA, Klaassen CD (1995) Alteration of thyroid homeostasis by UDPglucuronosyltransferase inducers in rats: A dose–response study. *Journal of Pharmacology and Experimental Therapeutics*, **273**:977–985.

Masubuchi N, Hakusui H, Okazaki O (1997) Effects of proton pump inhibitors on thyroid hormone metabolism in rats: A comparison of UDP-glucuronyltransferase induction. *Biochemical Pharmacology*, **54**(11):1225–1231.

Mazzaferri EL (2000) Thyroid cancer and Graves' disease: The controversy ten years later. *Endocrine Practice*, **6**:221–225.

McClain RM (1992) Thyroid gland neoplasia: Non-genotoxic mechanisms. *Toxicology Letters*, **64/65**:397–408.

McClain RM (1995) Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research*, **333**(1–2):131–142.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**(6):591–654.

Naylor MW, McDonald MM (1992) *Chronic study of MON 13200 administered in feed to albino rats. Project No. ML-88-247/EHL 88148.* Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-24).

Naylor MW, Raju NR (1992) Chronic study of MON 13200 administered in feed to albino mice. Project No. ML-88-248/EHL 88147. Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-23).

Olsen JH, Wallin H, Boice JD, Rask K, Schulgen G, Fraumaen FF Jr (1993) Phenobarbital, drug metabolism and human cancer. *Cancer Epidemiology, Biomarkers and Prevention*, **5**:449–452.

Oropeza-Hernandez LF, Lopez-Romero R, Albores A (2003) Hepatic CYP1A, 2B, 2C, 2E and 3A regulation by methoxychlor in male and female rats. *Toxicology Letters*, **144**(1):93–103.

Ron E, Kleinerman RA, Boice JD, LiVolsi VA, Flannery JT, Fraumeni JF Jr (1987) A population-based case–control study of thyroid cancer. *Journal of the National Cancer Institute*, **79**:1–12.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Sundseth SS, Waxman DJ (1992) Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid omega-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. *Journal of Biological Chemistry*, **267**(6):3915–3921.

Thomas GA, Williams ED (1999) Thyroid stimulating hormone (TSH)-associated follicular hypertrophy and hyperplasia as a mechanism of thyroid carcinogenesis in mice and rats. In: Capen CC, Dybing E, Rice JM, Wilbourn JD, eds. *Species differences in thyroid gland, kidney and urinary bladder carcinogenesis*. Lyon, International Agency for Research on Cancer, pp. 45–59 (IARC Scientific Publications No. 147).

USEPA (1998) *Assessment of thyroid follicular cell tumors*. Washington, DC, United States Environmental Protection Agency, Office of Research and Development, Risk Assessment Forum (EPA/630/R-97/002; http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=13102; accessed 22 November 2004).

# 4-AMINOBIPHENYL AND DNA REACTIVITY: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

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The International Programme on Chemical Safety (IPCS) Human Relevance Framework (HRF) was evaluated for a DNA-reactive (genotoxic) carcinogen, 4-aminobiphenyl, based on a wealth of data in animals and humans. The mode of action (MOA) involves metabolic activation by *N*-hydroxylation, followed by *N*-esterification leading to the formation of a reactive electrophile, which binds covalently to DNA, principally to deoxyguanosine, leading to an increased rate of DNA mutations and ultimately to the development of cancer. In humans and dogs, the urinary bladder urothelium is the target organ, whereas in mice, it is the bladder and liver; in other species, other tissues can be involved. Differences in organ specificity are thought to be due to differences in metabolic activation versus inactivation. Based on qualitative and quantitative considerations, the MOA is possible in humans. Other biological processes, such as toxicity and regenerative proliferation, can significantly influence the dose–response of 4-aminobiphenyl-induced tumours. Based on the IPCS HRF, 4-aminobiphenyl would be predicted to be a carcinogen in humans, and this is corroborated by extensive epidemiological evidence. The IPCS HRF is useful in evaluating DNA-reactive carcinogens.

4-Aminobiphenyl is carcinogenic when administered to several species by a variety of routes (IARC, 1972, 1986, 1987). It was selected as a chemical for a case-study for the International Programme on Chemical Safety (IPCS) Human Relevance Framework (HRF) as a representative DNA-reactive carcinogen because of its established mode of action (MOA) in animal models, based on substantial data available evaluating its metabolic activation, DNA reactivity, genotoxicity, and carcinogenicity. It is also similar to numerous known animal and human carcinogens belonging to the chemical class of aromatic amines (structure–activity relationships), and there are extensive epidemiological, metabolic, and biochemical data in humans. This case-study illustrates the nature of data that are helpful in delineating MOAs for DNA-reactive carcinogens. Distinction between modulating factors and key events in an MOA analysis is also presented.

Based on the strong animal evidence and extensive epidemiological data, the International Agency for Research on Cancer (IARC) has classified 4-aminobiphenyl as a known human carcinogen (IARC, 1972, 1987). Although initially identified as a human urinary bladder carcinogen in individuals exposed to high levels occupationally, it has subsequently been demonstrated as a major component of cigarette smoke, leading to an increased risk of urinary bladder cancer in cigarette smokers (Del Santo et al., 1991; Curigliano et al., 1996). Additional research has shown that it is a ubiquitous environmental chemical occurring naturally when organic material containing nitrogen undergoes combustion.

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### **CARCINOGENICITY OF 4-AMINOBIPHENYL IN ANIMALS**

Experimental studies indicate that 4-aminobiphenyl is carcinogenic in mice, rats, rabbits, and dogs, although significant target tissue differences and susceptibility have been observed (IARC, 1972). By most routes of exposure, 4-aminobiphenyl is primarily a carcinogen of the liver and, to a lesser extent, the urinary bladder in mice, whereas in dogs (and humans), the urinary bladder appears to be the target organ. Many of the studies were conducted a number of years ago, and published accounts include only limited details. In addition, potential precursor lesions at interim periods were rarely documented, and none of the studies included protocols, such as stop/recovery, which might be informative in the context of MOA. Nonetheless, results indicate clear species and individual differences in response (e.g. Block et al., 1978), characteristic of MOAs entailing competing metabolic activation and deactivation processes (Table 1).

Species	Route/dose	Incidence	Comment	Reference
Mice	Gavage; 1 mg/week for 38 weeks	Bladder carcinomas in 2/12 mice surviving to 90 weeks		Clayson et al. (1965)
Mice	Gavage; 0 or 1.5 mg/week for 52 weeks	Bladder carcinomas in 1/21 exposed males vs 0/19 in controls; increased incidence of hepatomas in males and females		Clayson et al. (1967)
Mice	Subcutaneous injection of 200 µg for up to 52 weeks	Hepatomas in 19/20 males and 6/23 females after 48–52 weeks		Gorrod et al. (1968)
Mice	0–220 mg/l in	Significant increases in	Hyperplasia of the	Schieferstein
(BALB/ cStCrlfC3Hf/ Nctr)	drinking-water (males), 0–300 mg/l (females), for up to 96 weeks	urinary bladder carcino- mas (males only), hepatocellular carcino- mas (females only), and angiosarcomas (males and females)	bladder in most mice of both sexes receiving 75 mg/l (females) and 55 mg/l (males) or greater, but none in controls	et al. (1985)
Mice	Different regi-	Liver tumours		Dooley et al.
(newborn B6C3F1)	mens; injected prior to weaning			(1988, 1992); Von Tungeln et al. (1996); Parsons et al. (2005)
Rats	Subcutaneous injection in arachis oil of total dose of 3.6–5.8 g/kg bw	Mammary and intestinal tumours		Walpole et al. (1952)

#### Table 1. Carcinogenicity studies of 4-aminobiphenyl in various species.

Species	Route/dose	Incidence	Comment	Reference
Rabbits	Oral administra- tion of unspecified dose	Bladder papillomas in 1 animal and carcinomas in 3 animals	Earliest carcinoma observed 4 years after start of treat- ment	Bonser (1962)
Dogs (2)	Gelatin capsules 6 times weekly for life for a total dose of 30 or 34 g	Carcinoma of the bladders appeared in 33 months		Walpole et al. (1954)
Dogs	Gelatin capsules 0.3 g 3 times weekly (total dose: 94.5–144 g per dog)	Bladder carcinomas after 21–34 months		Deichmann et al. (1958)
Dogs (6)	1.0 mg/kg bw 5 times weekly for 34 or 37 months (total dose 5.5–7.0 g per dog)	3 bladder papillomas and 3 bladder carcinomas (transitional cell type)		Deichmann et al. (1965)
Dogs	Single dose	Ineffective in inducing bladder tumours over a 5-year period		Deichmann & MacDonald (1968)
Dogs (24 beagles)	Oral administra- tion 5 days/week for 3 years	Negative or minimal disease in 4 dogs, with no neoplasia in 2; neoplasia developed slowly in 11 dogs, while a rapidly progressive pattern was observed in the remaining 9 dogs		Block et al. (1978)

#### Table 1 (Contd)

bw, body weight

Following its oral administration by gavage (1 mg per mouse per week for 38 weeks), 2/12 mice surviving to 90 weeks developed bladder carcinoma (Clayson et al., 1965). In a separate but similar experiment, dosing mice with 1.5 mg of 4-aminobiphenyl for 52 weeks resulted in bladder carcinoma in 1/21 male mice as compared with 0/19 in controls. In this experiment, the frequency of hepatomas in both male and female mice was significantly higher than that in the controls (Clayson et al., 1967). Three subcutaneous injections of mice with 200 µg of 4-aminobiphenyl produced hepatomas in 19/20 males and 6/23 females after 48–52 weeks (Gorrod et al., 1968). Oral administration of 4-aminobiphenyl in drinking-water at concentrations of up to 220 and 300 mg/l to male and female BALB/cStCrlfC3Hf/Nctr mice, respectively, for up to 96 weeks induced dose-related, significant increases in angiosarcomas (males only). Hyperplasia of the bladder was observed in most of the mice of both sexes in groups of about 118 receiving concentrations of 75 mg/l (females) and 55 mg/l (males) or greater, whereas none was reported in the control groups of similar size (Schieferstein et al., 1985). In a number of experiments, newborn B6C3F1 mice were primarily susceptible to

liver carcinogenesis following 4-aminobiphenyl administration (Dooley et al., 1988, 1992; Von Tungeln et al., 1996; Parsons et al., 2005).

Daily subcutaneous injection of rats with 4-aminobiphenyl in arachis oil to a total dose of 3.6–5.8 g/kg body weight (bw) resulted in significant increases in the incidence of mammary gland and intestinal tumours (Walpole et al., 1952).

Among seven rabbits given commercial 4-aminobiphenyl orally (dose unstated), bladder papillomas were found in one and carcinomas in three animals. The earliest carcinoma was observed 4 years after the start of treatment (Bonser, 1962).

Two dogs fed 4-aminobiphenyl in gelatin capsules 6 times weekly for life (total dose per dog: 30, 34 g) developed carcinoma of the bladder in 33 months (Walpole et al., 1954). This was confirmed by similarly feeding capsules containing 4-aminobiphenyl (0.3 g per dog) 3 times weekly. Bladder carcinomas were observed after 21–34 months (total dose: 94.5–144.0 g per dog) (Deichmann et al., 1958). When the dose of 4-aminobiphenyl was reduced to 1.0 mg/kg bw and given to six dogs 5 times weekly for 34 months or 37 months (total dose: 5.5–7.0 g per dog), three bladder papillomas and three bladder carcinomas (transitional cell type) were observed (Deichmann et al., 1965). A single dose was not effective in inducing bladder tumours over a period of 5 years (Deichmann & MacDonald, 1968). Among 24 beagles that received 4-aminobiphenyl orally 5 days per week for 3 years, three basic patterns of bladder carcinogen responses were seen. Negative or minimal disease was seen in four dogs, of which two remained completely free of neoplasia. Neoplasia developed slowly in 11 dogs, while a rapidly progressive pattern was observed in the remaining 9 dogs (Block et al., 1978).

# IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH A MODE OF ACTION (MOA) IN ANIMALS?

The first question of the IPCS HRF is an evaluation of the animal MOA itself. This is based on the process delineated by the MOA Framework developed by IPCS and published in 2001 (Sonich-Mullin et al., 2001), which evolved from the Bradford Hill criteria for causality in epidemiology studies (Hill, 1965).

#### A. Postulated mode of action

4-Aminobiphenyl is metabolized by hepatic enzymes to *N*-hydroxy-4-aminobiphenyl, which can be *N*-esterified (*N*-acetylated, *N*-glucuronidated, or *N*-sulfated) in hepatic and other tissues (Miller et al., 1961; Kadlubar et al., 1977, 1991; Miller & Miller, 1977; Delclos et al., 1987; Chou et al., 1995) (Figure 1). *O*-Esterification and ring hydroxylation are competing enzymatic reactions leading to detoxification. Tissue and species differences in the activity of these reactions dictate, at least in part, variations in susceptibility to the carcinogenic effects of 4-aminobiphenyl and differences in organ specificity in the development of tumours. Ultimately, a reactive electrophilic nitrenium ion is formed in the target tissue following *N*-esterification, and this is capable of forming DNA adducts. The principal DNA adduct is *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (Talaska et al., 1990; Kadlubar et al., 1991; Flammang et al., 1992; Hatcher & Swaminathan, 1995, 2002). As a consequence of the

mutations that can result from these reactions at critical sites of critical genes, neoplastic cells eventually develop.

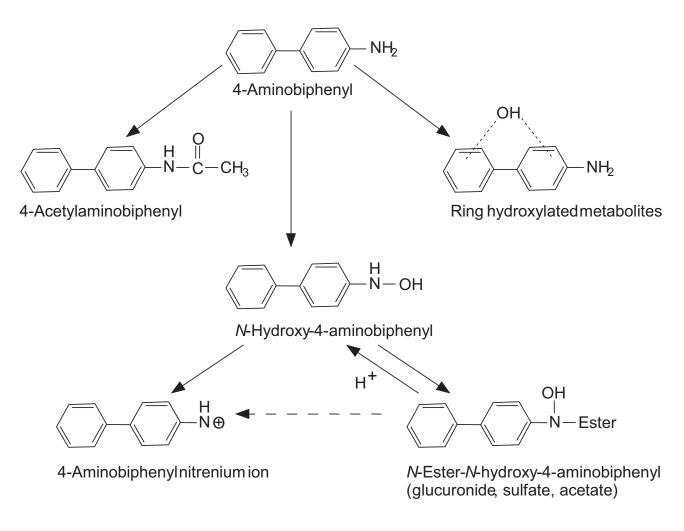


Figure 1. Metabolism of 4-aminobiphenyl

# **B. Key events**

The major route of hepatic activation of 4-aminobiphenyl begins with its *N*-hydroxylation, catalysed, the balance of evidence indicates, by CYP1A2, at least in rats and humans (Butler et al., 1989b). In mice, there is evidence that CYP1A2 is not the only, or even the primary, form of cytochrome P-450 involved (Kimura et al., 1999). The *N*-hydroxylamine can also be produced by reaction with a variety of oxidases and peroxidases, such as by the prostaglandin synthase component of cyclo-oxygenase (Kadlubar et al., 1982). Whether any of these non-cytochrome P-450 reactions occur in vivo and are of toxicological significance remains unclear. The *N*-hydroxylamine undergoes *N*-acetylation by *N*-acetyltransferase-1 (NAT1) (Flammang & Kadlubar, 1986; Oda, 2004), resulting in an *N*-acetoxy ester that is unstable in acidic conditions, forming an arylnitrenium ion that can react directly with DNA, forming a DNA adduct at the C-8 position of guanine (Hammons et al., 1985; Flammang & Kadlubar, 1986; Hatcher & Swaminathan, 2002). Additionally, the *N*-hydroxylamine generated in liver can serve as a substrate for uridine diphosphate (UDP) glucuronosyltransferase (UGT),

yielding an *N*-glucuronide conjugate that is transported to the urinary bladder (Kadlubar et al., 1977). The glucuronide can either be excreted in urine or, under acidic conditions, serve as an additional source of the *N*-hydroxylamine in the urinary bladder, following hydrolysis. There are a number of reactions that can compete with this reaction scheme, including *N*-acetylation of 4-aminobiphenyl by *N*-acetyltransferase-2 (NAT2), but the resulting arylacetamide is a poor substrate for CYP1A2, and it is considered to be primarily a detoxification reaction. As a consequence, *N*-acetylation of the parent amine is considered a deactivating process. Rates of acetylation can thus affect the balance between activation and deactivation. Humans phenotypically are either rapid or slow acetylators (Lower et al., 1979). Mouse strains exist that are analogous to human slow and rapid acetylators. Thus, C57BL/6 is a rapid acetylator strain, while A/J is a slow acetylator (Hein, 1988). Interest in these differences in response. As a consequence of the DNA adducts formed, mutations can be produced. The key events are summarized in Table 2.

#### Table 2. Key events in the carcinogenicity of 4-aminobiphenyl in animals.

- 1. Metabolic activation
  - a) N-Hydroxylation
  - b) N-Esterification (glucuronide, acetyl, sulfate)
  - c) Hydrolysis to nitrenium ion
- 2. DNA adduct formation (dG-C8, dA-C8, dG-N2) in pluripotential cell of target organ
- 3. DNA mutation in critical gene(s) leading to cancer
- 4. Cancer

dA, deoxyadenosine; dG, deoxyguanosine

# C. Dose-response relationship

In view of the fact that many of the relevant studies were conducted a number of years ago, data on concordance of dose-response for precursor lesions for tumours are restricted to hyperplasia in the mouse urinary bladder. Dogs do not develop bladder tumours after a single dose of 4-aminobiphenyl (Deichmann & MacDonald, 1968), and there do not appear to have been studies of dose-response relationships in this species following multiple exposures. In the only study in which information on the incidence of precursor lesions was reported, male BALB/c mice were treated with drinking-water containing 4-aminobiphenyl at concentrations of 0, 7, 14, 28, 55, 110, or 220 mg/l for up to 96 weeks (Schieferstein et al., 1985). These treatments were associated with bladder carcinoma incidences of 0/116, 1/117, 1/118, 0/118, 6/115, 5/118, and 23/118, respectively. The incidences in the 55 mg/l group and higher were statistically significantly higher than in controls. Female mice were exposed to drinkingwater concentrations of 4-aminobiphenyl of 0, 7, 19, 38, 75, 150, and 300 mg/l. The corresponding incidences of bladder carcinomas were 0/118, 0/118, 0/119, 1/118, 0/118, 5/117, and 1/117. Incidences of hyperplasia were much higher, although severity was not indicated. In males, the incidences of hyperplasia were 0/116, 4/117, 9/118, 71/118, 108/115, 107/118, and 102/118 for doses of 0, 7, 14, 28, 55, 110, and 220 mg/l, respectively, and for females, 0/118, 0/118, 3/119, 53/119, 106/118, 97/117, and 83/117 for doses of 0, 7, 19, 38, 75, 150, and 300 mg/l, respectively. Thus, the dose-response curves for tumours and hyperplasia were sigmoidal or hockey stick-shaped. In contrast, steady-state levels of urothelial C-8 guanine DNA adducts showed a linear dose–response (Poirier et al., 1995).

In this same study (Schieferstein et al., 1985), there was no increase in the incidence of liver tumours in the males, whereas in the females, the incidences of liver tumours (adenomas and carcinomas combined) were 0/117, 0/120, 2/120, 4/119, 11/119, 17/118, and 10/117 at doses of 0, 7, 19, 38, 75, 150, and 300 mg/l, respectively. The incidence of angiosarcomas of various tissues combined was also increased at the three highest doses in males and females, although the incidences were somewhat higher in females than in males.

### **D. Temporal relationship**

Establishing time sequences for events in a carcinogenic process is partially, but to an important extent, dependent upon the sensitivity of the available methods for their measurement. Thus, tumours must attain a size allowing their histological detection, while the measurement of mutations and DNA adducts requires not only time but sufficient tissue. Consequently, the latter are more usually studied in liver than in urinary bladder, where the paucity of tissue available in the urothelium, particularly in rodents, causes technical difficulties that have no connection with the frequency of the biochemical and biological events. The metabolism and formation of DNA adducts are early events, which can be observed within a few minutes or hours in vitro and within a day following in vivo treatment with 4aminobiphenyl (e.g. Kadlubar et al., 1991; Swaminathan & Reznikoff, 1992; al-Atrash et al., 1995; Hatcher & Swaminathan, 1995; Doerge et al., 1999; Tsuneoka et al., 2003). Many in vivo experiments, however, continue exposure for 3-4 weeks to allow an accumulation of adducts, achieve steady-state levels, and facilitate their detection (e.g. Talaska et al., 1990; Flammang et al., 1992; Poirier & Beland, 1992; Poirier et al., 1995; Underwood et al., 1997). Mutations can also be detected within a short time in vitro, but have generally not been detected in vivo in target tissues until after several weeks or months of exposure (e.g. H-ras in mouse liver; Parsons et al., 2002), although this comparatively long period may not be a true reflection of when mutations first arise. In one study, mutations were detected in a Muta<sup>TM</sup>Mouse urinary bladder assay 14 days after a single dose of 4-aminobiphenyl (Fletcher et al., 1998). Carcinomas and hyperplasia of the urinary bladder are apparently late-occurring lesions in mice and dogs; however, time course changes have not been systematically evaluated. Although mice were killed at intervals beginning at 13 weeks in one 2-year study, and hyperplastic lesions were induced in the urinary bladder, their incidences at different times were not presented (Schieferstein et al., 1985). Tumours in the urinary bladder are commonly not discovered until after about 2 years in mice (Schieferstein et al., 1985) and longer in dogs (Walpole et al., 1954; Deichmann et al., 1958, 1965). However, neoplastic transformation of human urothelial cells (infected with SV40) treated in vitro with 4aminobiphenyl followed by in vitro culture for 6 weeks was demonstrated upon their inoculation into nude mice (Bookland et al., 1992b).

# **E.** Strength, consistency, and specificity of association of the tumour response with key events

Evidence in support of the association of the tumour response with key events comes only in part from studies on bladder; considerable evidence is provided by studies on liver. DNA adduct formation has been demonstrated in both tissues.

There is an abundance of studies that demonstrate that 4-aminobiphenyl is a mutagen, including positive mutagenicity with certain frameshift mutation and base pair substitutionsensitive strains (TA1538, TA98, and TA100) of Salmonella typhimurium, but only in the presence of rodent liver S9 metabolic activating preparations. The requirement for S9 metabolic activation clearly demonstrates the lack of DNA reactivity and mutagenicity of the parent amine. In addition, 4-aminobiphenyl induces unscheduled DNA synthesis in rat liver cells in vitro (United States Environmental Protection Agency Genetic Activity Profiles). These in vitro studies provide evidence that 4-aminobiphenyl can cause genetic damage following metabolic activation. Bacterial mutation studies have also been conducted comparing metabolic activation systems based on liver homogenates from Aroclor 1254-induced male Sprague-Dawley rats and C57BL/6 mice, using S. typhimurium TA100 tester strains that expressed different levels of N- and O-acetyltransferase (OAT) activity (Dang & McQueen, 1999). TA100 has a single copy of the NAT/OAT gene; YG1029 has multiple copies of the NAT/OAT gene, and TA100/1,8DNP<sub>6</sub> is NAT/OAT-deficient. Effects with mouse and rat S9 were similar (but the effects of Aroclor 1254 treatment were not examined). Using either 4aminobiphenyl or 4-acetylaminobiphenyl as substrates, considerably more mutations were induced in YG1029 than in TA100 or TA100/1,8DNP<sub>6</sub>, in which mutation induction was similar. This supports a role for high acetylation activity in mutation induction by the Nhydroxylamine in these bacteria.

The non-enzymatic step to an arylnitrenium ion in the mechanism of mutagenesis in vivo is supported by the observation that *N*-hydroxy-4-aminobiphenyl mutagenesis in the high OAT-expressing *S. typhimurium* TG1024 strain is dependent on the pH of the medium, with an inverse relationship between mutant numbers and pH over the range 4.0–8.0 (Sarkar et al., 2002).

Administration of 4-aminobiphenyl in the drinking-water of BALB/c mice for 28 days resulted in higher levels of DNA adducts in liver than in urinary bladder of females, while the reverse occurred in males. Thus, in each sex, the DNA adduct level correlated with the susceptibility of the tissue to tumour induction by 4-aminobiphenyl (Poirier et al., 1995). However, the shape of the dose–response curve was linear for DNA adducts in both tissues (although it appears to saturate and is relatively flat in female mice), whereas the tumour dose–response curve was sigmoidal (Poirier et al., 1995).

Adduct levels were also highest in the urinary bladder of female Hsd:ICR(Br) mice that were dosed topically (the more usual exposure route in occupational settings) with 50 nmol 4-aminobiphenyl for 21 weeks. The principal adduct in all tissues examined (bladder, liver, lung, and skin) co-chromatographed with *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (Underwood et al., 1997).

One study of mutagenesis in male Muta<sup>TM</sup>Mouse transgenic mice (i.e. transgenic CD2F, [BALB/c × DBA/2]) treated orally with 4-aminobiphenyl at 10 mg/kg bw per day for 10 days reported that the mutation frequencies in urinary bladder, liver, and bone marrow were increased by 13.7-, 4.8-, and 2.4-fold, respectively (Fletcher et al., 1998).

Newborn B6C3F1 (C57BL/6 × C3H) mice responded to treatment with 4-aminobiphenyl by developing a high frequency of liver tumours, many of which carried H-*ras* codon 61 CAA  $\rightarrow$  AAA mutations (Parsons et al., 2005). In vivo, the level of one major DNA adduct [*N*-(deoxyguanosin-8-yl)-4-aminobiphenyl] was present at 5 adducts/10<sup>6</sup> nucleotides in newborn mice treated with 0.3 µmol 4-aminobiphenyl 24 h earlier. After 8 months, the CAA  $\rightarrow$  AAA mutation was detected in 67% of the treated mice and 50% of the vehicle (dimethyl sulfoxide, or DMSO) controls, but the average mutant fraction in treated mice was 45 × 10<sup>-5</sup> compared with only 2 × 10<sup>-5</sup> in controls. After 12 months, liver tumours had developed in 79% of the treated mice and in 8% of the controls. These tumours are not those of the human target organ, but the results of this study support the general MOA proposed for bladder carcinogenesis (i.e. DNA adduct formation, followed by mutation in a key gene and the subsequent emergence of tumours).

Dogs (sex not stated) killed 24 h after a single oral dose of 4-aminobiphenyl (5 mg/kg bw) had 5.4 fmol DNA adducts/µg liver DNA and 4.8 fmol DNA adducts/µg urinary bladder DNA, whereas no DNA adducts were detected in either the liver or bladder of a dog whose bladder had been instilled with 4-aminobiphenyl. In contrast, a dog bladder instilled with the reactive intermediate N-hydroxy-4-aminobiphenyl had 3.9 fmol DNA adducts/µg bladder DNA and no detectable adducts in liver DNA. Quantification was by an immunochemical method (Roberts et al., 1988). Examination of bitches treated with tritium-labelled 4aminobiphenyl (per os, intravenously, or intraurethrally), N-hydroxy-4-aminobiphenyl (intravenously or intraurethrally), or *N*-hydroxy-4-aminobiphenyl *N*-glucuronide (intravenously) demonstrated (1) the presence of 4-aminobiphenyl-haemoglobin adducts in blood erythrocytes; (2) that after per os dosing with 4-aminobiphenyl, the major portion of total Nhydroxy-4-aminobiphenyl entering the bladder lumen was free N-hydroxy-4-aminobiphenyl (0.7%), with lower concentrations of the acid-labile *N*-glucuronide (0.3%); (3) that urothelial DNA adducts following intraurethral instillation of N-hydroxy-4-aminobiphenyl were 60 times higher than after intraurethral instillation of 4-aminobiphenyl; and (4) that exposure to N-hydroxy-4-aminobiphenyl and subsequent 4-aminobiphenyl-DNA adduct formation are directly dependent on the frequency of urination and, to a lesser extent, on urinary pH (Kadlubar et al., 1991). The urinary pH of dogs may vary from about 4.5 to 7.5, depending upon the diet (Merck, 1998), time after eating, time of day, and amount of water consumed; these are factors that might influence the carcinogenic response (Cohen, 1995). Studies in vitro with microsomal preparations from dog liver and bladder have shown the presence of transacetylation activities in both organs, so that N-hydroxy-4-aminobiphenyl binding to RNA and DNA occurs in the presence of 4-acetylaminobiphenyl, N-hydroxy-4-acetylaminobiphenyl, or acetyl coenzyme A (CoA) as acetyl donors, although the levels of binding were less with bladder than with hepatic microsomes (Hatcher & Swaminathan, 1992).

Examination of urothelial cells exfoliated into urine of dogs treated with 4-aminobiphenyl showed that DNA adducts were identical to those from DNA modified in vitro with *N*-hydroxy-4-aminobiphenyl and from dog bladder urothelial DNA isolated from 4-aminobiphenyl-dosed dogs at autopsy. A dose-related increase in 4-aminobiphenyl–DNA adduct formation was demonstrated (Talaska et al., 1990).

#### F. Biological plausibility and coherence

The observations that 4-aminobiphenyl can form adducts with DNA and that it is mutagenic in organs in which tumours develop indicate, in general terms, that the proposed MOA is plausible (Fletcher et al., 1998). In addition, *N*-hydroxy-4-aminobiphenyl is able to cause neoplastic transformation of non-tumorigenic SV40-immortalized human urothelial cells (Bookland et al., 1992b). The findings with 4-aminobiphenyl are also consistent with the vast literature regarding the metabolic activation, DNA adduct formation, mutagenesis, and urinary bladder carcinogenesis in several species (including humans) of several related aromatic amine chemicals (Kadlubar et al., 1977; Miller & Miller, 1977; Delclos et al., 1987). The lack of DNA adduct formation and mutagenicity of the parent amine in various in vitro systems without metabolic activation clearly demonstrates the requirement for metabolic activation. The same DNA adducts are identified in tissues after administration of the amine or following exposure to the *N*-hydroxyl metabolite, with the structure of the adducts having been chemically confirmed. The mutagenic potential of the specific C-8 guanine DNA adduct has also been demonstrated, although the specific biophysical aspects have been better demonstrated for structurally related aromatic amines such as 2-aminofluorene (Kriek, 1992).

### **G. Other modes of action**

Alternatives of components of the already described MOA have been suggested. However, they do not detract from the overall described MOA but suggest either alternative specific aspects (such as other activating enzymes) or associative processes that could affect quantitative aspects. 4-Aminobiphenyl is oxidized by hepatic enzymes other than CYP1A2 (Kimura et al., 1999) to the *N*-hydroxylated metabolite that causes liver and urinary bladder toxicity and carcinogenesis, possibly including oxidases and peroxidases (Kadlubar et al., 1982, 1991). Although the specific enzymes involved in metabolic activation may vary, the ultimate sequence of generation of a reactive electrophile, DNA adduct formation, mutagenesis, and carcinogenesis is consistent. Furthermore, it is reasonable to believe that from this point in the MOA, the same sequence occurs as that involving CYP1A2-mediated activation, regardless of the activating enzyme.

In addition to bulky adducts, there is evidence to suggest that *N*-hydroxy-4-aminobiphenyl causes oxidative damage in urothelial DNA, possibly involving endogenous peroxidases (Burger et al., 2001). The relevance of this for the carcinogenic activity of 4-aminobiphenyl is unknown.

*N*-Hydroxy-4-aminobiphenyl and its further activated forms are cytotoxic to urothelial and other cells in vitro (Reznikoff et al., 1986), but the role that this plays in its carcinogenic effects is unclear (see below for discussion of a potentiating role in urothelial carcinogenesis, rather than causative role). It is likely that this process alters the dose–response relationship, but does not alter the fundamental MOA described above.

#### H. Assessment of the postulated mode of action

The early steps in the proposed MOA are well supported by the available evidence, and it has been judged that there is good and sufficient evidence that 4-aminobiphenyl is a urinary bladder carcinogen in dogs and mice, and in other tissues (primarily the liver) in rodents. Thus, it is metabolized to products that can form DNA adducts in the liver and in other target organs, and mutations have been demonstrated to arise. Although other organs can also be targets for 4-aminobiphenyl-induced neoplasia, the urinary bladder is the main target in dogs and in some strains of mice. Evidence for the intervening steps between general genotoxicity and the emergence of neoplasia is lacking. There is a notable lack of study of the effects of 4-aminobiphenyl on cell proliferation in the urinary bladder, but information on related aromatic amines and amides is available, particularly the analysis of the interaction between DNA reactivity (and mutagenesis) and cell proliferation induced by 2-acetylaminofluorene in mouse urinary bladder utilizing data from a megamouse, ED-01 study (Cairns, 1979; Gaylor, 1979; Littlefield et al., 1979). The reliance for mutagenicity on cell proliferation can provide an explanation for the sigmoidal shape of the tumour dose–response despite a linear dose–response for DNA adducts (Cohen & Ellwein, 1990). This link has significant implications for assessing potency and dose–response for 4-aminobiphenyl-induced urinary bladder cancer (see discussion below).

# I. Uncertainties, inconsistencies, and data gaps

Bacterial mutation studies of 4-aminobiphenyl with metabolic activation have shown that most mutations are frameshifts, whereas a single study of sequence analysis of 4-aminobiphenyl-induced mutations in the *lacZ* gene in single-stranded DNA from a bacterio-phage M13 cloning vector revealed exclusively base pair substitutions, with over 80% occurring at G sites:  $G \rightarrow T$  transversions predominated, followed by  $G \rightarrow C$  transversions and  $G \rightarrow A$  transitions. The major DNA adduct, *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl, was then inserted within the M13 genome, and the mutational frequency and specificity were measured after in vivo replication. The targeted mutational efficiency was approximately 0.01%, and the primary mutation was  $G \rightarrow C$  transversion. Thus, the observations are consistent with in vivo observations, but the mutagenic activity was weak (Verghis et al., 1997).

Most in vivo investigations have been in mice. Dogs, for understandable reasons, have received less attention, although this is the species that is more sensitive to bladder carcinogenesis. Mouse strain differences in response are evident: B6C3F1 and female BALB/cStCrlfC3Hf/Nctr are more susceptible to liver carcinogenesis, whereas male BALB/cStCrlfC3Hf/Nctr mice develop bladder tumours after exposure to 4-aminobiphenyl (Schieferstein et al., 1985; Dooley et al., 1988, 1992). Nevertheless, mouse strain effects have received relatively little attention in the available studies.

The enzyme considered as fundamental for the metabolism of 4-aminobiphenyl to a product that forms adducts with DNA in liver and bladder is CYP1A2 (Butler et al., 1989a, 1989b). However, comparison of responses in CYP1A2(+/+) wild-type mice with CYP1A2(-/-) knockout mice showed that, contrary to expectations, CYP1A2 expression was not associated with 4-aminobiphenyl-induced oxidative stress or with 4-aminobiphenyl–DNA adduct formation. Furthermore, prior treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which increased hepatic CYP1A2 protein expression 5-fold along with expression of other phase I and phase II enzymes, either did not change or actually decreased the level of adducts in liver. The specific quantitative effects of such induction will depend on the balance of the enzymes induced. These results suggest either that CYP1A2 is not the major metabolic activator of 4-aminobiphenyl or that other enzymes in mice activate the compound in the absence of CYP1A2 (Tsuneoka et al., 2003). Based on studies with other aromatic amines,

additional activating enzymes might include other P-450 enzymes, oxidases, or peroxidases (Lakshmi et al., 1990; Smith et al., 1991; Hughes et al., 1992).

Another reaction considered to be important for carcinogenesis induced by 4-aminobiphenyl is acetylation. Acetylation plays several roles in 4-aminobiphenyl carcinogenesis. *O*-Acetylation and *N*,*O*-acetyltransfer of *N*-hydroxy-4-aminobiphenyl are expected to increase risk in humans, whereas *N*-acetylation of 4-aminobiphenyl should reduce risk (Lower et al., 1979). Acetylation can be catalysed by NAT1 or NAT2, with the latter exhibiting a marked polymorphism within the population (Hein et al., 2000; Cascorbi et al., 2001). It is predicted that a slow acetylation phenotype will increase the risk of bladder cancer, since acetylation of the parent amine, 4-aminobiphenyl, is considered to be a detoxification process in humans, whereas a rapid acetylation phenotype should be associated with a decreased risk.

However, studies of acetylator phenotype in mice have produced conflicting results. In one study, male and female homozygous rapid acetylator or homozygous slow acetylator mice that were apparently identical in every other respect were administered 4-aminobiphenyl·HCl (55-300 mg/l) in drinking-water for 28 days. The levels of hepatic DNA adducts increased with dose in both sexes, with the levels being higher in females, but were independent of the mouse acetylator phenotype. In the urinary bladder, DNA adducts increased to a plateau at 100 mg/kg in male mice and were again independent of acetylator phenotype. In female mice, the DNA adduct levels were lower than in males and decreased at the highest dose; the DNA adduct levels were higher in the rapid acetylator phenotype, contrary to expectations (Flammang et al., 1992). These results were interpreted as suggesting that acetyltransferase activities are not rate determining for DNA adduct formation in mice. A similar conclusion that there was no correlation between murine NAT2 alleles and 4-aminobiphenyl-DNA adduct levels was reached by McQueen et al. (2003), using C57BL/6, B6.A, and A/J mouse strains and the transgenic strains hNAT1:A/J and hNAT1:C57, which carry the human NAT1 transgene. However, the differences in murine NAT2 activity were modest and probably not sufficient to affect 4-aminobiphenyl genotoxicity. Recent studies suggest that in humans, NAT1, not NAT2, is responsible for the O-acetylation of N-hydroxy-4-aminobiphenyl (Oda, 2004).

There are also mouse strain-specific mutations that require explanation. Thus, in B6C3F1, 4aminobiphenyl induces predominantly  $C \rightarrow A$  mutations (reflecting  $G \rightarrow T$  transversions in the non-coding strand) in H-*ras* codon 61, whereas in CD-1 mice, the predominant mutation in H-*ras* codon 61 was  $A \rightarrow T$  transversion (Manjanatha et al., 1996).

Cell proliferation is also required for neoplasia, but there have been few studies that have investigated cell proliferation at an early stage of the carcinogenic process of 4-aminobiphenyl. It is also notable that in the carcinogenicity experiment described previously (Schieferstein et al., 1985), although urinary bladder carcinomas developed only in males, a high prevalence of hyperplasia was reported in both males and females. Apparently this observation has not been investigated further (discussed below).

In summary, the evidence is strong for the sequence of key events including metabolic activation, DNA adduct formation, and gene mutation as the MOA for 4-aminobiphenyl-induced urinary bladder carcinogenesis. It is further strengthened by data from studies with structurally related aromatic amines. However, data gaps remain concerning details of the specific enzymes involved, the basis for differing organ specificity between species and details regarding potency, and the shape of the dose–response curve in humans. This is, perhaps, not unexpected in view of the complexity of the relevant competing metabolic pathways. While available data are considered sufficient to support the hypothesized MOA, the impact of these uncertainties needs to be considered quantitatively in the overall assessment (Table 3).

#### Table 3. Modulating factors affecting 4-aminobiphenyl urinary bladder carcinogenesis.

- 1. Competing activities of esterification enzymes
- 2. Genetic polymorphisms affecting enzymatic activation or inactivation (e.g. slow and fast acetylators)
- 3. Urinary pH (mainly affected by diet) and possibly other urinary constituents
- 4. Urothelial cell proliferation (induced by high doses of 4-aminobiphenyl or by co-administration with some other agent affecting urothelial proliferation)

# CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

There is considerable evidence in humans and human cell systems supporting each of the key events for 4-aminobiphenyl-induced urinary bladder cancer. Metabolic activation to the *N*-hydroxylamine has been demonstrated, with several different enzymes being suggested for activation and several others that might potentiate or reduce the effects of *N*-hydroxylation, such as *N*-acetylation. Genetic polymorphisms significantly affect activities of these enzymes, producing variations in the population that can affect susceptibility to the urinary bladder carcinogenesis response to 4-aminobiphenyl exposures. DNA adducts identical to those detected in DNA from mice and dogs have been identified in human urothelial cells, and consequently they have a similar mutagenic potential. Furthermore, extensive epidemiological evidence demonstrates the urinary bladder carcinogeneity of 4-aminobiphenyl in humans.

Bladder cancer is associated with smoking and occupational exposures to 4-aminobiphenyl. 4-Aminobiphenyl was manufactured in the United States of America from 1935 to 1955 (Melick et al., 1955) and was used as a highly efficient rubber antioxidant, but it is apparently no longer commercially produced. In epidemiological studies, which were confined to one series of workers occupationally exposed to commercial 4-aminobiphenyl, a high incidence of bladder carcinomas was reported (Melick et al., 1955, 1971; Melamed et al., 1960; Koss et al., 1965, 1969). Among 503 workers, 59 cases with positive cytology were identified, among which 35 cases of carcinoma of the urinary bladder were histologically verified; 7 remained cytologically positive at the time of publication, while 7 died from other causes and 10 were lost to follow-up (Koss et al., 1969). In addition to cigarette smoke, there also appear to be other, ill-defined environmental sources of exposure, possibly from other sources of combustion of substances containing carbon and nitrogen (Skipper et al., 2003). Cigarette smoking accounts for between 40% and 70% of the bladder cancer cases in the United States and Europe (IARC, 1986; Castelao et al., 2001). Black (air-cured) tobacco is a greater source of 4-aminobiphenyl than is blonde (flue-cured) tobacco (Bryant et al., 1988).

The key events demonstrated for 4-aminobiphenyl bladder carcinogenesis in mice and dogs have also been specifically evaluated for 4-aminobiphenyl in humans, primarily in individuals exposed to 4-aminobiphenyl in cigarette smoke, but also utilizing in vitro methods with human urothelial cells (see Table 4).

Table 4. Concordance evaluation of key events of 4-aminobiphenyl-induced urinary
bladder carcinogenesis between species.

Key event	Mouse	Dog	Human
1. Metabolic activation to reactive electrophile	+	+	+
2. DNA adduct formation	+	+	+
3. Mutagenesis	+	+	+
4. Carcinoma	+	+	+

Absorbed 4-aminobiphenyl is *N*-oxidized in the liver by CYP1A2, which, in spite of its rather high homology with CYP1A1, has an essentially different substrate specificity and is found only in liver (Lang & Pelkonen, 1999). Other enzymes have been suggested to be capable of supporting metabolic activation to the *N*-hydroxylamine.

NAT1 and NAT2 each catalyse three types of acetylation: the N-acetylation of arylamines, the O-acetylation of N-hydroxylamines, and the N,O-acetyltransfer of arylhydroxamic acids (Flammang & Kadlubar, 1986; Mattano et al., 1989; Fretland et al., 1997; Hein et al., 2000). It is believed that N-acetylation by N-acetyltransferases has a protective effect regarding bladder carcinogenicity, primarily because the acetamide of 4-aminobiphenyl formed is significantly less potent as a substrate for N-hydroxylation compared with the amine. Two genes, NAT1 and NAT2, code for the NAT isoforms, and allelic variation has been associated with susceptibility to urinary bladder cancer in humans (Hein et al., 2000). Most studies suggest that NAT2 slow acetylators are at increased risk of developing bladder cancer, whereas the contribution of the NAT1 genotype to aromatic amine bladder carcinogenesis is less clear (Cartwright et al., 1982; Hein et al., 2000). Among smokers, there is a higher level of 4-aminobiphenyl-haemoglobin adducts associated with the slow acetylator phenotype (Vineis et al., 1990). Interactions of NAT1 and NAT2 have been suggested (Cascorbi et al., 2001). In a study of 425 German bladder cancer patients, Cascorbi et al. (2001) found that there is (1) a partial linkage of the NAT1\*10 genotype to the NAT2\*4 genotype, (2) a clear underrepresentation of NAT1\*10 genotypes among rapid NAT2 genotypes in the cases studied, and (3) a gene-gene-environment interaction in that NAT2\*slow/NAT1\*4 genotype combinations with a history of occupational exposure were 5.96 (2.96-12.0) times more frequent in cancer cases than in controls without a risk from occupation (P < 0.0001). Hence, the data suggest that individuals with NAT2\*4 and NAT1\*10 are at a significantly lower risk for bladder cancer, particularly when exposed to environmental risk factors.

Polymorphisms in *CYP1A2* (Oscarson et al., n.d.) and *NAT2* (Hein et al., 2000) genes are associated with variations in the activities of these enzymes in human populations, although the extent to which variation in CYP1A2 activity is due to genetic factors has yet to be determined (Sachse et al., 2003). Moreover, expression of the *CYP1A2* gene is induced in cigarette smokers, leading to even higher CYP1A2 enzyme activities (Sesardic et al., 1988; Eaton et al., 1995). An individual exposed to 4-aminobiphenyl and expressing high levels of CYP1A2 and slow NAT2 activity would be expected to have increased levels of *N*-hydroxy-4-aminobiphenyl and, therefore, higher levels of 4-aminobiphenyl–haemoglobin adducts and 4-aminobiphenyl–DNA adducts in liver and urinary bladder than an individual expressing low levels of CYP1A2 and rapid NAT2 activity.

The tumour suppressor genes *RB1* and *TP53* appear to be involved in bladder cancer, especially high-grade urothelial carcinomas rather than low-grade papillary tumours. Both genes are involved in the regulation of the cell cycle. In addition, TP53 plays a role in response to DNA damage, cell death, and neovascularization (Hickman et al., 2002), and its gene product regulates the expression of multiple genes (Vousden & Lu, 2002). A strong association has been found between RB1 inactivation and muscle invasion (Cairns et al., 1991; Ishikawa et al., 1991; Presti et al., 1991; Primdahl et al., 2000). In one study of 45 bladder cancers, seven of nine TP53 mutations occurred in grade 3 tumours (i.e. invasion includes perivesicular tissue) (Martone et al., 1998). Inactivation of RB1 occurs in 30-80% of muscle-invasive bladder cancers (Cairns et al., 1991; Logothetis et al., 1992; Wright et al., 1995; Ioachim et al., 2000), most frequently as a consequence of heterozygous 13q deletions in combination with mutation of the remaining allele (Cordon-Cardo & Reuter, 1997). In studies investigating at least 30 tumours, TP53 mutations occurred in 40-60% of invasive bladder cancers (Tiguert et al., 2001; Lu et al., 2002). Although no specific mutational hotspots were identified, more than 90% of the mutations occurred in exons 4-9. In a study of the binding spectrum of N-hydroxy-4-aminobiphenyl in DNA fragments containing exons 5, 7, and 8 of TP53, preferential binding was identified at codon 285, a non-CpG site, and at codons 175 and 248, which are CpG sites, but only after C5 cytosine methylation had occurred (Feng et al., 2002). The authors concluded that the mutational spectrum in TP53 in bladder cancer strongly suggests a role of 4-aminobiphenyl in the etiology of this neoplasm.

Exposure to tobacco smoke, an environmental source of 4-aminobiphenyl, is associated with increased levels of 4-aminobiphenyl-haemoglobin adducts, in both adults and fetuses. In a study of smoking (n = 14) and non-smoking (n = 38) women, 4-aminobiphenyl-haemoglobin levels were  $183 \pm 108$  pg/g haemoglobin in smokers and  $22 \pm 8$  pg/g haemoglobin in non-smokers, whereas the levels in their respective fetuses were  $92 \pm 54$  pg/g haemoglobin and 17  $\pm 13$  pg/g haemoglobin (Coghlin et al., 1991), a difference that has also been observed in adults in studies of tumour tissue DNA (Curigliano et al., 1996). Haemoglobin adduct levels (used as a surrogate for exposure levels and indicator for DNA adduct potential) have been associated with levels of exposure to tobacco as a source of 4-aminobiphenyl (black tobacco > blonde tobacco > non-smokers) in a male study population from Turin, Italy; the risk of bladder cancer followed the same pattern (Bryant et al., 1988). There is a substantial gap in information linking the presence of adducts, primarily an indication of exposure, and the emergence of cancer.

In humans, 4-aminobiphenyl has been associated only with urinary bladder cancer, whereas in mice, liver and urinary bladder tumours are induced. Although the specific reasons for these species differences in organ specificity are not known, they appear to be due to variations in competing N-esterification enzymatic activations. Sulfation appears to be primarily associated with liver carcinogenesis by aromatic amines, whereas N-glucuronidation appears to be more associated with bladder carcinogenesis. Acetylation has mixed effects, but in humans appears to be principally a detoxification process that can be influenced significantly by N-acetyltransferase polymorphisms that result in fast versus slow acetylation. Human tissues have been studied for their possible involvement in the metabolism of 4aminobiphenyl and its metabolites. CYP1A2 is responsible for the metabolism of 4-aminobiphenyl to N-hydroxy-4-aminobiphenyl by human hepatic microsomal fraction (Butler et al., 1989b). N-Hydroxy-4-aminobiphenyl can be metabolized to a product that binds covalently to calf thymus DNA by cytosolic sulfotransferases from human liver and, to a lesser extent, colon, but not from pancreas or urinary bladder. In view of this lack of sulfotransferase activity in bladder, it has been suggested that hepatic sulfotransferase may actually decrease the bioavailability of N-hydroxy-4-aminobiphenyl in extrahepatic tissues and serve as a detoxification mechanism for the urinary bladder (Chou et al., 1995). On the other hand, Nacetyltransferases that are present in human urothelial cells (Frederickson et al., 1992; Swaminathan & Reznikoff, 1992) can metabolize N-hydroxy-4-aminobiphenyl, as well as the acetylated compounds N-hydroxy-4-acetylaminobiphenyl and N-acetoxy-4-acetylaminobiphenyl, to a DNA-reactive material. The major adduct co-chromatographs with N-(deoxyguanosin-8-yl)-4-aminobiphenyl. <sup>32</sup>P-postlabelling analysis of the DNA from cytosolmediated binding of N-hydroxy-4-aminobiphenyl revealed four radioactive spots. Five adducts were found when intact human urothelial cells were used, two of which were the same as two found using cytosol. This suggests the possibility of an activation pathway or pathways in addition to acetylation.

Experiments similar to those performed with dog tissues have shown that human urothelial cell microsomes possess transacetylation activity, so that *N*-hydroxy-4-aminobiphenyl binding to RNA and DNA occurs in the presence of 4-acetylaminobiphenyl, *N*-hydroxy-4-acetylaminobiphenyl, or acetylCoA as acetyl donors (Hatcher et al., 1993). These authors also found that <sup>32</sup>P-postlabelling of DNA adducts formed after reaction with *N*-hydroxy-4-aminobiphenyl, *N*-hydroxy-4-acetylaminobiphenyl, and *N*-acetoxy-4-aminobiphenyl showed similar profiles, suggesting that the arylnitrenium ion, arising from *N*-acetoxy-4-aminobiphenyl, might be the common reactive species. The structures of the adducts have been identified as the 3',5'-bisphospho derivatives of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl) (dG-C8-aminobiphenyl), *N*-(deoxyadenosin-8-yl)-4-aminobiphenyl (dA-C8-aminobiphenyl) (Frederickson et al., 1992; Hatcher & Swaminathan, 1995), and *N*-(deoxyguanosin-*N*(2)-yl)-4-azobiphenyl (Hatcher & Swaminathan, 2002).

The results available comparing tobacco smokers with non-smokers support the relevance to humans of the hypothesized MOA. In a study of 46 T1 bladder cancer cases, mean relative staining intensity for 4-aminobiphenyl–DNA adducts was significantly higher in current smokers ( $275 \pm 81$ , n = 24) than in non-smokers ( $113 \pm 71$ , n = 22) (Curigliano et al., 1996). Similar results have been reported for laryngeal tissue (Flamini et al., 1998) and for mammary tissue (Faraglia et al., 2003). Using 4-aminobiphenyl–haemoglobin adducts as an

indicator of exposure, it was found that bladder carcinoma patients had higher levels than controls (Del Santo et al., 1991), whereas lung cancer patients did not (Weston et al., 1991). The basis for this difference is unknown.

In addition to the evidence of genotoxicity generated with non-human test systems, 4-aminobiphenyl can be metabolized by human urothelial cell microsomal preparations to a mutagen in *S. typhimurium* YG1024 (a derivative of TA98 with elevated *O*-acetyltransferase activity) but not in strain TA98 itself (Hatcher et al., 1993). No other species or other human tissues were examined in this study.

6-Thioguanine-resistant mutants can be induced in a non-tumorigenic, SV40-immortalized human urothelial cell line by exposure to 4-aminobiphenyl itself or exposure to N-hydroxy-4aminobiphenyl, N-hydroxy-4-acetylaminobiphenyl, or N-acetoxy-4-acetylaminobiphenyl (Bookland et al., 1992a). No exogenous metabolic activation system was required for the observed activity. The lowest effective concentrations to produce a statistically significant increase in the mutant fraction were as follows: *N*-acetoxy-4-acetylaminobiphenyl, 2 µmol/l; *N*-hydroxy-4-acetylaminobiphenyl, 5 µmol/l; *N*-hydroxy-4-aminobiphenyl, 20 µmol/l; and 4aminobiphenyl, 100 µmol/l. Three of these substances were also tested for tumorigenic transformation using the same human immortalized urothelial cells in an in vitro-in vivo assay in which the end-point was carcinoma development when treated cells were injected subcutaneously into nude mice (Bookland et al., 1992b). Transformation was demonstrated after all treatments, the lowest concentrations being as follows: N-hydroxy-4-acetylaminobiphenyl, 0.5 µmol/l; N-hydroxy-4-aminobiphenyl, 0.5 µmol/l; and 4-aminobiphenyl, 20 µmol/l. The lower concentrations required for transformation in comparison with those for mutation are noted, but how this should be interpreted is not clear. It is consistent with the transformation being independent of mutation and with the transformation assay having a higher sensitivity, or it could merely reflect a difference in sensitivity of the methods.

In summary, on a qualitative basis, the key events in the MOA are the same in mice, dogs, and humans: metabolic activation to the *N*-hydroxylamine with subsequent formation of a reactive electrophile (presumably the nitrenium ion), formation of guanine adducts, gene mutation, and the ultimate formation of cancer. The intervening events between gene mutation and cancer, such as which genes are mutated and how cancer is induced, are not known. The MOA, nevertheless, has been clearly demonstrated and is the same in the animal models and in humans.

### CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETIC OR DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

As described in detail above, the metabolic activation, DNA adducts, and mutagenicity of 4aminobiphenyl are qualitatively the same in mice, dogs, and humans, leading to the induction of urothelial tumours of the urinary bladder in these three species and other tumours in mice, rats, and rabbits. Although detailed aspects of absorption, distribution, and excretion have not been reported, similarity in the levels of DNA adduct formation in the urothelium occurring in mice, dogs, and humans suggests that kinetic differences are not significant between these three species. Although similar enzymatic processes occur in the three species, quantitative differences are evident. These differences may explain some of the variations seen in target organ specificity among the species and might suggest possible quantitative differences in generation of the DNA adducts. Nevertheless, these differences do not negate the overall MOA for any of the species or the different target organs and are consistent with the complexity of the competing pathways for metabolic activation and deactivation.

Presumably there is a potential for repair of the different adducts, and quantitative differences might exist among species and even among tissues. However, the detection of relatively high numbers of adducts in all three species indicates that significant numbers of stable adducts are produced.

The target tissue common among mice, dogs, and humans, the urinary bladder urothelium, is similar morphologically (Pauli et al., 1983). The urothelium has a characteristic asymmetric unit membrane at the luminal surface that provides a major part of the barrier function to urine. It is composed of urothelium-specific proteins, the uroplakins, the sequence of which is highly conserved among species (Wu et al., 1994). In addition, the urothelium is metabolically active in all three species.

Modulating urinary factors have also been identified that can quantitatively affect the ultimate formation of urothelial DNA adducts, such as pH and frequency of urination (Cohen, 1995; Sarkar et al., 2002). Although the range of pH varies among species, the pH in mice, dogs, and humans readily reaches acidic and alkaline levels as well as neutral. Again, although quantitative differences occur, these do not preclude the existence of this MOA in humans.

There is no evidence implicating another MOA besides DNA reactivity. However, significant quantitative differences exist between species with regard to apparent potency of 4aminobiphenyl with respect to urinary bladder carcinogenesis. It is clear, however, that metabolites of 4-aminobiphenyl interact with proteins (e.g. haemoglobin) as well as with DNA and that metabolites of 4-aminobiphenyl are cytotoxic (Schieferstein et al., 1985; Reznikoff et al., 1986; Kadlubar et al., 1991). Interaction with urothelial cellular proteins might be responsible for the cytotoxicity and regenerative proliferation seen in the mouse bladder at higher doses of 4-aminobiphenyl. The interaction of DNA reactivity and consequent mutagenicity and cell proliferation provide an explanation for the sigmoidal shape of the dose-response curve for tumours despite a linear dose-response for DNA adducts (Cohen & Ellwein, 1990). The high concentrations of 4-aminobiphenyl found in the urine of mice that can produce urothelial cytotoxicity are generally not attained in humans exposed to cigarette smoke. However, other (unknown) substances appear to produce urothelial hyperplasia in cigarette smokers (Auerbach & Garfinkel, 1989). This increased cell proliferation significantly potentiates the effects of 4-aminobiphenyl on the bladder, providing a significantly greater number of DNA-replicating cell targets on which to act in comparison with the small number present in the normal, slowly replicating urothelium. Thus, the apparent greater potency of 4-aminobiphenyl in humans compared with mice is unlikely, but represents the synergy of DNA reactivity and cell proliferation produced by a single substance, 4-aminobiphenyl, in mice, but by different substances in the complex mixture of cigarette smoke.

Occupational exposure to 4-aminobiphenyl presumably resulted in greater doses of 4-aminobiphenyl than did exposure to cigarette smoke, since the incidence of bladder cancer in such populations was considerably higher than in smokers. However, quantitative measurements of metabolite concentrations or DNA adduct levels in urothelial cells could not be determined at the time these occupational exposures occurred, and cigarette smoking history in those individuals was not assessed (Koss et al., 1965, 1969).

In summary, although quantitative differences among species exist, they do not exclude the same MOA in mice and dogs occurring in humans.

# CONCLUSION: STATEMENT OF CONFIDENCE, ANALYSIS, AND IMPLICATIONS

The early steps in the proposed MOA are well supported by the available evidence, indicating that the key events of metabolic activation, DNA adduct formation, and mutation are the same qualitatively in mice, dogs, and humans. There is strong and sufficient evidence that 4-aminobiphenyl is a human urinary bladder carcinogen. Evidence for the intervening steps between mutation and cancer development is lacking. The associations described for adduct levels and *TP53* mutations are not compelling because these particular genetic alterations appear late in tumour progression and are often the result of endogenous causes (e.g. spontaneous depurination at methylated CpG sites). This aspect of *TP53* mutations in bladder cancer has been studied in a case–control study (Schroeder et al., 2003). In addition, most urothelial tumours in humans are low-grade papillary lesions, which generally do not have *TP53* mutations.

The mutational spectrum of N-hydroxy-4-acetylaminobiphenyl has been studied in embryonic fibroblasts of the Big Blue mouse (Besaratinia et al., 2002). Treatment of these cells for 24 h resulted in a dose-dependent increase in mutation frequency of the *cII* transgene of up to 12.8-fold over background. Single-base substitutions comprised 86% of the mutations in the treated cells and 74% of the mutations in the controls. Of these mutations, 63% and 36%, respectively, occurred at guanine residues along the *cII* gene. Whereas  $G \rightarrow T$  transversions accounted for 47% of the mutations in the treated *cII* gene, the most common mutations in untreated cells were insertions, which accounted for 19% of the mutations. Mapping of the induced adducts established five preferred DNA adduction sites, of which four were major mutation sites for N-hydroxy-4-acetoxyaminobiphenyl, especially  $G \rightarrow T$  transversions. In the TP53 gene in human bladder cancer, however,  $G \rightarrow A$  transitions predominate (53%) and are prevalent at all of its five mutational hotspots (codons 175, 248, 273, 280, and 285), three of which are at methylated CpG hotspots (175, 248, and 273). In cII, neither the preferred adduction sites nor the induced mutational hotspots are biased towards methylated CpG dinucleotides. It is concluded from this study that there is a serious discordance between the mutation pattern induced by N-hydroxy-4-acetoxyaminobiphenyl in the cII gene and the mutational pattern observed in TP53 in human bladder cancer. However, the role of methylation status and transcriptional activity on the mutation spectrum induced by 4aminobiphenyl has yet to be determined. It is also to be noted that the TP53 mutation spectrum is a reflection of a selection process during tumour development.

Based on the preceding analysis, it is clear that the MOA for 4-aminobiphenyl carcinogenesis is known in the animal model, and the MOA is relevant to humans both qualitatively and quantitatively. The conclusion based on this evaluation, even without epidemiological evidence, is that 4-aminobiphenyl poses a cancer hazard to humans.

To perform a full risk assessment requires additional information regarding the doseresponse and human exposures. Based on the information described above, it is clear that the data predict a cancer hazard for humans at expected exposures, at least for occupational (historical) and cigarette smoking exposures. Further analysis is required regarding the potential risk at ambient exposures in those who are not cigarette smokers. The MOA analysis provides the basis and foundation for such an assessment. The epidemiological evidence on 4-aminobiphenyl supports the conclusions suggested by the MOA HRF.

#### 4-AMINOBIPHENYL AND THE HUMAN RELEVANCE FRAMEWORK

4-Aminobiphenyl was evaluated using the proposed IPCS HRF based on an MOA analysis. The defined key events for this DNA reactivity MOA—metabolic activation, DNA adduct formation, mutagenicity, and cancer induction—clearly are the same in humans as in the animal (mice, dogs) models, indicating that 4-aminobiphenyl presents a cancer hazard for humans. The information for this MOA analysis provides a substantive foundation on which to build a complete cancer risk assessment for humans. For this chemical, there is also substantial epidemiological evidence to verify the conclusions derived from the HRF analysis.

The additional key events for this MOA—which genes are mutated and how do these genetic alterations lead to cancer—are not known for 4-aminobiphenyl. However, this does not detract from the conclusions, given the strength of evidence for the proposed MOA, based on the framework analysis presented here.

What data are necessary to conclude that a chemical produces cancer by a DNA-reactive MOA? Our suggestion is that at the very least there be a demonstration that DNA adducts are produced, preferably in the target tissue, and that the chemical is mutagenic (either with or without metabolic activation). Mutagenicity is used here in a more specific, restricted sense than the broader term genotoxicity. Demonstration of DNA adducts and mutagenicity in the target tissue after in vivo exposure increases confidence in the proposed MOA. Identification of the specific metabolic pathway and specific DNA adducts induced provides a significantly better basis for extrapolating between the animal model and humans.

This case demonstrates the potential utility of data on surrogate compounds in MOA analysis. However, the relevance of data on related compounds, whether in vivo or in vitro, needs to be adequately justified. Weight-of-evidence analysis of structure–activity relationships, which have been well developed for DNA reactivity and mutagenicity, should also contribute to framework analysis.

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#### REFERENCES

al-Atrash J, Zhang YJ, Lin D, Kadlubar FF, Santella RM (1995) Quantitative immunohistochemical analysis of 4-aminobiphenyl–DNA cultured cells and mice: Comparison to gas chromatography/mass spectroscopy analysis. *Chemical Research in Toxicology*, **8**:747–752.

Auerbach O, Garfinkel L (1989) Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer*, **64**:983–987.

Besaratinia A, Bates SE, Pfeifer GP (2002) Mutational signature of the proximate bladder carcinogen N-hydroxy-4-acetylaminobiphenyl: Inconsistency with the p53 mutational spectrum in bladder cancer. *Cancer Research*, **62**:4331–4338.

Block NL, Sigel MM, Lynne CM, Ng AB, Grosberg RA (1978) The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. *Investigative Urology*, **16**:50–54.

Bonser GM (1962) Precancerous changes in the urinary bladder. In: Severi L, ed. *The morphological precursor of cancer*. Perugia, University of Perugia, p. 435.

Bookland EA, Reznikoff CA, Lindstrom M, Swaminathan S (1992a) Induction of thioguanine-resistant mutations in human uroepithelial cells by 4-aminobiphenyl and its *N*-hydroxy derivatives. *Cancer Research*, **52**:1615–1621.

Bookland EA, Swaminathan S, Oyasu R, Gilchrist KW, Lindstrom M, Reznikoff CA (1992b) Tumorigenic transformation and neoplastic progression of human uroepithelial cells after exposure in vitro to 4-aminobiphenyl or its metabolites. *Cancer Research*, **52**:1606–1614.

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR (1988) Hemoglobin adducts of aromatic amines: Associations with smoking status and type of tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, **85**:9788–9791.

Burger MS, Torino JL, Swaminathan S (2001) DNA damage in human transitional cell carcinoma cells after exposure to the proximate metabolite of the bladder carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **38**:1–11.

Butler MA, Guengerich FP, Kadlubar FF (1989a) Metabolic oxidation of the carcinogens 4aminobiphenyl and 4,4'-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Research*, **49**:25–31. Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989b) Human cytochrome P-450PA (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proceedings of the National Academy of Sciences of the United States of America*, **86**:7696–7700.

Cairns P, Proctor AJ, Knowles MA (1991) Loss of heterozygosity at the *RB* locus is frequent and correlates with muscle invasion in bladder carcinoma. *Oncogene*, **6**:2305–2309.

Cairns T (1979) The  $ED_{01}$  study: Introduction, objectives, and experimental design. *Journal of Environmental Pathology and Toxicology*, **3**:1–7.

Cartwright RA, Rogers HJ, Barham-Hall D, Glashan RW, Ahmad RA, Higgins E, Kahn MA (1982) Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **16**:842–846.

Cascorbi I, Roots I, Brockmoller J (2001) Association of *NAT1* and *NAT2* polymorphisms to urinary bladder cancer: Significantly reduced risk in subjects with *NAT1\*10. Cancer Research*, **61**:5051–5056.

Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, Ross RK, Yu MC (2001) Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute*, **93**:538–545.

Chou HC, Lang NP, Kadlubar FF (1995) Metabolic activation of the *N*-hydroxy derivative of the carcinogen 4-aminobiphenyl by human tissue sulfotransferases. *Carcinogenesis*, **16**:413–417.

Clayson DB, Lawson TA, Santana S, Bonser GM (1965) Correlation between the chemical induction of hyperplasia and of malignancy in the bladder epithelium. *British Journal of Cancer*, **19**:297–310.

Clayson DB, Lawson TA, Pringle JAS (1967) The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of  $C57 \times IF$  mouse. *British Journal of Cancer*, **1**:755–762.

Coghlin J, Gann PH, Hammond SK, Skipper PL, Taghizadeh K, Paul M, Tannenbaum SR (1991) 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, **83**:274–280.

Cohen SM (1995) The role of urinary physiology and chemistry in bladder carcinogenesis. *Food and Chemical Toxicology*, **33**:715–730.

Cohen SM, Ellwein LB (1990) Proliferative and genotoxic cellular effects in 2acetylaminofluorene bladder and liver carcinogenesis: Biological modeling of the  $ED_{01}$  study. *Toxicology and Applied Pharmacology*, **104**:79–93. Cordon-Cardo C, Reuter VE (1997) Alterations of tumor suppressor genes in bladder cancer. *Seminars in Diagnostic Pathology*, **14**:123–132.

Curigliano G, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A, Santella RM (1996) Immunohistochemical quantitation of 4-aminobiphenyl–DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis*, **17**:911–916.

Dang LN, McQueen CA (1999) Mutagenicity of 4-aminobiphenyl and 4-acetylbiphenyl in *Salmonella typhimurium* strains expressing different levels of *N*-acetyltransferase. *Toxicology and Applied Pharmacology*, **159**:77–82.

Deichmann WB, MacDonald WE (1968) The non-carcinogenicity of a single dose of 4-aminobiphenyl in the dog. *Food and Cosmetics Toxicology*, **6**:143–146.

Deichmann WB, Radomski JL, Anderson WAD, Coplan MM, Woods FM (1958) The carcinogenic action of *p*-aminobiphenyl in the dog; final report. *Industrial Medicine and Surgery*, **27**:25–26.

Deichmann WB, Radomski JL, Glass E, Anderson WAD, Coplan M, Woods FM (1965) Synergism among oral carcinogens. Simultaneous feeding of four bladder carcinogens to dogs. *Industrial Medicine and Surgery*, **34**:640–649.

Delclos KB, Miller DW, Lay JO Jr, Casciano DA, Walker RP, Fu PP, Kadlubar FF (1987) Identification of C8-modified deoxyinosine and N2- and C8-modified deoxyguanosine as major products of the in vitro reaction of *N*-hydroxy-6-aminochrysene with DNA and the formation of these adducts in isolated rat hepatocytes treated with 6-nitrochrysene and 6-aminochrysene. *Carcinogenesis*, **8**:1703–1709.

Del Santo P, Moneti G, Salvadori M, Saltutti C, Delle RA, Dolara P (1991) Levels of the adducts of 4-aminobiphenyl to hemoglobin in control subjects and bladder carcinoma patients. *Cancer Letters*, **60**:245–251.

Doerge DR, Churchwell MI, Marques MM, Beland FA (1999) Quantitative analyses of 4aminobiphenyl–C8-deoxyguanosyl DNA adducts produced in vitro and in vivo using HPLC-ES-MS. *Carcinogenesis*, **6**:1055–1061.

Dooley KL, Stavenuiter JF, Westra JG, Kadlubar FF (1988) Comparative carcinogenicity of the food pyrolysis product, 2-amino-5-phenylpyridine, and the known human carcinogen, 4-aminobiphenyl, in the neonatal B6C3F1 mouse. *Cancer Letters*, **41**:99–103.

Dooley KL, Von Tungeln LS, Bucci T, Fu PP, Kadlubar FF (1992) Comparative carcinogenicity of 4-aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1 male mouse. *Cancer Letters*, **62**:205–209.

Eaton DL, Gallagher EP, Bammler TK, Kunze KL (1995) Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. *Pharmacogenetics*, **5**:259–274.

Faraglia B, Chen SY, Gammon MD, Zhang Y, Teitelbaum SL, Neugut AI, Ahsan H, Garbowski GC, Hibshoosh H, Lin D, Kadlubar FF, Santella RM (2003) Evaluation of 4-aminobiphenyl–DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis*, **24**:719–725.

Feng Z, Hu W, Rom WN, Beland FA, Tang MS (2002) *N*-Hydroxy-4-aminobiphenyl–DNA binding in human *p53* gene: Sequence preference and the effect of C5 cytosine methylation. *Biochemistry*, **41**:6414–6421.

Flamini G, Romano G, Curigliano G, Chiominto A, Capelli G, Boninsegna A, Signorelli C, Ventura L, Santella RM, Sgambato A, Cittadini A (1998) 4-Aminobiphenyl–DNA adducts in laryngeal tissue and smoking habits: An immunohistochemical study. *Carcinogenesis*, **19**:353–357.

Flammang TJ, Kadlubar FF (1986) Acetyl coenzyme A-dependent metabolic activation of *N*-hydroxy-3,2'-dimethyl-4-aminobiphenyl and several carcinogenic *N*-hydroxy arylamines in relation to tissue and species differences, other acyl donors, and arylhydroxamic acid-dependent acyltransferases. *Carcinogenesis*, 7:919–926.

Flammang TJ, Couch LH, Levy GN, Weber WW, Wise CK (1992) DNA adduct levels in congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. *Carcinogenesis*, **13**:1887–1891.

Fletcher K, Tinwell H, Ashby J (1998) Mutagenicity of the human bladder carcinogen 4aminobiphenyl to the bladder of Muta<sup>™</sup>Mouse transgenic mice. *Mutation Research*, **400**:245–250.

Frederickson SM, Hatcher JF, Reznikoff CA, Swaminathan S (1992) Acetyl transferasemediated metabolic activation of *N*-hydroxy-4-aminobiphenyl by human uroepithelial cells. *Carcinogenesis*, **13**:955–961.

Fretland AJ, Doll MA, Gray K, Feng Y, Hein DW (1997) Cloning, sequencing, and recombinant expression of NAT1, NAT2, and NAT3 derived from the C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: Functional characterization of the activation and deactivation of aromatic amine carcinogens. *Toxicology and Applied Pharmacology*, **142**:360–366.

Gaylor DW (1979) The  $ED_{01}$  study: Summary and conclusions. *Journal of Environmental Pathology and Toxicology*, **3**:179–183.

Gorrod JW, Carter RL, Roe FJ (1968) Induction of hepatomas by 4-aminobiphenyl and three of its hydroxylated derivatives administered to newborn mice. *Journal of the National Cancer Institute*, **41**:403–410.

Hammons GJ, Guengerich FP, Weis CC, Beland FA, Kadlubar FF (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. *Cancer Research*, **45**:3578–3585.

Hatcher JF, Swaminathan S (1992) Microsome-mediated transacetylation and binding of *N*-hydroxy-4-aminobiphenyl to nucleic acids by hepatic and bladder tissues from dog. *Carcinogenesis*, **13**:1705–1711.

Hatcher JF, Swaminathan S (1995) Detection of deoxyadenosine-4-aminobiphenyl adduct in DNA of human uroepithelial cells treated with *N*-hydroxy-4-aminobiphenyl following nuclease P1 enrichment and <sup>32</sup>P-postlabeling analysis. *Carcinogenesis*, **16**:295–301.

Hatcher JF, Swaminathan S (2002) Identification of *N*-(deoxyguanosin-8-yl)-4-azobiphenyl by <sup>32</sup>P-postlabeling analyses of DNA in human uroepithelial cells exposed to proximate metabolites of the environmental carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **39**:314–322.

Hatcher JF, Rao KP, Swaminathan S (1993) Mutagenic activation of 4-aminobiphenyl and its *N*-hydroxy derivatives by microsomes from cultured human uroepithelial cells. *Mutagenesis*, **8**:113–120.

Hein DW (1988) Acetylator genotype and arylamine-induced carcinogenesis. *Biochimica et Biophysica Acta*, **948**:37–66.

Hein DW, Grant DM, Sim E (2000) *Arylamine* N-*acetyltransferase (NAT) nomenclature* (http://louisville.edu/medschool/pharmacology/NAT.html).

Hickman ES, Moroni MC, Helin K (2002) The role of p53 and pRB in apoptosis and cancer. *Current Opinion in Genetics and Development*, **12**:60–66.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Hughes MF, Smith BJ, Eling TE (1992) The oxidation of 4-aminobiphenyl by horseradish peroxidase. *Chemical Research in Toxicology*, **5**:340–345.

IARC (1972) 4-Aminobiphenyl. In: *Some inorganic substances, chlorinated hydrocarbons, aromatic amines,* N-*nitroso compounds, and natural products*. Lyon, International Agency for Research on Cancer, pp. 74–79 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 1).

IARC (1986) *Tobacco smoking*. Lyon, International Agency for Research on Cancer, 421 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 38).

IARC (1987) 4-Aminobiphenyl (Group 1). In: *Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42.* Lyon, International Agency for Research on Cancer, pp. 91–92 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

Ioachim E, Charchanti A, Stavropoulos NE, Skopelitou A, Athanassiou ED, Agnantis NJ (2000) Immunohistochemical expression of retinoblastoma gene product (Rb), p53 protein, MDM2, c-erbB-2, HLA-DR and proliferation indices in human urinary bladder carcinoma. *Histology and Histopathology*, **15**:721–727.

Ishikawa J, Xu HJ, Hu SX, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R (1991) Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Research*, **51**:5736–5743.

Kadlubar FF, Miller JA, Miller EC (1977) Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Research*, **37**:805–814.

Kadlubar FF, Frederick CB, Weis CD, Zenser TV (1982) Prostaglandin endoperoxide synthetase-mediated metabolism of carcinogenic aromatic amines and their binding to DNA and protein. *Biochemical and Biophysical Research Communications*, **108**:253–258.

Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, Bailey JR, Young JF, Skipper PW, Tannenbaum SR (1991) Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Research*, **51**:4371–4377.

Kimura S, Kawabe M, Ward JM, Morishima H, Kadlubar FF, Hammons GJ, Fernandez-Salguero P, Gonzalez FJ (1999) CYP1A2 is not the primary enzyme responsible for 4-aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis*, **20**:1825–1830.

Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE (1965) Carcinogenesis in the human urinary bladder. Observations after exposure to *para*-aminodiphenyl. *New England Journal of Medicine*, **272**:767–770.

Koss LG, Melamed MR, Kelly RE (1969) Further cytologic and histologic studies of bladder lesions in workers exposed to *para*-aminodiphenyl: Progress report. *Journal of the National Cancer Institute*, **43**:233–243.

Kriek E (1992) Fifty years of research on *N*-acetyl-2-aminofluorene, one of the most versatile compounds in experimental research. *Journal of Cancer Research and Clinical Oncology*, **118**:481–489.

Lakshmi VM, Mattammal MB, Zenser TV, Davis BB (1990) Mechanism of peroxidative activation of the bladder carcinogen 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT): Comparison with benzidine. *Carcinogenesis*, **11**:1965–1970.

Lang M, Pelkonen O (1999) Metabolism of xenobiotics and chemical carcinogenesis. *IARC Scientific Publications*, **148**:13–22.

Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG (1979) Effects of dose and time in a long-term, low-dose carcinogenic study. *Journal of Environmental Pathology and Toxicology*, **3**:17–34.

Logothetis CJ, Xu HJ, Ro JY, Hu SX, Sahin A, Ordonez N, Benedict WF (1992) Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. *Journal of the National Cancer Institute*, **84**:1256–1261.

Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT (1979) *N*-Acetyltransferase phenotype and risk in urinary bladder cancer: Approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environmental Health Perspectives*, **29**:71–79.

Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C (2002) Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. *Clinical Cancer Research*, **8**:171–179.

Manjanatha MG, Li EE, Fu PP, Heflich RH (1996) H- and K-*ras* mutational profiles in chemically induced liver tumours from B6C3F1 and CD-1 mice. *Journal of Toxicology and Environmental Health*, **47**:195–208.

Martone T, Airoldi L, Magagnotti C, Coda R, Randone D, Malaveille C, Avanzi G, Merletti F, Hautefeuille A, Vineis P (1998) 4-Aminobiphenyl–DNA adducts and *p53* mutations in bladder cancer. *International Journal of Cancer*, **75**:512–516.

Mattano SS, Land S, King CM, Weber WW (1989) Purification and biochemical characterization of hepatic arylamine *N*-acetyltransferase from rapid and slow acetylator mice: Identity with arylhydroxamic acid *N*,*O*-acyltransferase and *N*-hydroxyarylamine *O*-acetyltransferase. *Molecular Pharmacology*, **68**:599–609.

McQueen CA, Chau B, Erickson RP, Tjalkens RB, Philbert MA (2003) The effects of genetic variation in *N*-acetyltransferases on 4-aminobiphenyl genotoxicity in mouse liver. *Chemico-Biological Interactions*, **146**:51–60.

Melamed MR, Koss LG, Ricci A, Whitmore WF Jr (1960) Cytohistological observations on developing carcinoma of urinary bladder in man. *Cancer (Philadelphia)*, **13**:67–74.

Melick WF, Escue HM, Naryka JJ, Mezera RA, Wheeler EP (1955) The first reported cases of human bladder tumors due to a new carcinogen—Xenylamine. *Journal of Urology (Baltimore)*, **74**:760–766.

Melick WF, Naryka JJ, Kelly RE (1971) Bladder cancer due to exposure to *para*-aminobiphenyl: A 17-year follow-up. *Journal of Urology (Baltimore)*, **106**:220–226.

Merck (1998) Merck veterinary manual. Whitehouse Station, NJ, Merck & Co., Inc.

Miller JA, Miller EC (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of human cancer*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 605–627.

Miller JA, Wyatt CS, Miller EC, Hartmann HA (1961) The *N*-hydroxylation of 4-acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of *N*-hydroxy-4-acetylaminobiphenyl in the rat. *Cancer Research*, **21**:1465–1473.

Oda Y (2004) Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. *Mutation Research*, **554**:399–406.

Oscarson M, Ingelman-Sundberg M, Daly AK, Nebert DW (n.d.) *Human Cytochrome P450 (CYP) Allele Nomenclature Committee* (http://www.cypalleles.ki.se/).

Parsons BL, Culp SJ, Manjanatha MG, Heflich RH (2002) Occurrence of H-*ras* codon 61 CAA to AAA mutation during mouse liver tumor progression. *Carcinogenesis*, **23**:943–948.

Parsons BL, Beland FA, Von Tungeln LS, Delongchamp RR, Fu P, Heflich RH (2005) Levels of 4-aminobiphenyl-induced somatic H-*ras* mutation in mouse liver correlate with potential for liver tumor development. *Molecular Carcinogenesis*, **42**:193–201.

Pauli BU, Alroy J, Weinstein RS (1983) The ultrastructure and pathobiology of urinary bladder cancer. In: Bryan GT, Cohen SM, eds. *The pathology of bladder cancer, Vol. II.* Boca Raton, FL, CRC Press, pp. 41–140.

Poirier MC, Beland FA (1992) DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications for DNA adduct-based human cancer risk assessment. *Chemical Research in Toxicology*, **5**:749–755.

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995) DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis*, **16**:2917–2921.

Presti JCJ, Reuter VE, Galan T, Fair WR, Cordon-Cardo C (1991) Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Research*, **51**:5405–5409.

Primdahl H, von der Maase H, Christensen M, Wolf H, Orntoft TF (2000) Allelic deletions of cell growth regulators during progression of bladder cancer. *Cancer Research*, **60**:6623–6629.

Reznikoff CA, Loretz LJ, Johnson MD, Swaminathan S (1986) Quantitative assessments of the cytotoxicity of bladder carcinogens towards cultured normal human uroepithelial cells. *Carcinogenesis*, 7:1625–1632.

Roberts DW, Benson RW, Groopman JD, Flammang TJ, Nagle WA, Moss AJ, Kadlubar FF (1988) Immunochemical quantitation of DNA adducts derived from the human bladder carcinogen 4-aminobiphenyl. *Cancer Research*, **48**:6336–6342.

Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ, Colorectal Cancer Study Group (2003) Polymorphisms in the cytochrome P450 CYP1A2 gene (*CYP1A2*) in colorectal cancer patients and controls: Allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *British Journal of Clinical Pharmacology*, **55**:68–76.

Sarkar MA, Nseyo UO, Zhong B-Z (2002) Mutagenic outcome of the urinary carcinogen 4aminobiphenyl is increased in acidic pH. *Environmental Toxicology and Pharmacology*, **11**:23–26.

Schieferstein GJ, Littlefield NA, Gaylor DW, Sheldon WG, Burgers GT (1985) Carcinogenesis of 4-aminobiphenyl in BALB/cStCrlfC3Hf/Nctr mice. *European Journal of Cancer and Clinical Oncology*, **21**:865–873.

Schroeder JC, Conway K, Li Y, Mistry K, Bell DA, Taylor JA (2003) *p53* mutations in bladder cancer: Evidence for exogenous versus endogenous risk factors. *Cancer Research*, **63**:7530–7538.

Sesardic D, Boobis AR, Edwards RJ, Davies DS (1988) A form of cytochrome P450 in man, orthologous to form *d* in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *British Journal of Clinical Pharmacology*, **26**:363–372.

Skipper PL, Tannenbaum SR, Ross RK, Yu MC (2003) Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **12**:503–507.

Smith BJ, Curtis JF, Eling TE (1991) Bioactivation of xenobiotics by prostaglandin H synthase. *Chemico-Biological Interactions*, **79**:245–264.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Krose D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Swaminathan S, Reznikoff CA (1992) Metabolism and nucleic acid binding of *N*-hydroxy-4-acetylaminobiphenyl and *N*-acetoxy-4-acetylaminobiphenyl by cultured human uroepithelial cells. *Cancer Research*, **52**:3286–3294.

Talaska G, Dooley KL, Kadlubar FF (1990) Detection and characterization of carcinogen– DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by <sup>32</sup>Ppostlabelling and subsequent thin-layer and high-pressure liquid chromatography. *Carcinogenesis*, **11**:639–646.

Tiguert R, Bianco FJJ, Oskanian P, Li Y, Grignon DJ, Wood DPJ, Pontes JE, Sarkar FH (2001) Structural alteration of p53 protein in patients with muscle invasive bladder transitional cell carcinoma. *Journal of Urology*, **166**:2155–2160.

Tsuneoka Y, Dalton TP, Miller ML, Clay CD, Shertzer HG, Talaska G, Medvedovic M, Nebert DW (2003) 4-Aminobiphenyl-induced liver and urinary bladder DNA adduct formation in Cyp1a2(-/-) and Cyp1a2(+/+) mice. *Journal of the National Cancer Institute*, **95**:1227–1237.

Underwood PM, Zhou Q, Jaeger M, Reilman R, Pinney S, Warshawsky D, Talaska G (1997) Chronic, topical administration of 4-aminobiphenyl induces tissue-specific DNA adducts in mice. *Toxicology and Applied Pharmacology*, **144**:325–331.

Verghis SBM, Essigmann JM, Kadlubar FF, Morningstar ML, Lasko DD (1997) Specificity of mutagenesis by 4-aminobiphenyl: Mutations at G residues in bacteriophage M13 DNA and  $G \rightarrow C$  transversions at a unique dG<sup>8-ABP</sup> lesion in single-stranded DNA. *Carcinogenesis*, **18**:2403–2414.

Vineis P, Caporaso N, Tannenbaum SR, Skipper PL, Glogowski J, Bartsch H, Coda M, Talaska G, Kadlubar FF (1990) Acetylation phenotype, carcinogen–hemoglobin adducts, and cigarette smoking. *Cancer Research*, **50**:3002–3004.

Von Tungeln LS, Bucci TJ, Hart RW, Kadlubar FF, Fu PP (1996) Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) in the CD1 newborn mouse bioassay. *Cancer Letters*, **104**:133–136.

Vousden KH, Lu X (2002) Live or let die: The cell's response to p53. *Nature Reviews. Cancer*, **2**:594–604.

Walpole AL, Williams MHC, Roberts DC (1952) The carcinogenic action of 4aminodiphenyl and 3:2'-dimethyl-4-aminodiphenyl. *British Journal of Industrial Medicine*, **9**:255–263.

Walpole AL, Williams MHC, Roberts DC (1954) Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl. *British Journal of Industrial Medicine*, **11**:105–109.

Weston A, Caporaso NE, Taghizadeh K, Hoover RN, Tannenbaum SR, Skipper PL, Resau JH, Trump BF, Harris CC (1991) Measurement of 4-aminobiphenyl–hemoglobin adducts in lung cancer cases and controls. *Cancer Research*, **51**:5219–5223.

Wright C, Thomas D, Mellon K, Neal DE, Horne CH (1995) Expression of retinoblastoma gene product and p53 protein in bladder carcinoma: Correlation with Ki67 index. *British Journal of Urology*, **75**:173–179.

Wu XR, Lin JH, Walz T, Haner M, Yu J, Aebi U, Sun TT (1994) Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. *Journal of Biological Chemistsy*, **269**:13716–13724.

#### FORMALDEHYDE AND GLUTARALDEHYDE AND NASAL CYTOTOXICITY: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

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Formaldehyde and glutaraldehyde cause toxicity to the nasal epithelium of rats and mice upon inhalation. In addition, formaldehyde above certain concentrations induces dose-related increases in nasal tumours in rats and mice, but glutaraldehyde does not. Using the 2006 International Programme on Chemical Safety (IPCS) human framework for the analysis of cancer mode of action (MOA), an MOA for formaldehyde was formulated and its relevance tested against the properties of the non-carcinogenic glutaraldehyde. These compounds produce similar patterns of response in histopathology and in genotoxicity tests (although formaldehyde has been much more extensively studied). The MOA is based on the induction of sustained cytotoxicity and reparative cell proliferation induced by formaldehyde at concentrations that also induce nasal tumours upon long-term exposure. Data on dose dependency and temporal relationships of key events are consistent with this MOA. While a genotoxic MOA can never be ruled out for a compound that is clearly genotoxic, at least in vitro, the non-genotoxic properties fundamental to the proposed MOA can explain the neoplastic response in the nose and may be more informative than genotoxicity in risk assessment. It is not yet fully explained why glutaraldehyde remains non-carcinogenic upon inhalation, but its greater inherent toxicity may be a key factor. The dual aldehyde functions in glutaraldehyde are likely to produce damage resulting in fewer kinetic possibilities (particularly for proteins involved in differentiation control) and lower potential for repair (nucleic acids) than would be the case for formaldehyde. While there have been few studies of possible glutaraldehyde-associated cancer, the evidence that formaldehyde is a human carcinogen is strong for nasopharyngeal cancers, although less so for sinonasal cancers. This apparent discrepancy could be due in part to the classification of human nasal tumours with tumours of the sinuses, which would receive much less exposure to inhaled formaldehyde. Evaluation of the human relevance of the proposed MOA of formaldehyde in rodents is restricted by human data limitations, although the key events are plausible. It is clear that the human relevance of the formaldehyde MOA in rodents cannot be excluded on either kinetic or dynamic grounds.

#### INTRODUCTION

Formaldehyde and glutaraldehyde are aliphatic mono- and dialdehydes, respectively, that undergo reactions typical of aldehydes to form acetals, cyanohydrins, oximes, hydrazones, and bisulfite complexes. They are highly reactive chemicals and produce covalently crosslinked complexes with DNA and proteins. Their metabolism has some commonality in that they are both oxidized by aldehyde dehydrogenases. Several studies have demonstrated that inhalation exposure to formaldehyde causes nasal tumours in rats, whereas no nasal tumours were observed in the only 2-year inhalation study of rats exposed to glutaraldehyde.

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#### Formaldehyde

Formaldehyde has been tested for carcinogenicity by the inhalation route in mice, rats, and Syrian hamsters, by oral administration (drinking-water) in rats, by skin application in mice, and by subcutaneous injection in rats. There is conclusive evidence from the inhalation studies that formaldehyde is a carcinogen in rats.

There is considerable evidence that prolonged inhalation exposure to formaldehyde induces highly non-linear dose-related increases in the incidence of tumours of the anterior and posterior lateral meatus of rats (Morgan et al., 1986; Feron et al., 1988; Woutersen et al., 1989; Monticello et al., 1996; Kamata et al., 1997; CIIT, 1999). There are sharp increases in tumour incidence at formaldehyde concentrations equal to and greater than 7.2 mg/m<sup>3</sup>. Exposure to concentrations of 2.4 mg/m<sup>3</sup> and lower induced no malignant nasal tumours. Table 1 combines the data from two published rat studies (Kerns et al., 1983a; Monticello et al., 1996) conducted at the same laboratory and some additional information from one of these studies on a number of rats that had not been examined at the time of the publications (Schlosser et al., 2003). The majority of formaldehyde-induced neoplasms were squamous cell carcinomas.

Formaldehyde concentration (mg/m <sup>3</sup> )	Number of rats at risk <sup>a</sup>	Actual number of tumours <sup>b</sup>
0	122	0
0.84	27	0
2.4	126	0
7.2	113	3
12	34	22
18	182	157

Table 1. Combined incidence of nasal squamous cell carcinomas in rats exposed to
formaldehyde.

Note: Adapted from Schlosser et al. (2003).

<sup>a</sup> Rats at risk are those that survived to 2 years and were examined at that time plus those that died before 2 years in which tumours were found.

<sup>b</sup> Rats in which tumours were found at or before 2 years.

In contrast, inhalation studies in Syrian hamsters showed no carcinogenic effect at a single dose of 12.3 mg/m<sup>3</sup> (Dalbey, 1982), and one of two inhalation studies in mice showed no effect in females and squamous cell carcinomas in 2/17 males killed at 2 years at a high-dose concentration of 17.6 mg/m<sup>3</sup> (Kerns et al., 1983a, 1983b), whereas the other was inadequate for evaluation (Horton et al., 1963).

Studies on rats using other routes of exposure produced no significant results in two of four drinking-water studies (Takahashi et al., 1986; Tobe et al., 1989), forestomach papillomas in one study (Til et al., 1989), and leukaemia and gastrointestinal tract tumours in another (Soffritti et al., 1989), but the interpretation of the last study has been questioned (Feron et al., 1990). Mouse skin application and subcutaneous injection studies were not suitable for evaluation. In no study in rodents was there a significant increase in nasal tumours other than in the five inhalation exposure studies in rats—that is, at the entry portal.

#### Glutaraldehyde

Glutaraldehyde has been tested for carcinogenicity by the inhalation route in mice and rats and by oral administration (drinking-water) in rats. Inhalation studies showed no carcinogenic effect in either B6C3F1 mice exposed to a single concentration of 400  $\mu$ g/m<sup>3</sup> for 78 weeks (Zissu et al., 1998) or multiple concentrations up to 1000  $\mu$ g/m<sup>3</sup> for 2 years (NTP, 1999) or F344 rats exposed to concentrations of up to 3000  $\mu$ g/m<sup>3</sup> for 2 years (NTP, 1999). In a drinking-water study in which male and female F344 rats were exposed to glutaraldehyde concentrations of up to 4000 mg/m<sup>3</sup> for 2 years, increased incidences of large granular cell lymphatic leukaemia were found in the spleen of females at all exposure concentrations (Ballantyne, 1995; Van Miller et al., 1995).

## 1. IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH A MODE OF ACTION (MOA) IN ANIMALS?

#### A. Postulated mode of action

Prolonged exposure to formaldehyde above a critical concentration induces sustained cytotoxicity and cell proliferation. As a result of genetic changes within this proliferating cell population, neoplasia emerges. The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by formaldehyde. Formal-dehyde is a genotoxic substance in vitro and forms DNA–protein cross-links (DPX). DPX are a well established indicator of formaldehyde exposure, but it is not clear whether they are premutational lesions required to produce neoplasia (by initiating DNA replication errors, resulting in mutation). Apart from the abundance of DPX observations in rats, there is little evidence that formaldehyde is mutagenic to mammalian cells in vivo.

This postulated MOA is mainly based on observations of consistent, non-linear dose–response relationships for all three key events (sustained cell proliferation, DPX, and tumours) and concordance of incidence of these effects across regions of the nasal passages.

#### **B. Key events**

#### Formaldehyde

Limitation of damage to the entry portal following exposure to formaldehyde is clearly important, with metabolism playing a significant role in the process. The importance of the entry portal for formaldehyde-induced nasal tumours is supported by the observation that the principal non-neoplastic effect in rats exposed orally to formaldehyde solutions is the development of histological changes within the forestomach and glandular stomach (Til et al., 1989; Tobe et al., 1989).

Formaldehyde is an endogenous metabolic product of *N*-, *O*-, and *S*-demethylation reactions within cells (Hardman et al., 2001), and circulating concentrations of about 2.0–2.6  $\mu$ g/g blood are normal in unexposed mammals (Heck et al., 1982, 1985; Casanova et al., 1988). Exogenous formaldehyde is rapidly detoxified upon absorption. It has a half-life in plasma of about 1 min in rats exposed intravenously (Rietbrock, 1965), and it readily and spontaneously combines with reduced glutathione to form *S*-hydroxymethylglutathione, the substrate for alcohol dehydrogenase 3 (ADH3, also known as glutathione-dependent formaldehyde

dehydrogenase) (Uotila & Koivusalo, 1974; Koivusalo et al., 1989), to form S-formylglutathione, which is further metabolized to formic acid and reduced glutathione by S-formylglutathione hydrolase (Uotila & Koivusalo, 1997). The  $K_M$  for initial binding of hydroxymethylglutathione with ADH3 is about 0.004 mmol/l, and the concentration of free formaldehyde is likely to be even lower (Uotila & Koivusalo, 1997; Hedberg et al., 1998). It may be toxicologically significant that formaldehyde also combines with thiols such as cysteine and cysteinylglycine (Holmquist & Vallee, 1991). In addition to this efficient metabolic detoxification mechanism, the mucociliary apparatus provides protection of the underlying epithelium from gases and vapours. Thus, in order to attain free formaldehyde concentrations that may be cytotoxic to the target tissue, relatively high concentrations of formaldehyde vapour must be delivered to the target site to overcome these protective mechanisms. Mechanistic events of clear significance for carcinogenicity occur at dose levels where formaldehyde detoxification mechanisms are saturated in rats (Casanova & Heck, 1987).

The predominant non-neoplastic and preneoplastic events that have been measured and associated with nasal cancer formation following inhalation exposure of the nasal epithelium to formaldehyde include cytotoxicity, DPX formation, nasal epithelial cell regenerative proliferation, squamous metaplasia, and inflammation, which are site-specific, highly non-linear response processes in concordance with the incidence of nasal tumours.

The relative magnitude of an increase in cell proliferation is dependent upon the size of the target cell population within specific regions of the nasal cavity and not always directly related to the length of exposure, or total cumulative exposure (Swenberg et al., 1983, 1986; Monticello et al., 1991, 1996; Monticello & Morgan, 1994). These factors have been well defined and measured in a number of studies in rat, monkey, and human epithelial cells. In a 24-month carcinogenicity assay with interim sacrifices at 3, 6, 12, and 18 months, cell proliferation was demonstrated in rats exposed to 7.2, 12, and 18 mg/m<sup>3</sup> at all times (Monticello et al., 1991, 1996).

An immunohistochemical technique was used to assess the presence of p53 protein, a marker of cell proliferation (proliferating cell nuclear antigen, or PCNA), and tumour growth factor (TGF)- $\alpha$  in the histopathological sections of the same tumours. In addition to the p53-positive immunostaining in squamous cell carcinomas, especially in cells with keratinization, p53-positive immunostaining was observed in preneoplastic hyperkeratotic plaques, while normal nasal mucosa did not stain. A correlation was found between the distribution of immunostaining of PCNA and that of p53 (Wolf et al., 1995).

The formation of DPX in rats is a non-linear function of concentration (Casanova & Heck, 1987; Casanova et al., 1989, 1994; Heck & Casanova, 1995) and correlates with the site specificity of tumours (Casanova et al., 1994). Cross-links were not detected in the olfactory mucosa or in the bone marrow of rats (Casanova-Schmitz et al., 1984; Casanova & Heck, 1987). DPX have been found in rhesus monkeys following inhalation exposure to formal-dehyde, with the highest concentrations in the middle turbinates, followed by the anterior lateral wall septum and nasopharynx (Casanova et al., 1991).

Studies of rats, mice, Syrian hamsters, and rhesus monkeys exposed to formaldehyde for 13 (mice) or 26 weeks found that squamous metaplasia in the nasal turbinates developed in rats and rhesus monkeys at 3.7 mg/m<sup>3</sup>, but not in Syrian hamsters or, at 4.9 mg/m<sup>3</sup>, in mice (Rusch et al., 1983; Maronpot et al., 1986). Cell replication is also a feature of the more tumour-susceptible areas of the nasal epithelium of rats (Casanova et al., 1994).

#### Glutaraldehyde

Inhalation exposure to glutaraldehyde at 400  $\mu$ g/m<sup>3</sup> for 78 weeks resulted in non-neoplastic lesions in the nasal vestibule of female mice, consisting of hyperplasia of the squamous epithelium lining the dorsal wall and the lateral aspect of the atrioturbinate (Zissu et al., 1998).

In the United States National Toxicology Program (NTP) studies of glutaraldehyde, the nasal changes observed in male and female rats included the following:

- 1. In the squamous epithelium in the most rostral part of the nasal passage, behind the external nares, there were increased incidences of hyperplasia and inflammation. The hyperplasia was a minimal to marked change characterized by variable thickening of the epithelium due to an increase in the number of cell layers and, in the more severe cases, varying degrees of keratin accumulation.
- 2. In the respiratory epithelium, there was hyperplasia, minimal goblet cell hyperplasia (primarily along the nasal septum and ventral meatus), inflammation, and squamous metaplasia, with accumulation of keratin on the epithelial surface in the more severe cases.
- 3. In the olfactory epithelium of the dorsal meatus, there were slightly increased incidences of hyaline degeneration.

The glutaraldehyde-associated inflammation that was observed in the squamous epithelial and respiratory epithelial regions was a minimal to marked change consisting of multifocal to locally extensive infiltrates of neutrophils, lymphocytes, and plasma cells. Occasionally, there were a few macrophages within the lamina propria and, in severe cases, within the epithelium itself. In male and female mice of this same study, the lesions were qualitatively similar to those found in rats. Females were more severely affected than male mice.

Glutaraldehyde induced DPX in a TK6 human lymphoblast cell line (St. Clair et al., 1991). In vivo, glutaraldehyde induced cell proliferation (S-phase nuclei) in nasal cells in rats and mice exposed by inhalation (Gross et al., 1994) and nasal instillation (St. Clair et al., 1990). In a parallel nasal instillation study by the same authors, formaldehyde induced the same level of cell proliferation at 20-fold higher molar concentrations.

#### C. Dose-response relationship

#### Formaldehyde

Available data from rats exposed to formaldehyde show a highly non-linear dose–response pattern for the key events, with no observed effects at 2.4 mg/m<sup>3</sup>, a minimal response at 7.2 mg/m<sup>3</sup>, and a sharp increase at 12 and 18 mg/m<sup>3</sup>.

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In rats exposed to formaldehyde, no increases in cell turnover or DNA synthesis were found in the nasal mucosa after subchronic or chronic exposure to concentrations of  $\leq 2.4 \text{ mg/m}^3$ (Rusch et al., 1983; Zwart et al., 1988; Monticello et al., 1991; Casanova et al., 1994). Small, site-specific increases in the rate of cell turnover were noted at 3.7 mg/m<sup>3</sup> (6 h/day, 5 days/week, for 13 weeks) in Wistar rats (Zwart et al., 1988) and in the rate of DNA synthesis at 7.2 mg/m<sup>3</sup> in Fischer 344 rats exposed for a similar period (Casanova et al., 1994). At these concentrations, however, an adaptive response would seem to occur in rat nasal epithelium, since cell turnover rates after 6 weeks (Monticello et al., 1991) or 13 weeks (Zwart et al., 1988) are lower than those after 1–4 days of exposure. The unit length labelling index (ULLI) method was used to establish the proliferation in male Fischer 344 rats exposed to formaldehyde concentrations of 0, 0.84, 2.4, 7.2, 12, or 18 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 3, 6, 12, 18, or 24 months. Significant increases in ULLI were present only in the 12 and 18 mg/m<sup>3</sup> groups, with the greater increases on the anterior lateral meatus and the medial maxilloturbinate. Elevated ULLI in the anterior dorsal septum developed later in the course of the exposure. This belated elevation of ULLI may have been secondary to changes in airflow patterns and thus local formaldehyde concentrations associated with growth of lesions and distortion of the airspace in those areas of the nose more susceptible to neoplasia (Monticello et al., 1996).

The non-linear relationships for formaldehyde-induced DPX formation, epithelial cell proliferation, and subsequently nasal tumours are demonstrated in Table 2. It is arguable that the designations of high- and low-tumour areas proposed by Casanova et al. (1994) are not the most appropriate, and consequently the truly high tumour incidence region DPX response may have been diluted by that of the intermediate tumour incidence (posterior lateral meatus) region.

Other studies showed that Fischer 344 rats exposed to 1.2 mg/m<sup>3</sup> (22 h/day, 7 days/week, for 26 weeks) developed no detectable nasal lesions, whereas at 3.6 mg/m<sup>3</sup>, the only histological change was squamous metaplasia in the nasal turbinates (Rusch et al., 1983). The development of mild squamous metaplasia was similarly demonstrated in the nasal turbinates of Fischer 344 rats exposed to 2.4 mg/m<sup>3</sup> (6 h/day, 5 days/week, for 24 months) (Kerns et al., 1983b). Epithelial dysplasia and rhinitis were also observed in these rats. The occurrence of squamous metaplasia appears to be the histological feature requiring the lowest formaldehyde concentration of any of the in vivo responses reported.

A rat, anatomically accurate computational fluid dynamics model was used to test whether the distribution of formaldehyde-induced squamous metaplasia was related to the location of high-flux regions posterior to the squamous epithelium. Squamous metaplasia was considered present when  $\geq$ 50% of a subsection was lined by squamous epithelium. No squamous metaplasia was present in sections of nose from rats exposed to 2.4 mg/m<sup>3</sup> or less. Squamous metaplasia was present on the lateral meatus after exposure to 7.2 mg/m<sup>3</sup> or more and on the lateral and medial walls of the airway after exposure to 12 or 18 mg/m<sup>3</sup> (Kimbell et al., 1997).

There is evidence that glutathione-mediated detoxification of formaldehyde within nasal tissues becomes saturated in rats at inhalation exposures above  $4.8 \text{ mg/m}^3$ . This saturation of

formaldehyde metabolism may contribute to the non-linearity of the dose–response relationships for DPX, cell proliferation, and tumour incidence at exposures above this level (Casanova & Heck, 1987).

Formalde- hyde	Cell proliferation ([ <sup>3</sup> H]thymidine-labelled . cells/mm basement membrane) <sup>a</sup>		DNA–protein cross-link formation (pmol [ <sup>14</sup> C]- formaldehyde bound/mg DNA) <sup>b</sup>		Incidence of nasal carcinoma <sup>c</sup>				
concen- tration (mg/m³)	Anterior lateral meatus	Posterior lateral meatus	Anterior mid- septum		"Low- tumour region"	All sites	Anterior lateral meatus	Posterior lateral meatus	Anterior mid- septum
0	10.11	7.69	6.58	0	0	0/90	0/90	0/90	0/90
0.84	10.53	7.82	8.04	5	5	0/90	0/90	0/90	0/90
2.4	9.83	11.24	12.74	8	8	0/96	0/96	0/96	0/96
7.2	15.68	9.96	4.15	30	10	1/90	1/90	0/90	0/90
12	76.79	15.29	30.01	_	_	20/90	12/90	2/90	0/90
18	93.22	59.52	75.71	150	60	69/147	17/147	9/147	8/147

### Table 2. Comparative effects of formaldehyde exposure upon cell proliferation, DNA– protein cross-linking, and tumour incidence.

<sup>a</sup> Cell proliferation measured in three locations of the nasal epithelium in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for 3 months (Monticello et al., 1996).

<sup>b</sup> Extent of DNA-protein cross-link formation measured in two regions of the nasal cavity (respiratory mucosa) in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for about 12 weeks; the complete lateral meatus was designated the "high-tumour region"; the "low-tumour region" comprised the medial aspects of naso- and maxilloturbinates, posterior lateral wall, posterior dorsal septum excluding olfactory region, and nasopharyngeal meatuses (Casanova et al., 1994). Data were derived from graphical representations in the reference cited.

<sup>c</sup> Incidence of nasal tumours within the entire nasal cavity or the anterior lateral meatus, posterior lateral meatus, or anterior mid-septum in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for 24 months (Monticello et al., 1996).

#### Glutaraldehyde

A series of repeated-dose experiments with rats and mice exposed to glutaraldehyde has been summarized by NICNAS (1994). Among these, the lowest concentration producing lesions of the nasal cavity of rats was 1000  $\mu$ g/m<sup>3</sup> (6 h/day, 5 days/week, for 13 weeks) (NTP, 1993). The most severe lesions occurred in the anterior portions of the nasal passages and involved both the respiratory and olfactory epithelium. Hyperplasia and squamous metaplasia were most commonly noted on the lateral wall of the nasal cavity and on the tips of the nasoturbinates. Lesions were most extensive in rats exposed to 4000  $\mu$ g/m<sup>3</sup>, but were also noted in the 1000 and 2000  $\mu$ g/m<sup>3</sup> groups and in one male exposed to 500  $\mu$ g/m<sup>3</sup> delivered for 14 weeks (Bushy Run, 1983).

Mice appeared to be more sensitive to glutaraldehyde inhalation in a 13-week study, with inflammation of the nasal cavity being observed in female mice even at the lowest concentration of 250  $\mu$ g/m<sup>3</sup> and in male mice at 1000  $\mu$ g/m<sup>3</sup>. The species difference in

sensitivity is probably due to the smaller airways of mice being more prone to blockage by debris (NTP, 1993). Histopathological lesions in the respiratory tract were most severe in mice in the 4000  $\mu$ g/m<sup>3</sup> group and consisted of minimal to mild squamous metaplasia of the laryngeal epithelium, suppurative inflammation in the anterior parts of the nasal cavity, and minimal squamous metaplasia on the tips of the nasoturbinates. Necrosis and inflammation were noted at lower concentrations, primarily in the anterior portion of the nasal passage.

In the NTP (1993) 13-week studies with glutaraldehyde, there were significant, exposurerelated increases in ULLI in the squamous epithelium of the nasal vestibule and, to a lesser extent, the respiratory epithelium of the atrioturbinate of the dorsal meatus. The exposurerelated increase in cell replication was generally greater in rats than in mice. Upon examining the results in individual mice, it was found that there was an increased rate of cell replication in the squamous epithelium of the nasal vestibule only of those mice in which there was also neutrophilic infiltration of the mucosa; however, the severity of the infiltrate did not correlate with the degree of cell proliferation. These observations were clearest at 13 weeks, particularly in female mice. In rats, in addition to increased replication in the squamous epithelium of the dorsal atrioturbinate, whereas in mice, the response in this area was weak.

#### **D. Temporal association**

#### Formaldehyde

A number of short-, medium-, and long-term studies of the effect of formaldehyde exposure on cell proliferation within the respiratory epithelium of rats have indicated a sustained increase in proliferation of nasal epithelial cells following exposure to concentrations greater than 2.4 mg/m<sup>3</sup>, irrespective of the exposure period. Cell proliferation was observed in rats exposed to formaldehyde for periods from as short as 3 days. In the ULLI study already described, the magnitude of increased cell proliferation generally decreased over time but remained significantly increased by approximately 2- to 10-fold over controls, for certain nasal locations, up to and including the 18-month observation period when this effect was last examined (Monticello et al., 1996).

Data relating to temporal associations for DPX are limited, as most formaldehyde inhalation studies of DPX formation are of short duration (i.e. exposure duration up to 1 day). Formaldehyde-induced DPX in the nasal epithelium of rats and rhesus monkeys was shown consistently in these studies (Casanova et al., 1991). However, a well conducted study investigating both acute and cumulative DPX yields in rats exposed to formaldehyde for about 12 weeks (Casanova et al., 1994) found that the acute DPX yield in the lateral meatus (a high tumour yield site) of previously exposed rats was about half that in naive rats at concentrations greater than 7.2 mg/m<sup>3</sup>, while there were no differences in the medial and posterior meatuses (low tumour yield sites). No significant accumulation of DPX occurred in previously exposed rats.

Regenerative cell proliferation following formaldehyde-induced cytotoxicity increases the number of DNA replications and thus increases the probability of DPX-initiated DNA

replication errors, resulting in mutations. This hypothesis is supported by the observed inhibition of DNA replication in the rat nose at elevated concentrations (Heck & Casanova, 1995) and increased p53 expression in preneoplastic lesions (Wolf et al., 1995). In 5 of 11 squamous cell carcinomas from rats exposed to 18 mg/m<sup>3</sup> for up to 2 years, there were point mutations at the GC base pairs in the p53 complementary DNA (cDNA) sequence (Recio et al., 1992).

#### Glutaraldehyde

The study of cell replication in the 13-week rat and mouse inhalation studies with glutaraldehyde (NTP, 1993) showed that, in contrast to the results obtained for mice, the increased cell replication (ULLI) in the nasal vestibule of rats occurred early (within a few days) and either remained elevated or decreased slightly through the course of the study. Increases in ULLI in the nasal vestibule of mice tended to develop with time. In an inhalation study with mice (Zissu et al., 1994), the earliest lesions were observed in the respiratory epithelium of the septum and the naso- and maxilloturbinates after 4 days of exposure to 1.2 mg/m<sup>3</sup>. Severe histopathological changes were still observed 2 weeks after the end of the exposure to 4.0 mg/m<sup>3</sup>. No exposure-related histological abnormalities were detected in the trachea and lungs.

### **E.** Strength, consistency, and specificity of association of tumour response with key events

#### Formaldehyde

There are extensive studies investigating formaldehyde-induced neoplasia. Available data revealed formaldehyde-induced DPX formation and increased epithelial cell proliferation within the upper respiratory tract in a range of species including rats and monkeys and a variety of rat and human cells in vitro. It was found that at similar levels of exposure, concentrations of DPX were approximately an order of magnitude lower in rhesus monkeys than in rats. Increased human epithelial cell proliferation following in situ exposure to formaldehyde was reported in a model system in which rat tracheae populated with human tracheobronchial epithelial cells were xenotransplanted into athymic mice.

There is good correlation between key events and regional tumour incidence and tumour sites. Cell proliferation, metaplasia, and increased DPX were seen in the regions of the nasal cavity where tumours have been observed. The highly non-linear dose–response relationships for DPX, cytotoxicity, cell proliferation, metaplasia, and tumours are consistent, with significant increases in metaplasia occurring at 2.4 mg/m<sup>3</sup> in one study and all end-points being observed at concentrations of greater than 4.8 mg/m<sup>3</sup>. This is also in good correlation with the concentration at which mucociliary clearance is inhibited and glutathione-mediated metabolism is saturated—that is, 4.8 mg/m<sup>3</sup>. The study by Morgan et al. (1986) examining effects of inhaled formaldehyde on the nasal mucociliary apparatus in male rats also included 18-h recovery groups following days 1, 9, and 14 of exposure to concentrations of 2.4 mg/m<sup>3</sup>, 7.2 mg/m<sup>3</sup>, and 18 mg/m<sup>3</sup>. Inhibition of mucociliary clearance was progressively more extensive with increasing duration of exposure, but showed little or no evidence of recovery 18 h after cessation of exposure.

Mice appear to be less susceptible than rats to the development of nasal tumours following exposure to a given concentration of formaldehyde. However, it is well known that mice decrease their minute volume in response to inhalation of noxious chemicals (Brown et al., 1986, in CIIT, 1999).

#### Glutaraldehyde

In comparison with formaldehyde, the glutaraldehyde-induced lesions were located in a more anterior part of the nose, involving the squamous epithelium. Also, they were of a different character, with none of the focal hyperkeratosis and hyperplasia with cellular atypia and dysplasia found in animals receiving formaldehyde for 13 weeks (Monticello, 1990; Morgan & Monticello, 1990).

#### F. Biological plausibility and coherence

#### Formaldehyde

Evidence supporting the hypothesis that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis continues to accumulate (IPCS, 2002). This proposed MOA for formaldehyde-induced nasal tumours in animals exposed by inhalation is consistent with biological plausibility and the available data. Sustained increased cell proliferation has been observed in the nasal cavity in extensive short- and medium-term toxicity studies in rats and a few studies in other species. Histopathological effects in the nasal cavity (epithelial cell dysplasia and metaplasia) were consistent in a range of sub-chronic and chronic animal studies. It should be noted, however, that the respective roles of DPX, mutation, and cellular proliferation in the induction of nasal tumours in the rat have not been fully elucidated.

#### Glutaraldehyde

Effects of inhaled glutaraldehyde have not been as extensively studied as those of formaldehyde. In inhalation studies, glutaraldehyde did not induce nasal tumours in rats and mice. However, the same key events that are considered key events in the nasal carcinogenicity of formaldehyde—cytotoxicity and cell proliferation—have been demonstrated in rats and mice exposed to glutaraldehyde. This might appear to reduce the plausibility of these processes being important for formaldehyde.

#### G. Possible alternative modes of action

#### Formaldehyde

There is the possibility that mutagenicity could play a role in the development of formaldehyde-induced tumours. Evaluation of the available data indicates that formaldehyde is genotoxic in vitro, but is generally not genotoxic in standard in vivo assays, although there are many studies demonstrating that it produces DPX.

Formaldehyde has been extensively studied for genotoxicity in vitro, with positive results in studies with bacterial and mammalian cells (Ames test, gene mutation), and produced DNA single-strand breaks and DPX (reviewed in IARC, 2005). In vivo, formaldehyde has reproducibly induced mutations in *Drosophila*, but there is no convincing evidence of its genotoxic activity in rodent bone marrow cell tests. There is limited evidence that formaldehyde expo-

sure is associated with increased chromosomal aberration and micronucleus frequencies in human nasal and buccal cells and peripheral blood lymphocytes (reviewed in IARC, 2005; see Appendix).

It is unclear to what extent DPX contributes to the mutagenesis and carcinogenicity of formaldehyde (Recio, 1997; Merk & Speit, 1998; Speit et al., 2000; Liteplo & Meek, 2003). The presence of DPX has been considered mainly as an indicator of exposure, although some have also seen these lesions as premutagenic in character and therefore evidence of a direct genotoxic mechanism. DPX are, however, potentially damaging to the afflicted cell, and cell death is a likely outcome should they occur at high frequency. They also indicate that protein–protein cross-linkage (PPX) may occur, with potentially less serious effects for the cell. Should key proteins be involved in the PPX formation, this could have consequences on the regulatory machinery of the cell, including the regulation of differentiation. Such a change clearly occurs in the nasal epithelium of rats exposed to formaldehyde, since areas of metaplasia emerge. Neoplasia could be viewed as simply a different kind of metaplasia, unless there is compelling evidence for a genotoxic mode of action.

A different interpretation of the data has been offered by Gaylor et al. (2004), who analysed the concentration–response relationship for formaldehyde-induced cell proliferation in rats using statistical methods designed to identify J-shaped concentration curves. Cell proliferation data were used because there were insufficient quantal data on cancer incidence to perform the analysis. Their analysis supports the hypothesis that the threshold-type dose–response for nasal tumour incidence is the result of a minor genotoxicity at low dose that is superimposed by a J-shaped dose–response for cell proliferation at high cytotoxic dose levels (Lutz, 1998). At low doses, the effect of incremental DNA damage may be cancelled out by a reduction in cell proliferation; therefore, in spite of the apparent threshold, the data remain consistent with a genotoxic mechanism.

In rats exposed to formaldehyde, point mutations at GC base pairs in the cDNA sequence of the evolutionarily conserved regions II–V of the p53 gene were found in 5 of 11 primary nasal squamous cell carcinomas (Recio et al., 1992). This observation may be interpreted to indicate genotoxic processes induced by formaldehyde in the carcinogenic process; however, the presence of specific mutations in the emergent tumour is not evidence that they were present in the early stages of neoplasia or that they were directly induced by the chemical. While there is the possibility of a direct mutagenic event occurring, it is also possible that these mutations arose indirectly of exposure as a result of functional changes in chromatin proteins induced by the chemical. At what stage in the life history of the tumour these observed mutations occurred is also open to speculation: they are relatively common events, it is clear, but it is also clear that they are not essential events (since they do not occur in all tumours that are apparently of the same type). The occurrence of these mutations indicates that a genotoxic mechanism has not been excluded, but this evidence does not necessarily support one.

Specific changes in gene expression have also been observed in vivo. The results indicated that exposure to formaldehyde can cause alteration in the expression levels of genes involved in several functional categories, including xenobiotic metabolism, cell cycle regulation, DNA synthesis and repair, oncogenes, and apoptosis (Hester et al., 2003). It is not clear at present

how specific these changes are to formaldehyde or what their role is, if any, in carcinogenicity.

#### Glutaraldehyde

Glutaraldehyde has been less extensively tested than formaldehyde for genotoxicity in vitro and in vivo. It produces weak and inconsistent positive findings in tests in vitro and is not active in the vast majority of in vivo studies. The genetic toxicity of glutaraldehyde has been recently reviewed (Zeiger et al., 2005).

Glutaraldehyde induced DNA repair systems in bacterial cells and was a weak mutagen in *Salmonella* and *Escherichia coli*. Unscheduled DNA synthesis (UDS), DPX, and double-strand breaks were seen in human cell lines, but not in primary rat cells. There were weak and inconsistent responses in chromosomal aberration and sister chromatid exchange (SCE) studies with mammalian cells, and glutaraldehyde did not induce transformation in cultured Syrian hamster embryo (SHE) cells.

In vivo, glutaraldehyde induced S-phase DNA synthesis in nasal cells in rats and mice following direct nasal administration. Glutaraldehyde did not produce DNA damage in rat liver or cross-links in rat testes DNA or sperm cells. Tests for induction of chromosomal aberration in bone marrow cells in rats and mice were generally negative. Glutaraldehyde did not induce micronuclei in bone marrow cells or dominant lethal mutations in mice. Thus, glutaraldehyde does possess genotoxic potential, and, although the database is not as extensive as it is for formaldehyde, it might be anticipated that site of contact genotoxicity would occur. Consequently, if genotoxicity is a major carcinogenic MOA for formaldehyde, it remains to be explained why glutaraldehyde is not active.

#### H. Uncertainties, inconsistencies, and data gaps

#### Formaldehyde

In most of the cancer bioassays for formaldehyde, data on intermediate end-points such as proliferative response as a measure of cytotoxicity and DPX are limited. Consequently, direct comparison of the incidence of intermediate lesions and tumours is restricted. Additionally, information on a direct relationship between DPX and mutation induction and the probability of converting a DPX into a mutation is desirable, while the mode by which regenerative cell proliferation is involved in the production of mutations required for tumour development needs to be determined.

Studies on the *hprt* mutation spectrum in formaldehyde-exposed human cells revealed that 50% of the mutations are deletions, whereas 50% are due to point mutation at the A:T base pair (Crosby et al., 1988; Liber et al., 1989). The finding of deletions as part of the formaldehyde mutation spectrum may explain the homozygous nature of base pair mutations observed in p53 in formaldehyde-induced squamous cell carcinomas. However, there is an inconsistency with regard to the base pair that is mutated. It was found to be A:T in *hprt* in human and mammalian cell lines and G:C at p53 in formaldehyde-induced squamous cell carcinomas are induced by formaldehyde in vitro, these types of mutation may not be fundamental to its carcinogenicity.

#### Glutaraldehyde

Glutaraldehyde is clearly much more cytotoxic than formaldehyde, perhaps because it is a bifunctional alkylating agent. Intranasal instillation studies have demonstrated that, on a molar basis, glutaraldehyde is 10- to 20-fold more toxic than formaldehyde when delivered to the nasal mucosa as a single treatment in aqueous solution (St. Clair et al., 1990). Comparison of results from a 13-week inhalation study of glutaraldehyde (NTP, 1993) with similar inhalation studies with formaldehyde (Heck et al., 1990; Monticello, 1990; Monticello et al., 1991) shows that glutaraldehyde is about 20-fold more toxic than formaldehyde by this route also. Pulmonary damage and mortality occur at much higher glutaraldehyde concentrations. Cytotoxicity is manifest closer to the external nares in the case of inhaled glutaraldehyde. This difference in the site of toxic action may be particularly important because, if the only difference was toxic potency, then glutaraldehyde would be expected to produce effects similar to those of formaldehyde, although only at lower doses.

#### I. Assessment of postulated mode of action

#### Formaldehyde

From a weight-of-evidence point of view, the hypothesized MOA for formaldehyde-induced nasal tumours satisfies several criteria, including consistency, concordance of dose–response relationships across all key events, and biological plausibility and coherence of the database. Given the extensive experimental data that address and are consistent with the proposed MOA of formaldehyde in the induction of tumours in the nasal cavity, a high degree of confidence may be ascribed to it.

#### Glutaraldehyde

The key events of cytotoxicity, cell proliferation, and DPX formation (in vitro) have been demonstrated with exposure to glutaraldehyde. However, glutaraldehyde has not produced nasal tumours in rats and mice. Therefore, if the proposed MOA for formaldehyde is to be maintained, an explanation for this discrepancy is necessary. A reason for the difference has not been identified, but a hypothesis can be proposed. The dialdehyde function of glutaraldehyde is an important factor that may inhibit the macromolecules with which it reacts from further reaction within the cellular environment. Should these macromolecules be proteins involved in the maintenance of survival, then their immobility perhaps more likely leads to cell death than to a change in differentiation state. This immobilization of macromolecules by glutaraldehyde is the property that makes it a better fixative for high-resolution microscopy (e.g. electron microscopy) than formaldehyde. It almost certainly contributes to the very much higher toxicity of the dialdehyde. The monoaldehyde function of formaldehyde also causes cellular damage, but a proportion of proteins involved in cellular differentiation may be able to continue in that role, although with an altered outcome that may be the beginning of a path to neoplasia. If, on the other hand, these aldehydes react with nucleic acids (the evidence for glutaraldehyde reacting in this way is not substantial), then the repair of the alkylated nucleotides may be more difficult or even impossible in the case of glutaraldehyde, whereas repair does occur following formaldehyde interaction with DNA. Thus, irrespective of whether the mode of formaldehyde action in carcinogenicity is as proposed or is primarily due to genetic toxicity, the different response to glutaraldehyde exposure can be explained.

#### 2. CAN HUMAN RELEVANCE OF THE MOA BE EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

#### A. Formaldehyde

In rhesus monkeys exposed to formaldehyde at 7.2 mg/m<sup>3</sup> for between 1 and 6 weeks, formaldehyde-induced lesions were associated with increases in cell proliferation rates of up to 18-fold over controls and remained significantly elevated after 6 weeks of exposure. Histological lesions and increases in cell proliferation were most extensive in the nasal passages and were minimal in the lower airways, whereas the maxillary sinuses showed no evidence of a response to formaldehyde exposure. Based on the extent of lesions and cell proliferation data, it appeared that rhesus monkeys are more sensitive than rats to the acute and subacute effects of formaldehyde at 7.2 mg/m<sup>3</sup> (Monticello et al., 1989). The absence of response in the maxillary sinuses in rhesus monkeys is an observation deserving special attention in the design of epidemiological studies (or, perhaps, in the reporting of tumour sites). Most epidemiological studies of sinonasal cancer have not distinguished tumours arising in the nose from those developing in the nasal sinuses. Thus, the risk for nasal cancer specifically would tend to be diluted if there was no corresponding risk for cancer in the sinuses and could go undetected through lack of statistical power.

Many epidemiological studies have investigated formaldehyde exposure and cancer of the respiratory tract. The strongest evidence of an association has been observed for nasopharyngeal cancers. A statistically significant excess of deaths from nasopharyngeal cancer has been observed in the largest cohort study of industrial workers (Hauptmann et al., 2004), with statistically significant exposure–response relationships for peak and cumulative exposure. An excess of deaths from nasopharyngeal cancer was observed in a proportionate mortality analysis of the largest cohort of embalmers in the United States (Hayes et al., 1990). An excess of cases of nasopharyngeal cancer was observed in a Danish study of proportionate cancer incidence among workers at companies that manufactured or used formaldehyde (Hansen & Olsen, 1995). Other cohort studies reported fewer cases of nasopharyngeal cancer than expected (Walrath & Fraumeni, 1983; Coggon et al., 2003; Pinkerton et al., 2004). Of seven case–control studies of nasopharyngeal cancer, five found elevations of risk for exposure to formaldehyde.

Several case–control studies have investigated the association between exposure to formaldehyde and sinonasal cancer. A pooled analysis of 12 studies showed an increased risk of adenocarcinoma in men and women thought never to have been exposed to wood dust or leather dust, with an exposure–response trend for an index of cumulative exposure (Luce et al., 2002). One other case–control study (Olsen & Asnaes, 1986) and a proportionate incidence study (Hansen & Olsen, 1995) showed an increased risk of sinonasal cancer, particularly squamous cell carcinoma. However, the three most informative cohort studies of industrial workers showed no excesses of sinonasal cancer (Coggon et al., 2003; Hauptmann et al., 2004; Pinkerton et al., 2004).

In evaluating this body of evidence, the International Agency for Research on Cancer (IARC) concluded that there was sufficient epidemiological evidence that formaldehyde causes

nasopharyngeal cancer in humans; only limited epidemiological evidence that formaldehyde causes sinonasal cancer in humans; and strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde (Cogliano et al., 2005).

There are no publications describing DPX in nasal cells from formaldehyde-exposed personnel. Assessment of DPX in peripheral lymphocytes from formaldehyde-exposed workers demonstrated an association with overall exposure (Shaham et al., 2003). The single DPX study involved 399 workers from 14 hospital pathology departments, and formaldehyde exposure categories were low-level (mean 0.5 mg/m<sup>3</sup>, range 0.05–0.8 mg/m<sup>3</sup>) and high-level (mean 2.7 mg/m<sup>3</sup>, range 0.86–6.7 mg/m<sup>3</sup>). Adjusted mean DPX were significantly higher in the exposed groups. There appear to be some doubts regarding the sensitivity and reproducibility of the physical separation method used in this study (Heck & Casanova, 2004).

Some studies have investigated the histological changes within the nasal epithelium of workers occupationally exposed to formaldehyde; however, the extent to which nasal epithelial cell regenerative proliferation occurs is unresolved because the results are mixed and there was co-exposure to wood dust in some studies (Berke, 1987; Edling et al., 1988; Holmström et al., 1989; Boysen et al., 1990; Ballarin et al., 1992).

Mucociliary clearance in the anterior portion of the nasal cavity was reduced following exposure of volunteers to formaldehyde at  $0.30 \text{ mg/m}^3$  (Andersen & Mølhave, 1983).

The concordance of animal and human key events for formaldehyde is summarized in Table 3.

Evidence in animals	Evidence in humans
Positive in vivo (target cells)	Plausible
Positive in vivo (target cells)	Plausible (some evidence but confounded by co-exposure)
DPX (target cells in vivo)	DPX (non-target cells, i.e. lymphocytes)
Positive in vitro; unconvincing in vivo	Positive (? cells)
Positive (mainly anterior lateral meatus)	Positive (nasopharyngeal) ? (sinonasal)
	Positive in vivo (target cells) Positive in vivo (target cells) DPX (target cells in vivo) Positive in vitro; unconvincing in vivo Positive (mainly anterior lateral

Table 3. Formaldehyde concordance table	
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#### **B. Glutaraldehyde**

There are few epidemiological studies for exposure to glutaraldehyde and human cancer. No increase in the number of cancer deaths was observed among 186 male glutaraldehyde production workers. The average time since first exposure to glutaraldehyde was 20.6 years, and the period of exposure was 3–7 years. During periods of monitoring exposure, glutaraldehyde concentrations in air ranged from 0.04 to 1.4 mg/m<sup>3</sup> (NICNAS, 1994). Studies of embalmers, pathologists, and members of the American Association of Anatomists for possible effects of glutaraldehyde have all shown increases in risk of cancer; however, all of

these groups were also exposed to formaldehyde (Walrath & Fraumeni, 1983; Consensus Workshop on Formaldehyde, 1984; Stroup et al., 1986).

There are no studies examining glutaraldehyde exposure and DPX formation, cytotoxicity, and cell proliferation in human nasal tissues.

The concordance of animal and human key events for glutaraldehyde is summarized in Table 4.

Key event	Evidence in animals	Evidence in humans
Cytotoxicity	Positive	Plausible
Proliferation	Positive in vivo	Plausible
Genotoxicity	DPX in vitro	Unknown
Mutations	Positive in vitro	Unknown
Nasal tumours	Negative (no evidence at any site)	Unknown

Table 4.	Glutaraldeh	vde conco	rdance	table.
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#### 3. CAN HUMAN RELEVANCE OF THE MOA BE EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETIC OR DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

#### A. Formaldehyde

Quantitative differences between experimental animals and humans for the postulated MOA will be a function of the concentration of formaldehyde at the target tissue. It is formaldehyde per se, and not its metabolites, that causes cytotoxicity. Exogenous inhaled formaldehyde is rapidly metabolized upon absorption, to formate, by a number of widely distributed cellular enzymes, particularly formaldehyde dehydrogenase. In addition to this efficient metabolic detoxification mechanism, the mucociliary apparatus provides protection of the underlying epithelium from gases and vapours. Thus, in order to attain free formaldehyde concentrations that may be cytotoxic to the target tissue, relatively high concentrations of formaldehyde vapour must be delivered to the target site to overcome these protective mechanisms. Mechanistic events of clear significance for carcinogenicity occur at dose levels where formaldehyde detoxification mechanisms are saturated in rats (Casanova & Heck, 1987).

It is critical to take dosimetry into consideration when considering quantitative species differences for formaldehyde-induced toxicity in the respiratory tract. Inhaled formaldehyde is predominantly deposited and readily absorbed in the regions of the upper respiratory tract with which it comes into initial contact, owing to its high reactivity with biological macromolecules (Heck et al., 1983; Swenberg et al., 1983). A complex relationship between nasal anatomy, ventilation, and breathing patterns (nasal or oronasal) determines where in the upper respiratory tract formaldehyde absorption occurs in species. In rodents, which are obligate nasal breathers, deposition and absorption occur primarily in the nasal passage. In contrast, primates are oronasal breathers; although absorption and deposition are likely to occur primarily in the oral mucosa and nasal passages, they can also occur in the trachea and bronchus (Monticello et al., 1991). This hypothesis is supported by effects (histopathological changes, increased epithelial cell proliferation, and DPX formation) being observed farther along within the upper respiratory tract in monkeys.

Species differences in dosimetry have been taken into account in a two-stage clonal growth model that has been developed to predict the nasal carcinogenic risk of formaldehyde in humans (Conolly et al., 2004). The model also incorporates data on normal growth curves for rats and humans, cell cycle times, and cells at risk in the different regions of the respiratory tract.

Mice are better able to reduce both their respiratory rate and tidal volume upon repeated exposures; therefore, mice have less formaldehyde available for deposition than rats, resulting in less tissue damage and a lower rate of cell turnover in the nasal epithelium (Chang et al., 1981, 1983). These are characteristics that may help explain the lack of neoplastic response in the nose of mice.

Although there are likely to be quantitative differences between animal species and humans due to differences in dosimetry in the respiratory tract, there do not appear to be fundamental differences that would indicate that the proposed MOA does not occur in humans.

#### **B. Glutaraldehyde**

Much less is known of the kinetics of glutaraldehyde in experimental animals compared with formaldehyde. Inhalation studies do not appear to have been conducted. The terminal halflives for elimination are long for both intravenous injection (rat 10 h, rabbit 15–30 h) and dermal application (rat 40–110 h, rabbit 20–100 h), probably due to the binding of glutaraldehyde to protein and the slow excretion of metabolites. The metabolites have not been identified, but it has been proposed that the metabolism of glutaraldehyde probably involves initial oxidation to the corresponding carboxylic acids by aldehyde dehydrogenase. The glutaric acid formed by oxidation is probably further metabolized by reaction with coenzyme A (CoA) to give glutaryl CoA, which is then oxidized by glutaryl CoA dehydrogenase to glutaconyl CoA, leading to its eventual degradation to carbon dioxide via acetate (Beauchamp et al., 1992; NTP, 1993; NICNAS, 1994; Ballantyne, 1995).

Glutaraldehyde reacts readily with proteins as a cross-linking agent, mainly between amino groups. The reaction is rapid and pH dependent (rate increases at pH >9), to give Schiff bases. Further reaction occurs to give a number of complex reaction products, with the mechanism of the cross-linking process not yet fully understood.

Little information is available on the interaction between glutaraldehyde and DNA, but it has been reported (Hopwood, 1975) that glutaraldehyde reacts with DNA only at >60 °C (summarized by NICNAS, 1994), and there are data implying that there is no reaction under physiological conditions (Sewell et al., 1984; Douglas & Rogers, 1998; Vock et al., 1999).

#### 4. STATEMENT OF CONFIDENCE, ANALYSIS, AND IMPLICATION

#### A. Formaldehyde

Sustained cytotoxicity and cell proliferation are key events in the proposed MOA for the induction of several types of animal tumours. There are substantial data to support this postulated MOA for formaldehyde-induced nasal tumours in rats. Cytotoxicity, DPX formation, nasal epithelial cell regenerative proliferation, squamous metaplasia, and inflammation have been measured in rat studies and are site-specific, highly non-linear concentration–response processes in concordance with the incidence of nasal tumours.

Based on the weight of evidence, it is likely that the MOA is relevant to humans, at least qualitatively. Increased cell proliferation and DPX formation within epithelia of the upper respiratory tract have been observed in monkeys exposed to formaldehyde vapour. Increased human epithelial cell proliferation following in situ exposure to formaldehyde has also been observed in a model system in which rat tracheae populated with human tracheobronchial epithelial cells were xenotransplanted into athymic mice. Limited evidence on histopathological lesions in the nose of humans exposed primarily to formaldehyde in the occupational environment is consistent with a qualitatively similar response of the upper respiratory tract in experimental animals. In addition, several epidemiological studies have indicated an increased risk of nasal cancers with formaldehyde exposure.

Therefore, the MOA is considered relevant to humans, and animal nasal tumour and other supporting data should be taken forward to evaluate human risk. This process would include consideration of the data suggesting that formaldehyde induces tumours in a non-linear, dose-dependent manner. There may also be quantitative differences in response between species for the proposed MOA due to differences in dosimetry.

#### **B.** Glutaraldehyde

The epidemiological studies for glutaraldehyde are very limited and do not show an association with nasal tumours. In animal studies, glutaraldehyde has been shown to cause cytotoxicity, cell proliferation, and DPX production, but not nasal tumours, in inhalation studies in rats and mice. The fact that glutaraldehyde is clearly more toxic than formaldehyde should not constitute a reason for the difference in carcinogenic potential. Although, dose for dose, glutaraldehyde exposure may tend to result in more cell death than formaldehyde exposure, if glutaraldehyde is a carcinogen, this should be demonstrable at doses lower than those used for formaldehyde.

The MOA postulated for formaldehyde—that is, sustained cytotoxicity and cell proliferation—would appear to be relevant to glutaraldehyde, but tumour formation has not been demonstrated. It has been tentatively suggested here that the difference in pathological responses to these aldehydes is due to formaldehyde being a monoaldehyde whereas glutaraldehyde is a dialdehyde. This difference may result in a different form of cross-linking so that glutaraldehyde cross-link products are neither likely to retain any biological function nor likely to be repairable. The case-study highlights the difficulties in applying the HRF when the animal tumour data are inadequate.

#### REFERENCES

Andersen I, Mølhave L (1983) Controlled human studies with formaldehyde. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 155–165.

Ballantyne B (1995) *Toxicology of glutaraldehyde: Review of studies and human health effects.* Bound Brook, NJ, Union Carbide Corporation.

Ballarin C, Sarto F, Giacomelli L, Bartolucci GB, Clonfero E (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. *Mutation Research*, **280**:1–7.

Beauchamp ROJ, St Clair MB, Fennell TR, Clarke DO, Morgan KT, Kari FW (1992) A critical review of the toxicology of glutaraldehyde. *Critical Reviews in Toxicology*, **22**:143–174.

Berke JH (1987) Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. *Journal of Occupational Medicine*, **29**:681–684.

Boysen M, Zadig E, Digernes V, Abeler V, Reith A (1990) Nasal mucosa in workers exposed to formaldehyde: A pilot study. *British Journal of Industrial Medicine*, **47**:116–121.

Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma*, **48**:144–147.

Burgaz S, Erdem O, Cakmak G, Erdem N, Karakaya A, Karakaya AE (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, **7**:151–161.

Bushy Run (1983) *Glutaraldehyde vapour subchronic inhalation study on rats*. Export, PA, Bushy Run Research Center (Project Report 46-101).

Casanova M, Heck Hd'A (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [<sup>3</sup>H]- and [<sup>14</sup>C]formaldehyde in Fischer-344 rats: Effects of glutathione depletion. *Toxicology and Applied Pharmacology*, **89**:105–121.

Casanova M, Heck Hd'A, Everitt JI, Harrington WW Jr, Popp JA (1988) Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food and Chemical Toxicology*, **26**:715–716.

Casanova M, Deyo DF, Heck Hd'A (1989) Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundamental and Applied Toxicology*, **12**:397–417.

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck Hd'A (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Fundamental and Applied Toxicology*, **17**:409–428.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck Hd'A (1994) DNA–protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Fundamental and Applied Toxicology*, **23**:525–536.

Casanova-Schmitz M, Starr TB, Heck H (1984) Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [<sup>14</sup>C]- and [<sup>3</sup>H]formaldehyde. *Toxicology and Applied Pharmacology*, **76**:26–44.

Chang JCF, Steinhagen WH, Barrow CS (1981) Effects of single or repeated formaldehyde exposures on minute volume of B6C3F1 mice and F344 rats. *Toxicology and Applied Pharmacology*, **61**:451–459.

Chang JCF, Gross EA, Swenberg JA, Barrow CS (1983) Nasal cavity deposition, histopathology and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicology and Applied Pharmacology*, **68**:161–176.

CIIT (1999) Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation, rev. ed. Research Triangle Park, NC, Chemical Industry Institute of Toxicology.

Coggon D, Harris EC, Poole J, Palmer KT (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *Journal of the National Cancer Institute*, **21**:1608–1614.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F (2005) Meeting report: Summary of IARC Monographs on formaldehyde, 2-butoxyethanol and 1-*tert*-butoxy-2-propanol. *Environmental Health Perspectives*, **113**(9):1205–1208.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2004) Human respiratory tract cancer risks of inhaled formaldehyde: Dose–response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicological Sciences*, **82**:279–296.

Consensus Workshop on Formaldehyde (1984) Report on the consensus workshop on formaldehyde. *Environmental Health Perspectives*, **58**:323–381.

Crosby RM, Richardson KK, Craft TR, Benforado KB, Liber HL, Skopek TR (1988) Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and *E. coli*. *Environmental and Molecular Mutagenesis*, **12**:155–166. Dalbey WE (1982) Formaldehyde and tumors in hamster respiratory tract. *Toxicology*, **24**:9–14.

Douglas MP, Rogers SO (1998) DNA damage caused by common cytological fixatives. *Mutation Research*, **401**:77–88.

Edling C, Hellquist H, Ödkvist L (1988) Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. *British Journal of Industrial Medicine*, **45**:761–765.

Feron VJ, Bruyntes JP, Woutersen RA, Immel HR, Appelman LM (1988) Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Letters*, **39**:101–111.

Feron VJ, Til HP, Woutersen RA (1990) Letter to the editor. *Toxicology and Industrial Health*, **6**:637–639.

Gaylor DW, Lutz WK, Connolly RB (2004) Statistical analysis of nonmonotonic doseresponse relationships: Research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. *Toxicological Sciences*, **77**:158–164.

Gross EA, Mellick PW, Kari FW, Miller FJ, Morgan KT (1994) Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. *Fundamental and Applied Toxicology*, **23**:348–362.

Hansen J, Olsen JH (1995) Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes and Control*, **6**:354–360.

Hardman JG, Limbird LE, Gilman AG, eds (2001) Goodman & Gilman's The pharmacological basis of therapeutics, 10th ed. The McGraw-Hill Companies, Inc., 2025 pp.

Hauptmann A, Lubin JH, Stewart PA, Hayes RB, Blair A (2004). Mortality from solid cancers among workers in formaldehyde industries. *American Journal of Epidemiology*, **159**:1117–1130.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H (1990) Mortality of U.S. embalmers and funeral directors. *American Journal of Industrial Medicine*, **18**:641–652.

He J-L, Jin L-F, Jin H-Y (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomedical and Environmental Sciences*, **11**:87–92.

Heck H, Casanova M (1995). Nasal dosimetry of formaldehyde: Modelling site specificity and the effects of pre-exposure. In: Miller JF, ed. *Nasal toxicity and dosimetry of inhaled xenobiotics: Implications for human health.* Washington, DC, Taylor & Francis, pp. 159–175.

Heck H, Casanova M (2004) The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. *Regulatory Toxicology and Pharmacology*, **40**:92–106.

Heck Hd'A, White EL, Casanova-Schmitz M (1982) Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomedical Mass Spectrometry*, **9**:347–353.

Heck Hd'A, Chin TY, Schmitz MC (1983) Distribution of [<sup>14</sup>C]formaldehyde in rats after inhalation exposure. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 26–37.

Heck Hd'A, Casanova-Schmitz M, Dodd PB, Schachter EN, Witek TJ, Tosun T (1985) Formaldehyde (CH<sub>2</sub>O) concentrations in the blood of humans and Fischer-344 rats exposed to CH<sub>2</sub>O under controlled conditions. *American Industrial Hygiene Association Journal*, **46**:1–3.

Heck Hd'A, Casanova M, Starr TB (1990) Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**:397–426.

Hedberg JJ, Strömberg P, Höög JO (1998) An attempt to transform class characteristics within the alcohol dehydrogenase family. *FEBS Letters*, **436**:67–70.

Hester SD, Benavides GB, Yoon L, Morgan KT, Zou F, Barry W, Wolf DC (2003) Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium. *Toxicology*, **187**:13–24.

Holmquist B, Vallee BL (1991) Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. *Biochemical and Biophysical Research Communications*, **178**:1371–1377.

Holmström M, Wilhelmsson B, Hellquist H, Rosén G (1989) Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Oto-laryngologica*, **107**:120–129.

Hopwood D (1975) The reactions of glutaraldehyde with nucleic acids. *Journal of Histochemistry*, **7**:267–276.

Horton AW, Tye R, Stemmer KL (1963) Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *Journal of the National Cancer Institute*, **30**:31–43.

IARC (2005) *Formaldehyde, 2-butoxyethanol and 1-*tert-*butoxypropan-2-ol.* Lyon, International Agency for Research on Cancer, 478 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88).

IPCS (2002) *Formaldehyde*. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document No. 40).

Kamata E, Nakadate E, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. *Journal of Toxicological Sciences*, **22**:239–254.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA (1983a) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Research*, **43**:4382–4392.

Kerns WD, Donofrio DJ, Pavkov KL (1983b) The chronic effects of formaldehyde inhalation in rats and mice: A preliminary report. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 111–131.

Kimbell JS, Gross EA, Richardson RB, Conolly RB, Morgan KT (1997) Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutation Research*, **380**:143–154.

Koivusalo M, Baumann M, Uotila L (1989) Evidence for the identity of glutathionedependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Letters*, **257**:105–109.

Liber HL, Benforado K, Crosby RM, Simpson D, Skopek TR (1989) Formaldehyde-induced and spontaneous alterations in human *hprt* DNA sequence and mRNA expression. *Mutation Research*, **226**:31–37.

Liteplo RG, Meek ME (2003) Inhaled formaldehyde: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health*, **B6**:85–114.

Luce D, Leclerc A, Begin D, Demers PA, Gerin M, Orlowski E, Kogevinas M, Belli S, Bugel I, Bolm-Audorff U, Brinton LA, Comba P, Hardell L, Hayes RB, Magnani C, Merler E, Preston-Martin S, Vaughan TL, Zheng W, Boffetta P (2002) Sinonasal cancer and occupational exposures: A pooled analysis of 12 case–control studies. *Cancer Causes and Control*, **13**:147–157.

Lutz WK (1998) Dose–response relationships in chemical carcinogenesis: Superposition of different mechanisms of action, resulting in linear–nonlinear curves, practical thresholds, J-shapes. *Mutation Research*, **405**:117–124.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR, Moss OR (1986) Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology*, **41**:253–266.

Merk O, Speit G (1998) Significance of formaldehyde-induced DNA–protein crosslinks for mutagenesis. *Environmental and Molecular Mutagenesis*, **32**:260–268.

Monticello TM (1990) *Formaldehyde induced pathology and cell proliferation: A thesis.* Durham, NC, Duke University.

Monticello TM, Morgan KT (1994) Cell proliferation and formaldehyde-induced respiratory carcinogenesis. *Risk Analysis*, **14**:313–319.

Monticello TM, Morgan KT, Everitt JI, Popp JA (1989) Effects of formaldehyde gas on the respiratory tract of rhesus monkeys. Pathology and cell proliferation. *American Journal of Pathology*, **134**:515–527.

Monticello TM, Miller FJ, Morgan KT (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subacute inhalation of formaldehyde. *Toxicology and Applied Pharmacology*, **111**:409–421.

Monticello TM, Swenberg JA, Gross EA, Leiniger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Research*, **56**:1012–1022.

Morgan KT, Monticello TM (1990) Formaldehyde toxicity: Respiratory epithelial injury and repair. In: Thomassen DG, Nettesheim P, eds. *Biology, toxicology, and carcinogenesis of the respiratory epithelium*. Washington, DC, Hemisphere Publishing, pp. 155–171.

Morgan KT, Jiang X-Z, Starr TB, Kerns WD (1986) More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicology and Applied Pharmacology*, **82**:264–271.

NICNAS (1994) *Glutaraldehyde. Full public report.* Canberra, Australian Government Publishing Service, National Industrial Chemicals Notification and Assessment Scheme, July (Priority Existing Chemical No. 3).

NTP (1993) *NTP technical report on toxicity studies on glutaraldehyde (CAS No. 111-30-8) administered by inhalation to F344/N rats and B6C3F1 mice*. Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Toxicity Report No. 25; NIH Publication No. 93-3348).

NTP (1999) *Toxicology and carcinogenesis studies of glutaraldehyde (CAS No. 111-30-8) in F344/N rats and B6C3F1 mice (inhalation studies)*. Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 490; NIH Publication No. 99-3980).

Olsen JH, Asnaes S (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *British Journal of Industrial Medicine*, **43**:769–774.

Pinkerton L, Hein M, Stayner L (2004). Mortality among a cohort of garment workers exposed to formaldehyde: An update. *Occupational and Environmental Medicine*, **61**:193–200.

Recio L (1997) Oncogene and tumor suppressor gene alterations in nasal tumors. *Mutation Research*, **380**:27–31.

Recio L, Sisk S, Pluta L, Bermudez E, Gross EA, Chen Z, Morgan K, Walker C (1992) *p53* mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. *Cancer Research*, **52**:6113–6116.

Rietbrock N (1965) [Formaldehyde oxidation in the rat.] *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie*, **251**:189–190 (in German).

Rusch GM, Clary JJ, Rinehart WE, Bolte HF (1983) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicology and Applied Pharmacology*, **68**:329–343.

Schlosser PM, Lilly PD, Conolly RB, Janszen DB, Kimbell JS (2003) Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA-protein cross-link dosimetry model to estimate human equivalent doses. *Risk Analysis*, **23**:473–487.

Sewell BT, Bouloukos C, von Holt C (1984) Formaldehyde and glutaraldehyde in the fixation of chromatin for electron microscopy. *Journal of Microscopy*, **136**:103–112.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003) DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occupational and Environmental Medicine*, **60**:403–409.

Soffritti M, Maltoni C, Maffei F, Biagi R (1989) Formaldehyde: An experimental multipotential carcinogen. *Toxicology and Industrial Health*, **5**:699–730.

Speit G, Schutz P, Merk O (2000) Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. *Mutagenesis*, **15**:85–90.

St Clair MB, Gross EA, Morgan KT (1990) Pathology and cell proliferation induced by intranasal instillation of aldehydes in the rat: Comparison of glutaraldehyde and formaldehyde. *Toxicologic Pathology*, **18**:353–361.

St Clair MB, Bermudez E, Gross EA, Butterworth BE, Recio L (1991) Evaluation of the genotoxic potential of glutaraldehyde. *Environmental and Molecular Mutagenesis*, **18**:113–119.

Stroup NE, Blair A, Erikson GE (1986) Brain cancer and other causes of deaths in anatomists. *Journal of the National Cancer Institute*, **77**:1217–1224.

Swenberg JA, Gross EA, Martin J, Popp JA (1983) Mechanisms of formaldehyde toxicity. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 132–147.

Swenberg JA, Gross EA, Martin J, Randall HA (1986) Localization and quantitation of cell proliferation following exposure to nasal irritants. In: Barrow CS, ed. *Toxicology of the nasal passages*. Washington, DC, Hemisphere Publishing, pp. 291–300.

Takahashi M, Hasegawa R, Furukawa F, Toyoda K, Sato H, Hayashi Y (1986) Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine. *Japanese Journal of Cancer Research*, **77**:118–124.

Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE (1989) Two-year drinking-water study of formaldehyde in rats. *Food and Chemical Toxicology*, **27**:77–87.

Titenko-Holland N, Levine AJ, Smith MT, Quintana PJ, Boeniger M, Hayes R, Suruda A, Schulte P (1996) Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutation Research*, **371**:237–248.

Tobe M, Naito K, Kurokawa Y (1989) Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology*, **56**:79–86.

Uotila L, Koivusalo M (1974) Formaldehyde dehydrogenase from human liver. Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *Journal of Biological Chemistry*, **249**:7653–7663.

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and *S*-formylglutathione hydrolase activities in different rat tissues. *Advances in Experimental Medicine and Biology*, **414**:365–371.

Van Miller JP, Hermansky SJ, Neptun DA, Loscoa PE, Ballantyne B (1995) Combined chronic toxicity/oncogenicity study with glutaraldehyde (GA) in the drinking water of rats. *Toxicologist*, **15**:203 (abstract).

Vargová M, Janota S, Karelová J, Barancokova M, Šulcová M (1992) Analysis of the health risk of occupational exposure to formaldehyde using biological markers. *Analysis*, **20**:451–454.

Vock EH, Lutz WK, Ilinskaya O, Vamvakas S (1999) Discrimination between genotoxicity and cytotoxicity for the induction of DNA double-strand breaks in cells treated with aldehydes and diepoxides. *Mutation Research*, **441**:85–93.

Walrath J, Fraumeni JF Jr (1983) Mortality patterns among embalmers. *International Journal of Cancer*, **31**:407–411.

Wolf DC, Gross EA, Lycht O, Bermudez E, Recio L, Morgan KT (1995) Immunohistochemical localization of p53, PCNA, and TGF-α proteins in formaldehydeinduced rat nasal squamous cell carcinomas. *Toxicology and Applied Pharmacology*, **132**:27–35. Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron VJ (1989) Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *Journal of Applied Toxicology*, **9**:39–46.

Ying C-J, Yan W-S, Zhao M-Y, Ye X-L, Xie H, Yin S-Y, Zhu X-S (1997) Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomedical and Environmental Science*, **10**:451–455.

Zeiger E, Gollapudi B, Spencer P (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde—A review. *Mutation Research*, **589**:136–151.

Zissu D, Gagnaire F, Bonnet P (1994) Nasal and pulmonary toxicity of glutaraldehyde in mice. *Toxicology Letters*, **71**:53–62.

Zissu D, Bonnet P, Binet S (1998) Histopathological study in B6C3F1 mice chronically exposed by inhalation to glutaraldehyde. *Toxicology Letters*, **95**:131–139.

Zwart A, Woutersen RA, Wilmer JWGM, Spit BJ, Feron VJ (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. *Toxicology*, **51**:87–99.

		End-		
Reference	Target tissue	point	Response (control vs exposed)	Comments and exposures
Vargová et al. (1992)	PBL	CA	3.6% vs 3.08%	<ul> <li>n = 20; high frequency in controls; wood splinter manufacture; formaldehyde 8-h TWA 0.55–10.36 mg/m<sup>3</sup> 5–&gt;16 years</li> </ul>
Ballarin et al. (1992)	Nasal mucosa	NM	0.25 ± 0.22% vs 0.90 ± 0.47% ( <i>P</i> < 0.01)	Concurrent exposure to wood dust; no dose- response
Burgaz et al. (2001)	Nasal mucosa	NM	0.61 ± 0.27% vs 1.01 ± 0.62% ( <i>P</i> < 0.01)	Exposed, $n = 23$ ; non-exposed, $n = 27$ ; no dose-response
Burgaz et al. (2002)	Oral mucosa	N	0.33 ± 0.30% vs 0.71 ± 0.56% pathology laboratory ( <i>P</i> < 0.05) 0.33 ± 0.30% vs 0.62 ± 0.45% shoe factory ( <i>P</i> < 0.05)	Exposed, <i>n</i> = 22 variable exposures; <i>n</i> = 28 exposed to formaldehyde; non-exposed, <i>n</i> = 28; correlation with duration of exposure
Titenko-Holland et al. (1996)	Oral mucosa	NM	0.6 ± 0.5% vs 2.0 ± 2.0% ( <i>P</i> = 0.007)	Exposed, $n = 28$ ; pre-versus post-exposure; no details on smoking habits; formaldehyde
	Nasal mucosa	NM	2.0 ± 1.3% vs 2.5 ± 1.3% (NS)	concentrations: Oral: 1.2 mg/m³-h vs 18 mg/m³-h, 90 days Nasal: 2.4 mg/m³-h vs 20 mg/m³-h, 90 days
Ying et al. (1997)	Nasal mucosa Oral mucosa PBL	N N N N N N	1.20 ± 0.67 vs 3.84 ± 1.48 ( <i>P</i> < 0.001) 0.57 ± 0.32 vs 0.86 ± 0.56 ( <i>P</i> < 0.001) 0.91 ± 0.39 vs 1.11 ± 0.54 (NS)	Exposed, $n = 25$ ; pre- versus post-exposure; questions about controlling for age, sex, and smoking habits; formaldehyde concentrations 0.508 ± 0.299 mg/m <sup>3</sup> vs 0.012 ± 0.0025 mg/m <sup>3</sup>
He et al. (1988)	PBL	CA MN	3.40 ± 1.57% vs 5.96 ± 2.40% ( <i>P</i> < 0.01) 3.15 ± 1.46% vs 6.38 ± 2.50% ( <i>P</i> < 0.01)	Chromosomal aberrations included breaks and gaps, which renders interpretation difficult

### IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS

#### PREFACE

Following completion of the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (see Part 1), an expert meeting was convened in Geneva in 2006 to explore the question as to whether the IPCS framework could be applied in chemical risk assessment generally (i.e. to develop a non-cancer framework). The participants at this expert meeting concluded that the framework should be applicable to all end-points and proceeded to author a draft publication out of session. The draft was sent for peer review by the members of the Harmonization Project Steering Committee and subsequently revised by the authors, taking into account the peer review comments received.

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#### LIST OF ACRONYMS AND ABBREVIATIONS

ACE	angiotensin-converting enzyme
CSAF	chemical-specific adjustment factor
EMS	eosinophilia-myalgia syndrome
HBOC	haemoglobin-based oxygen carriers
HRF	Human Relevance Framework
ILO	International Labour Organization
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
MOA	mode of action
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
RSI	Risk Science Institute (ILSI)
SLE	systemic lupus erythematosus
UNEP	United Nations Environment Programme
WHO	World Health Organization

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# IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Alan R. Boobis, John E. Doe, Barbara Heinrich-Hirsch, M.E. (Bette) Meek, Sharon Munn, Mathuros Ruchirawat, Josef Schlatter, Jennifer Seed, & Carolyn Vickers

Structured frameworks are extremely useful in promoting transparent, harmonized approaches to the risk assessment of chemicals. One area where this has been particularly successful is in the analysis of modes of action (MOAs) for chemical carcinogens in experimental animals and their relevance to humans. The International Programme on Chemical Safety (IPCS) recently published an updated version of its MOA Framework in animals to address human relevance (cancer Human Relevance Framework, or HRF). This work has now been extended to noncancer effects, with the eventual objective of harmonizing framework approaches to both cancer and non-cancer end-points. As in the cancer HRF, the first step is to determine whether the weight of evidence based on experimental observations is sufficient to establish a hypothesized MOA. This comprises a series of key events causally related to the toxic effect, identified using an approach based on the Bradford Hill criteria. These events are then compared qualitatively and, next, quantitatively between experimental animals and humans. The output of the analysis is a clear statement of conclusions, together with the confidence, analysis, and implications of the findings. This framework provides a means of ensuring a transparent evaluation of the data, identification of key data gaps and of information that would be of value in the further risk assessment of the compound, such as on dose-response relationships, and recognition of potentially susceptible subgroups, for example, based on life stage considerations.

The framework described in this paper, a non-cancer Human Relevance Framework (HRF), was prepared by the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. This global "Harmonization Project" aims to harmonize global approaches to chemical risk assessment through both increased consistency of risk assessment methodologies and development of international guidance documents. The project enables the achievement of commitments on harmonization of chemical risk assessment methodologies agreed by the United Nations Conference on Environment and Development (United Nations, 1992), the Intergovernmental Forum on Chemical Safety (1994), the World Summit on Sustainable Development (UNEP, 2002), and the Strategic Approach to International Chemicals Management (WHO, 2006). The project involves experts from the different sectors where chemicals are assessed, and hence the documents produced can be applied in the assessment of industrial chemicals, biocides, pesticides, veterinary chemicals, pharmaceuticals, cosmetics, natural toxicants, food additives, and environmental contaminants in food, water, air, and consumer products.

A main outcome of the Harmonization Project is the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (Sonich-Mullin et al., 2001) and

<sup>&</sup>lt;sup>1</sup> This article, to which WHO owns copyright, was published in 2008 in *Critical Reviews in Toxicology*, Volume 38, pages 87–96. It has been edited for this WHO publication.

its subsequent development into an IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (IPCS cancer HRF) (Boobis et al., 2006; see also Part 1 of this document). The mode-of-action (MOA) analysis utilizes a weight-of-evidence approach based on the Bradford Hill criteria for causality (Hill, 1965). It aims to determine whether it is possible to establish an MOA for a carcinogenic response observed in an experimental animal study, through application of a weight-of-evidence approach that requires identification of key events along the causal pathway to cancer. When an MOA has been established in experimental animals, the cancer HRF provides an analytical tool to enable the transparent evaluation of the data in order to consider the human relevance of the MOA.

Following on from this, IPCS decided to consider whether the framework for cancer could be applied, with modifications, if necessary, to other end-points and their associated MOAs. Recognizing the work that the Risk Science Institute (RSI) of the International Life Sciences Institute (ILSI) had conducted in parallel to develop a similar framework and apply it to non-cancer risk assessment, IPCS convened an international meeting in Geneva in March 2006 to review and consider the ILSI publication (Seed et al., 2005), along with the IPCS cancer HRF (Boobis et al., 2006; see also Part 1 of this document), in order to explore the question as to whether the IPCS framework could be applied in chemical risk assessment generally. In summary, this IPCS meeting recognized that the framework should be applicable to all endpoints, both cancer and non-cancer, and recommended further work to put this into practice, including documenting the rationale for application of the framework more generally, which appears in the present paper, and steps to facilitate uptake and use of the framework.

The IPCS meeting recognized that the non-cancer HRF would have multiple uses in chemical risk assessment:

- It would provide an internationally harmonized approach to the establishment of an MOA in experimental animals and its relevance to humans.
- It would generate criteria for the MOA against which subsequent cases could be considered—that is, to show whether a compound shares an established MOA.
- It would enable clarification of key information relating to the human relevance of the MOA, and this would inform the assessment of other chemicals that share the MOA.
- In general, application of the framework would enable critical data deficiencies and research needs to be identified and inform qualitative and quantitative assessment.

# THE NEED FOR A NON-CANCER HUMAN RELEVANCE FRAMEWORK

The non-cancer HRF is a tool that provides a structured approach to the assessment of human relevance of a postulated MOA in animals in a weight-of-evidence context. Subsequently, it includes explicit consideration of the relevance of the proposed MOA to humans, often based on consideration of more generic information, such as anatomical, physiological, and biochemical variations among species. In this manner, the framework encourages maximum use of both chemical-specific and more generic information in a transparent and analytical fashion.

Pivotal to transparency in determining human relevance using the framework are the delineation and consideration of the nature of evidence in various species of key events—that is, those in a postulated MOA that are measurable and critical to the induction of the toxicological response. Evaluation of the concordance of key events based on explicit consideration of variations between experimental animals and humans constitutes the principal basis of transparency in consideration of weight of evidence for human relevance.

While principally relevant to hazard characterization, the non-cancer HRF additionally contributes more generally to transparency in risk assessment through explicit delineation and consideration of data on appropriate key events that are also relevant to subsequent dose–response analysis for MOAs deemed relevant to humans. If the MOA in experimental animals is judged to be qualitatively relevant to humans, a more quantitative assessment is required that takes into account any kinetic and dynamic information that is available from both the experimental animals and humans in order to determine whether human relevance might be precluded on this basis.

These same data are critical to subsequent dose–response analysis for MOAs considered relevant in considering the adequacy of, for example, available information as a basis for replacement of default uncertainty factors in the development of chemical-specific adjustment factors (CSAFs) (IPCS, 2005). This information could, for example, constitute an adequate basis to consider interspecies variation in rates of formation of reactive metabolites in the target tissue, for replacement of the default subfactor for interspecies differences in toxicokinetics with a CSAF (IPCS, 2005).

Use of this non-cancer HRF also promotes harmonization of approaches to risk assessment for all end-points, bridging previously distinct approaches on, for example, cancer and noncancer effects. Harmonization in this context refers to a biologically consistent approach to risk assessment for all end-points, for which exploration of biological linkages is critical to ensuring maximal use of relevant information. Often, for example, organ toxicity is a critical key event in postulated MOAs for induction of tumours at the same site. The non-cancer HRF, then, sets the stage for identification of critical precursor non-cancer key events for which subsequent quantification of interspecies differences and interindividual variability in dose–response analysis is relevant. In other cases, a postulated MOA may lead to toxic effects in multiple organs, and these would be considered in the same non-cancer HRF analysis.

In addition, consideration in a transparent framework may identify factors that, while not themselves essential for the toxicological effect (and hence not key events), may modulate key events and, as a result, contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology.

Such an analysis may also provide an indication of those components of a proposed MOA that may operate only over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in an MOA, then the relevance to human

risk becomes a matter of exposure. Thus, the exposure assessment step of the risk assessment is critical to a comprehensive evaluation.

Importantly, then, application of the non-cancer HRF contributes to identification of any specific subpopulations (e.g. those with genetic predisposition) who are at increased risk and provides information relevant to consideration of relative risks at various life stages. In many cases, this is based not on chemical-specific information but rather on inference, based on knowledge of the MOA, as to whether specific age groups may be at increased or decreased risk. This requires explicit consideration of comparative developmental and ageing processes and events in humans and animal models. These considerations are critical to determination of focus in the remaining stages of risk assessment, such as dose–response analysis.

The transparent delineation of the weight of evidence for postulated MOAs and their relevance to humans (requiring explicit consideration of the strengths and weaknesses of the available database, as well as highlighting qualitative and quantitative similarities and differences among species and related uncertainties) also identifies any inconsistencies in the available data and defines critical data gaps and research needs. This derives from the requirement in each step to explicitly assess confidence in the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database—that is, that studies are not contradictory of each other—and the nature and extent of the concordance analysis.

Iterative application of the non-cancer HRF, even before all of the data are available, to the analysis of a postulated MOA and its relevance to humans are beneficial as a basis for developing and refining research strategies as additional information becomes available. In this context, the framework should prove helpful in facilitating discussion between risk assessors and research scientists in jointly understanding the nature of data that would support human relevance analysis of a postulated MOA in animals and defining next steps in data acquisition. Iterative consideration of MOA in designing research strategies is also expected to increase efficiency by focusing resources in critical areas in more tiered and targeted approaches.

As knowledge advances, MOAs will become less chemical specific and based even more on the key biological processes involved, allowing greater generalization of human relevance from one compound to another. The need for chemical-specific data for established MOAs will be less, although it will always be necessary to establish rigorously that the key events comprising the MOA occur.

The transparency in the human relevance of a postulated MOA that results from application of the non-cancer HRF should promote confidence in the conclusions reached, through the use of a defined procedure that encourages clear and consistent documentation supporting the analysis and reasoning, highlights inconsistencies and uncertainties in the available data, and identifies critically important data gaps that, when filled, would increase confidence in outcome. This transparency not only is anticipated to facilitate discussion between the risk assessment and research communities, but may also contribute to greater convergence among different regulatory agencies. The non-cancer HRF also provides the basis for improved process and content for scientific peer input and peer review, specifying minimum criteria of clarity and transparency as a basis to acquire input and acceptance of postulated MOAs and their relevance to humans. Adherence to these criteria enables others to determine the basis of the conclusions reached with respect to the key events, the exclusion of other MOAs, and the analysis of human relevance.

# WHEN WOULD THE NON-CANCER HRF BE APPLIED?

The non-cancer HRF provides a valuable tool to assess an MOA, but it requires significant amounts of effort and experimental work, so it is not something that would be used during the course of the assessment of every chemical. Its main purpose would be to determine whether to apply the default assumption that all effects seen in animals are relevant to humans. This question increases in importance when the application of the default assumption during the course of a risk assessment indicates that adverse effects are likely to occur—for example, where there is a low margin of exposure between the point of departure for the effect under consideration and the estimated human exposure, especially if the human exposure estimate has already been refined. It then becomes important to know whether risk management measures will be required. This is of most concern when new data emerge, such as those identifying a new effect, additional data on the dose–response relationship of the chemical, or changes in use pattern or exposure estimation, which change the risk assessment of a chemical that is already in use.

Use of the non-cancer HRF may also be of value in the situation where the effects in animals would have potentially serious consequences if they occurred in humans, such as neuro-toxicity or teratogenesis. These effects are subject to very rigorous risk assessment procedures, so they comparatively frequently suggest the need for risk management measures.

Another situation in which use of the non-cancer HRF should be considered is where there are interspecies differences in either the type of effect or the dose levels at which an effect occurs. In these cases, it will be important to understand which species is the most appropriate upon which to base extrapolation to humans. This indication would also apply to differences between sexes or strains in the same species.

These situations indicate that further consideration is required, and the non-cancer HRF provides a way of doing this. The framework can be applied at any stage in the process of considering an effect. It should be applied in an iterative way during the course of investigating an effect to help guide the scientist. When an effect has first been observed and gives rise to concern, the framework allows the investigator to structure the work programme by prompting the questions to be addressed. As the investigation develops, it guides the investigator in assessing the data as they are generated and provides pointers in deciding whether and what other data would be required.

In situations where there is a large body of data, the framework allows the evaluator to weight the evidence according to its significance as well as its volume.

The non-cancer HRF can also be useful when a chemical is observed to cause an effect suspected of being caused by an MOA that has already been established using the framework or shares structural similarity to a chemical or class of chemicals with an established MOA. The earlier use of the non-cancer HRF to establish this MOA will have identified the key steps that need to be investigated in order to ascribe the MOA to the new chemical. This will prove valuable both in a prospective way in designing new research or testing programmes and retrospectively in evaluating a data set.

# **CONSIDERATION OF THE NON-CANCER HRF**

The non-cancer HRF is an analytical tool that enables a structured approach to the assessment of the overall weight of the evidence for the postulated MOA and its relevance to humans. The framework is not designed to provide an absolute answer on sufficiency of the information, as this will vary, depending on the circumstance. It must be emphasized that it is not a checklist of criteria but an approach to data evaluation and presentation. The output from the application of the framework serves as the basis for the continuation of the risk assessment of the compound.

It is envisaged that the non-cancer HRF will be applicable to a wide range of toxicological end-points, encompassing changes in structure and function of organs, tissues, and cells, including physiological and neurobehavioural effects. The types of toxicity that could be addressed using the framework include, but are not limited to:

- *Organ toxicity*: Examples include benzene-induced haematotoxicity (aplastic anaemia), paraquat-induced lung toxicity, chloroquine-induced ocular toxicity.
- *Reproductive toxicity*: Examples include phthalate-induced male infertility, dioxininduced dysregulation of female fertility.
- *Developmental toxicity*: Examples include methylmercury-induced developmental neurotoxicity, retinoid-induced teratogenesis.
- *Neurotoxicity*: Examples include lead-induced peripheral neuropathy, acrylamide-induced axonopathy, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson disease.
- *Immunotoxicity*: Examples include organotin-induced immunosuppression, isoniazidinduced systemic lupus erythematosus (SLE)-like syndrome, contaminated L-tryptophaninduced eosinophilia-myalgia syndrome (EMS).

# **Introduction to MOA**

Prior to embarking on a non-cancer HRF analysis, there needs to be careful evaluation of the weight of evidence for a toxicological response on exposure to a chemical in experimental animals. The nature of the non-cancer HRF is such that only one MOA is analysed at a time; hence, for example, different toxicological effects associated with chemical administration, even if observed in the same animals, will require separate framework analyses to discern the MOA for each effect. Consistent with species- and tissue-specific variation in metabolic activation and detoxication, there may be poor site concordance for some toxicants. This will need to be kept in mind when comparing animal and human data.

# Postulated mode of action (theory of the case)

This comprises a brief outline of the sequence of events in the MOA postulated to be responsible for the toxicological effect of the test substance. This description leads into the next section, which identifies the events considered "key" (i.e. necessary and measurable) in the MOA.

## Key events

The "key events" in the MOA are briefly identified and described. Key events are those events that are critical to the induction of the toxicological response as hypothesized in the postulated MOA and are also measurable. To support an event as key, there needs to be a body of experimental data in which the event is characterized and consistently measured. The types of information that might be relevant include, for example, toxicological response and relevant key events in the same cell type, sites of action logically related to the event(s), specific biochemical events, changes in the expression or activity of enzymes, receptor–ligand interactions, effects on cofactor levels, specific changes in histology, changes in cell proliferation (increased or decreased), perturbations in hormone homeostasis or other signalling pathways (either intracellular or extracellular), second messengers, or ion fluxes, increased degradation of macromolecules, and changes in membrane permeability or integrity.

# Concordance of dose–response relationships

The dose–response relationships for each of the key events and for the toxicological response should be characterized and their interrelationships discussed with respect to the Bradford Hill criteria (Hill, 1965). Ideally, it should be possible to correlate the dose dependency of the increases in the magnitude (or frequency) of a key event with increases in the severity (e.g. lesion progression) of other key events occurring later in the process and with the ultimate toxicological response. Comparative tabular presentation of the magnitude of changes in key events and toxicological response is often helpful in examining dose–response concordance.

It is important to consider whether there are fundamental differences in the biological response (i.e. dose transitions) at different parts of the dose–response curve (Slikker et al., 2004). If so, key events relevant to the different parts of the dose–response curve will need to be defined and used in the framework analysis.

## Temporal association

The temporal relationships for each of the key events and for the toxicological response should be characterized. Key events should be observable before toxicity is apparent and should be consistent temporally with each other; this is an essential step in deciding whether the data support the postulated MOA. Observations of key events at the same time as the toxicological response (e.g. at the end of a study) do not permit conclusions as to temporal association, but can contribute to the analysis described in the next section.

# Strength, consistency, and specificity of association of toxicological response with key events

The weight of evidence linking the key events, any precursor lesions, and the toxicological response should be addressed (see Weed [2005] for a discussion of what is meant by weight of evidence). Stop/recovery studies showing absence or reduction of toxicity when a key

event is blocked or reduced are particularly useful tests of the association. Consistent observations in a number of studies, with different experimental designs, increase support for the MOA, since different designs can reduce any unknown bias or confounding. Consistency, which is the repeatability of the key events in the postulated MOA in different studies, is distinct from coherence, however, which addresses the relationship of the postulated MOA with observations more broadly (see next point). Observations that may be of value here include toxicological response and relevant key events in the same cell type, sites of action logically related to event(s), and results from stop/recovery studies.

## Biological plausibility and coherence

One should consider whether the MOA is consistent with what is known about the biology of the target process/site in general (biological plausibility) and also in relation to what is known specifically about the overall biological effects of the substance (coherence). For the postulated MOA and its associated key events to be biologically plausible, they need to be consistent with current understanding of biology. However, when using biological plausibility as a criterion against which weight of evidence is assessed, it is important to consider the potential for gaps in our knowledge. Coherence, which addresses the relationship of the postulated MOA for the toxicological response with that for other end-points—needs to be distinguished from consistency (addressed in the preceding point). In assessing coherence, information on structural analogues may be of value (i.e. structure–activity analysis). Information from other compounds that share the postulated MOA may also be helpful, such as sex, species, and strain differences in sensitivity and their relationship to key events. Additionally, this section should consider whether the database on the agent is internally consistent in supporting the proposed MOA.

## Other modes of action

Alternative MOAs that logically present themselves should be considered. If alternative MOAs are supported, they will need a separate non-cancer HRF analysis. These should be distinguished from additional components of a single MOA, since these would be addressed as part of the MOA under consideration.

# Uncertainties, inconsistencies, and data gaps

Uncertainties should be stated fully and explicitly. They should include those related to the biology of the toxicological response and those for the database on the compound being evaluated. Any inconsistencies should be noted and data gaps identified. It should be clearly stated whether the identified data gaps are critical in supporting the postulated MOA.

# Assessment of postulated mode of action

There should be a clear statement of the outcome of the analysis, indicating the level of confidence in the postulated MOA—for example, high, moderate, or low. If a novel MOA is being proposed, this should be clearly indicated. However, if the MOA is the same as one previously described, the extent to which the key events fit this MOA needs to be stated explicitly. Any major differences should be noted and their implications for acceptance of the MOA discussed.

# Life stage considerations

Since the response of an organism to a chemical exposure may vary through its lifespan, consideration of life stage is important for the MOA analysis of all toxic end-points. This is particularly true for effects that result from developmental exposures, since organ susceptibility may be restricted to critical periods of development, may depend on the ontogeny of key metabolic enzymes, or may depend on the interaction of the developing organism with its mother (see Zoetis & Walls, 2003). In addition, disruption of developmental processes may have downstream consequences.

Consideration of the ageing process is also important, for several reasons. First, developmental exposures can result in toxicities that are not detected until much later in life. In addition, there can be species-specific patterns of ageing for different organ systems. For example, reproductive senescence has a different etiology in rodents and humans and can even differ among different strains of rodents.

## Human relevance

If it is possible to establish an MOA in animals for a toxicological effect, the next stage is to evaluate its relevance to humans. The IPCS non-cancer HRF is presented as an approach to answering a series of three (or four) questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying the toxicological effect. The application of the guidance results in a narrative with four (or five) sections, which may be incorporated into the hazard characterization of a risk assessment.

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals? This question is addressed by performing an MOA analysis as described above, the steps of which are based on the Bradford Hill criteria for causality (Hill, 1965). The weight of evidence for possible alternative MOAs needs to be considered and a conclusion reached on the overall strength of evidence supporting the MOA under consideration. The approach also identifies any critically important data gaps that, when filled, would increase confidence in the proposed MOA. If the postulated MOA has already been described for other chemicals, its human relevance will already have been evaluated. If the proposed MOA is novel, human relevance will need to be assessed de novo.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? This step involves a qualitative assessment of the relevance of the MOA to humans. Listing the critical key events that occur in the animal MOA and directly evaluating whether or not each of the key events might occur in humans facilitate the evaluation and increase the transparency of the process. Presentation in tabular form, referred to as a concordance table, can be particularly useful. The information in such tables should be relatively brief, as a narrative explanation should always accompany the table. In one column, the effect on humans for each of the key events is evaluated. Another column for the results in a different strain, species, or sex or for a different route of administration that does not result in toxicity can be useful for comparative purposes. Factors may be identified that, while not key themselves, can modulate key events and so contribute to differences between species or individuals. Examples include genetic differences in pathways of metabolism, competing pathways of

metabolism, and effects induced by concurrent pathology. Any such factors identified should be noted in a footnote to the concordance table.

The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical specific. Later events will be more generic to the MOA. While information for evaluating the key events in humans can come from in vitro and in vivo studies on the substance itself, basic information on anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans can be of value.

In answering this question, a narrative describing the weight of evidence and an evaluation of the level of confidence for the human information should be prepared. Examples of specific types of information that can be useful include:

- where appropriate, background incidences of the effect at the anatomical site and cell type of interest, including age, sex, ethnic differences, and risk factors, including chemicals and other environmental agents;
- knowledge of the nature and function of the target site, including development, structure (gross and microscopic), and control mechanisms at the physiological, cellular, and biochemical levels;
- human and animal disease states that provide insight concerning target organ regulation and responsiveness;
- human and animal responses to the chemical under review or structural analogues following short-, intermediate-, or long-term exposure, including target organs and effects.

Obviously, a substantial amount of information is required to conclude that a given MOA is not relevant to humans. If such a conclusion is strongly supported by the data, exposure to chemicals producing toxicity only by that MOA would not pose a risk to humans, and no additional risk characterization for this end-point is required.

The question of relevance considers all groups and life stages. It is possible that the conditions under which an MOA operates occur primarily in a susceptible subpopulation or life stage—for example, in those with a pre-existing viral infection, hormonal imbalance, or disease state. Any information suggesting qualitative or quantitative differences in susceptibility is highlighted for use in risk characterization.

There are several aspects relating to life stage that should be considered in the non-cancer HRF analysis. First, the analysis should consider the comparative developmental processes and events that occur in humans and the animal model(s) (see Zoetis & Walls, 2003). This comparison will demonstrate the extent to which developmental processes are similar in humans and the animal model(s). In general, development is highly conserved; where this is the case, it would lead to a conclusion that the MOA in animals is also plausible in humans. However, there are some developmental processes that are unique to some species, which may therefore lead to a species-specific MOA that will not be plausible in humans.

Second, the analysis should consider the phase specificity or relative timing of the developmental processes or events in humans and the animal model(s). Critical developmental events may occur at different times during ontogeny. Some developmental events may occur early during the prenatal development of the animal and relatively late in human prenatal development. Other developmental events may occur prenatally in humans and postnatally in the animal, or vice versa. Differences in timing of the developmental events can have an impact on the dose metrics if there are substantial differences in placental versus lactational transfer. Similarly, a comparison of the ontogeny of key metabolic enzymes relative to the key developmental process may reveal substantial differences between humans and the animal model. Such considerations may lead to a conclusion that the animal MOA is not plausible in humans.

3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans? If the MOA in experimental animals cannot be judged to be qualitatively irrelevant to humans (no to question 2), a more quantitative assessment is undertaken, taking into account any kinetic and dynamic information that is available from experimental animals and humans. Such data will of necessity be both chemical and MOA specific and where possible should include the biologically effective doses required to produce the dynamic effects giving rise to the toxicity. Kinetic considerations include the rate and extent of absorption, tissue distribution, metabolism, and excretion. Differences in ontogeny can result in substantial species differences in placental and lactational transfers, which will affect the dose metrics. This may therefore result in a quantitative difference in the MOA between humans and experimental animals. Similarly, the differential ontogeny of key metabolic enzymes can result in substantial quantitative differences between humans and experimental animals. Dynamic considerations include the consequences of the interaction of the chemical with cells, tissues, and organs. Only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. Since quantitative exposure assessment is part of the subsequent risk characterization rather than the HRF, the difference would have to be of such a magnitude that human exposure could not possibly be envisaged to reach such levels. In most cases, it will not be possible to reach such a conclusion without undertaking formal exposure assessment in the subsequent risk characterization. Hence, the answer to the question will be no, but it may still be concluded that the risk is negligible in the subsequent risk characterization. Melamine-induced urinary bladder carcinogenesis provides a useful case-study illustrating this point (Meek et al., 2003). Again, tabular comparison of the data from experimental animals and humans can help in the evaluation. Information from studies of other compounds acting by the same or a similar MOA can be of value. As understanding of the basis for differences in responses between experimental animals and humans improves, differences in key events thought to be qualitative may be shown to be due to specific quantitative differences.

While it may not be possible to conclude that the MOA for toxicity is not relevant to humans on the basis of quantitative differences, during the evaluation it may become apparent that the magnitude of those differences is sufficient to impact markedly on the risk assessment. Hence, it is particularly important that the narrative for the answer to this question be comprehensive and capture as much quantitative information as possible. As with question 2, if the response to this question is *yes*, then exposure to chemicals producing toxicity only by this MOA would not pose a risk to humans, and no additional risk characterization is required.

The preceding three questions comprise a decision tree (see Figure 1).

		Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?	NO →	Continue with risk assessment
		$\downarrow$ YES		
MOA not relevant	YES ←	Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?		
		$\downarrow$ NO		
MOA not relevant	YES ←	Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?	$\mathbf{NO}$ $\rightarrow$	Continue with risk assessment

Figure 1. Decision tree for determining human relevance of an MOA for toxicity observed in experimental animals.

## Potential implications for dose-response assessment

Should it not be possible to exclude human relevance of the MOA for toxicity prior to proceeding with the risk assessment, a further question should be addressed. This is: *4. Are there any quantitative differences in the key events such that default values for uncertainty factors for species or individual differences could be modified?* Such information, on either kinetics or dynamics, could be used to calculate a CSAF, in which one or more of the default values for species or interindividual differences in kinetics or dynamics are replaced by a value based on chemical-specific information (IPCS, 2005). The other components of the adjustment factor would retain their default values. Such information may lead to either an increase or a decrease in the adjustment factor relative to the normal default.

## **Published case-studies**

In developing a framework for assessing the human relevance of MOAs for non-cancer endpoints, ILSI/RSI also developed a series of illustrative case-studies. These were on molinateinduced inhibition of spermatogenesis (Kavlock & Cummings, 2005a), renal and developmental effects of ethylene glycol (Corley et al., 2005), developmental neurotoxicity of nicotine (Slikker et al., 2005), phthalate ester effects on male reproductive development (Foster, 2005), vinclozolin-induced malformations (Kavlock & Cummings, 2005b), developmental effects of valproic acid (Wiltse, 2005), haemoglobin-based oxygen carriers (HBOC)-related congenital malformations (Holson et al., 2005), developmental effects of angiotensinconverting enzyme (ACE) inhibitors (Tabacova, 2005), developmental ototoxicity of polyhalogenated aromatic hydrocarbons (Crofton & Zoeller, 2005), and propylthiouracilinduced effects on neurological development (Zoeller & Crofton, 2005). While these cases covered a range of end-points, most involved effects during development. Hence, there is a need for additional case-studies on other end-points, such as those indicated above. As experience is obtained in using this framework, some of the published cases could be further refined to provide valuable illustrative examples for training in the application of the framework.

In general, the cases have been very useful in highlighting a number of the key issues on which this non-cancer HRF is based. Examples include the importance of the concordance analysis, the value of quantitative information identified during the application of the framework when it is not possible to exclude human relevance, the need for a transparent and comprehensive narrative when reporting the conclusions of a framework analysis, the importance in identifying key data gaps (e.g. case-study on molinate and HBOC), identification of research needs (e.g. case-study on vinclozolin), the importance of understanding the formation of a specific metabolite, and the importance of establishing a robust MOA through the application of the Bradford Hill criteria (Hill, 1965) to the key events.

# Statement of confidence, analysis, and implications

Following application of the non-cancer HRF and answering the three (or four) questions, a statement of confidence should be provided that addresses the quality and quantity of data underlying the analysis, the consistency of the analysis within the framework, the consistency of the database, and the nature and extent of the concordance analysis. Alternative MOAs should have been evaluated, when appropriate, with the same rigor. A critical outcome is the identification of specific data gaps that could be addressed experimentally to increase confidence in the analysis.

The output of the non-cancer HRF provides information that is useful for more than just determining whether or not the MOA for toxicity in experimental animals is relevant to humans. It can also provide much information that is critically important in subsequent steps in the risk characterization for relevant effects. For example, it may be possible to develop CSAFs on the basis of the information provided. Application of the framework can also provide information on relevant modulating factors that are likely to affect risk. In addition, it can identify those elements of a proposed MOA that operate only over a certain dose range. Where an obligatory step in an MOA occurs only following a high experimental dose of a compound, the relevance of the MOA to human risk is determined by the exposure. Thus, effective exposure assessment is particularly important to the evaluation of human risk from such toxicity.

The analysis also contributes to the identification of any specific subpopulations (e.g. those with genetic predisposition) who may be at increased risk and often provides information useful in considering relative risk at various life stages. This may be based not always on chemical-specific information but rather on inference, on the basis of knowledge of the MOA, as to whether the risk in specific age groups might be expected to differ.

The data and their analysis using the non-cancer HRF should be reported in a clear and comprehensive manner, so that others can determine the basis of the conclusions reached.

Although the specific form of presentation will vary with the type of data available, a structured report, including the key headings from the framework, should be provided where possible. Presentation should include sufficient details on the context and thought processes to ensure transparency of the conclusions reached. The inclusion of concordance tables is strongly encouraged. This increases transparency and facilitates peer engagement.

# **USE OF THE FRAMEWORK AND ITS OUTPUTS**

The IPCS non-cancer HRF, which is based principally on robust concordance analysis of key events in postulated MOAs, is envisaged to be of value to both the risk assessment and research communities as a basis to contribute to harmonization in several areas, including:

- adequacy and nature of weight of evidence for postulated MOAs in animals and their relevance to humans;
- MOA integration across end-points;
- criteria for transparency to ensure sufficiency of peer input and review.

Among the strengths of the non-cancer HRF are its flexibility, transparency, and general applicability across end-points. This includes determination of the nature and shape of the dose–response curve, the identification and location of biological thresholds for individual key events, and their consequences. In addition, consideration of the kinetic and dynamic factors involved in each key event is informative regarding the relevance or not to specific subpopulations—for example, in early life, in those with particular diseases, or in those with specific polymorphisms. Alternatively, application of the framework can provide quantitative information on the differences between such groups. Human relevance analysis may also indicate that a species is inappropriate for evaluating a potentially relevant end-point because of dose limitations.

# NEXT STEPS

To ensure effective adoption of the non-cancer HRF, there will be a need to train individuals in its application and in the interpretation of its outputs. Experience is being gained in the use of the cancer HRF, and the expertise gained would be applicable in the training of others in the use of the non-cancer HRF. Training would be facilitated by the availability of a number of suitable case-studies. Those published to date would be a sound basis for further development for this purpose (Seed et al., 2005). In addition, cases on a wider range of end-points need to be developed. It would be helpful if organizations with experience in non-cancer HRF analysis could develop courses and make the materials available to others with suitable expertise to help in training.

A database of generally accepted MOAs should be compiled and maintained, together with informative case-studies. Such a database would be of particular importance as experience continues to evolve in the development of MOAs and in determining whether the MOA for a compound is novel or has been described previously for other compounds.

The current non-cancer HRF, which arose out of the IPCS cancer HRF, is focused on noncancer end-points. However, there are marked similarities in the philosophy and strategy to evaluating cancer and non-cancer effects. It is strongly recommended that one of the next steps in harmonization of risk assessment of chemicals should be the preparation of a unified HRF that is applicable to all toxicological end-points, including cancer. The integration of framework approaches into the risk assessment process should be further elaborated, in which illustrative examples would be of value. Some guidance on problem formulation before embarking on an HRF analysis should be included in such a framework document, as should guidance on the use of the outputs of HRF analysis in risk assessment. For example, during application of the framework, a much deeper understanding of dose–response relationships is often developed, which should be taken forward into hazard characterization. As indicated above, knowledge of any dose transitions is invaluable in interpreting exposure data. Identification of key events in the MOA can provide insight into the sources and magnitude of interspecies and interindividual differences.

# REFERENCES

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Critical Reviews in Toxicology*, **36**:781–792.

Corley RA, Meek ME, Carney EW (2005) Mode of action: Oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—Renal and developmental effects of ethylene glycol. *Critical Reviews in Toxicology*, **35**:691–702.

Crofton KM, Zoeller RT (2005) Mode of action: Neurotoxicity induced by thyroid hormone disruption during development—Hearing loss resulting from exposure to PHAHs. *Critical Reviews in Toxicology*, **35**:757–769.

Foster PM (2005) Mode of action: Impaired fetal Leydig cell function—Effects on male reproductive development produced by certain phthalate esters. *Critical Reviews in Toxicology*, **35**:713–719.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Holson JF, Stump DG, Pearce LB, Watson RE, DeSesso JM (2005) Mode of action: Yolk sac poisoning and impeded histiotrophic nutrition—HBOC-related congenital malformations. *Critical Reviews in Toxicology*, **35**:739–745.

Intergovernmental Forum on Chemical Safety (1994) *The International Conference on Chemical Safety—Final report.* Geneva, World Health Organization (http://www.who.int/ifcs/documents/forum1/en/FI-report\_en.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in dose/concentration–response assessment.

Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document No. 2; http://whqlibdoc.who.int/publications/2005/9241546786\_eng.pdf).

Kavlock R, Cummings A (2005a) Mode of action: Reduction of testosterone availability— Molinate-induced inhibition of spermatogenesis. *Critical Reviews in Toxicology*, **35**:685–690.

Kavlock R, Cummings A (2005b) Mode of action: Inhibition of androgen receptor function— Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, **35**:721–726.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

Seed J, Carney E, Corley R, Crofton K, DeSesso J, Foster P, Kavlock R, Kimmel G, Klaunig J, Meek E, Preston J, Slikker W, Tabacova S, Williams G (2005) Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Critical Reviews in Toxicology*, **35**:663–672.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity. *Toxicology and Applied Pharmacology*, **201**:203–225.

Slikker W Jr, Xu ZA, Levin ED, Slotkin TA (2005) Mode of action: Disruption of brain cell replication, second messenger, and neurotransmitter systems during development leading to cognitive dysfunction—Developmental neurotoxicity of nicotine. *Critical Reviews in Toxicology*, **35**:703–711.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Tabacova S (2005) Mode of action: Angiotensin-converting enzyme inhibition— Developmental effects associated with exposure to ACE inhibitors. *Critical Reviews in Toxicology*, **35**:747–755.

UNEP (2002) *Plan of implementation of the World Summit on Sustainable Development.* New York, NY, United Nations Environment Programme (http://www.un.org/esa/ sustdev/documents/WSSD\_POI\_PD/English/WSSD\_PlanImpl.pdf). United Nations (1992) Agenda 21: United Nations Conference on Environment and Development. New York, NY, United Nations Division for Sustainable Development (http://www.un.org/esa/sustdev/documents/agenda21/english/Agenda21.pdf).

Weed DL (2005) Weight of evidence: A review of concept and methods. *Risk Analysis*, **25**:1545–1557.

WHO (2006) *Strategic Approach to International Chemicals Management (SAICM)*. Geneva, World Health Organization (http://www.who.int/ipcs/features/iccm\_crp.pdf).

Wiltse J (2005) Mode of action: Inhibition of histone deacetylase, altering WNT-dependent gene expression, and regulation of beta-catenin—Developmental effects of valproic acid. *Critical Reviews in Toxicology*, **35**:727–738.

Zoeller RT, Crofton KM (2005) Mode of action: Developmental thyroid hormone insufficiency—Neurological abnormalities resulting from exposure to propylthiouracil. *Critical Reviews in Toxicology*, **35**:771–781.

Zoetis T, Walls I, eds (2003) Principles and practices for direct dosing of preweaning mammals in toxicity testing and research. A report of the ILSI Risk Science Institute Expert Working Group on Direct Dosing of Preweaning Mammals in Toxicity Testing. Washington, DC, ILSI Press.

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ハーモナイゼーションプロジェクト文書第4号

第1部:

発がん MOA のヒトへの関連性を解析するための IPCS フレ ームワーク及び事例研究

第2部:

非発がん MOA のヒトへの関連性を解析するための IPCS フ レームワーク

本プロジェクトは、化学物質へのばく露によるリスク評価のアプローチの調和に関する IPCS プロジェクトの一環として実施された。

本文書は世界保健機関(WHO)、国際労働機関(ILO)、国連環境計画(UNEP)の共同支援のも とに出版され、化学物質の適正な管理に関する国際機関間プログラムのフレームワークの中で作 成された。 国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)は、1980年に設立された国連環境計画(UNEP)、国際労働機関(ILO)、世界保健機関(WHO)の共同事業である。IPCSの全体的な目的は、化学物質の安全性の向上のための必要条件として、国際的なピアレビューを通じて、化学物質へのばく露によるヒトの健康と環境へのリスクを評価するための科学的基盤を確立し、化学物質の健全な管理のために各国の管理能力を強化する技術支援を提供することである。

化学物質の適正な管理に関する国際機関間プログラム(IOMC)は、1992年の国連環境開発会議 (UNCED)において表明された化学物質の安全性の分野での協力及び連携の強化を目的とした勧告に 従い、UNEP、ILO、国連食糧農業機関(FAO)、WHO、国際連合工業開発機関(UNIDO)、国連訓練調 査研究所(UNITAR)、経済協力開発機構(OECD)(以上参加機関)によって1995年に設立された。 IOMCの目的は、ヒトの健康と環境に関連した化学物質の健全な管理を達成するために、参加機関が共 同で、あるいは個別に遂行する政策と活動の連携を促進することである。

WHO ライブラリ出版物目録データ

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4. 発がん性試験 5. 発がん物質 6. 腫瘍-化学的に誘発される腫瘍

I. 国際化学物質安全計画 II.シリーズ

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ハーモナイゼーションプロジェクト文書とは、国際化学物質安全性計画(IPCS)(WHO/ILO/UNEP) のもと世界保健機関(WHO)によって出版された出版物群である。ハーモナイゼーションプロジ ェクト文書は、化学物質のリスク評価方法に関する正式な文書として、環境保健クライテリア (EHC)の方法論シリーズ(黄表紙)を補完するものである。

有害化学物質の評価と管理に関する現在の国際的、地域的及び各国内の協調的な取り組みの主 なきっかけは、1992年の国連環境開発会議(UNCED)にあった。UNCEDアジェンダ21の第19 章において、有害化学物質の環境的に健全な管理のための「青写真」が示されている。各国政府 によるこのコミットメントは、2002年の持続可能な開発に関する世界首脳会議で、さらに2006年 の国際的な化学物質管理のための戦略的アプローチ(SAICM)でも再確認された。化学物質への ばく露によるリスク評価のアプローチの調和に関する IPCS プロジェクト(ハーモナイゼーショ ンプロジェクト)は、このアジェンダ21第19章の下で実施されており、SAICMの実施に貢献し ている。特に、本プロジェクトは、SAICMの目標である「リスク低減」とSAICM世界行動計画 の活動である「リスク評価のための新たな調和された手法の開発と利用」に対応している。

IPCS ハーモナイゼーションプロジェクトの目的は、共通の原則とアプローチを追求することに よって化学物質のリスク評価を世界的に改善し、持続可能性というフレームワークの中でヒトの 健康と環境をより良く保護するための国内及び国際的な管理手法を強化することである。ハーモ ナイゼーションプロジェクトは、特定の問題に関する国際的なガイダンス文書を作成することを 含め、化学物質リスク評価に対する世界各国のアプローチを調和させることを目的としている。 このガイダンスは、化学物質のリスク評価を実施する際に各国や国際機関で採用され、使用する ことを目的としている。このガイダンスは、世界中の専門家の協力を得て作成されている。本プ ロジェクトは、まず各国の手法や慣行について情報を共有し、理解を深め、異なるアプローチの 統一化が有益となる分野を特定した上で、調和のとれたアプローチの実施を可能にするガイダン スを作成するという段階的アプローチを用いて採用している。本プロジェクトでは、アプローチ の調和を図る上で、特に重要なリスク評価の側面に焦点を当てたビルディングブロック法を採用 している。

このプロジェクトにより、国際的に認められた手法を用いてリスク評価(またはその構成要素) を実施することを可能にし、これらの評価を共有することで重複を避け、リスク管理のための貴 重な資源を最適に利用することが可能となる。また、リスク管理の意思決定の基礎となる健全な 科学を促進し、リスク評価の透明性を高め、不必要な化学物質の試験を減らすことができる。ま た、科学的知識の発展が新たな調和のとれた手法の開発へとつながりうる。

この進行中のプロジェクトは、各地域の代表的なハーモナイゼーションプロジェクト運営委員 会と、詳細な作業を管理するいくつかの特別作業部会によって監督されている。最終的には、国 際的なピアレビュー及びパブリックコメントといった厳格なプロセスが含まれている。

第1部

発がん MOA のヒトへの関連性を解析するための IPCS フレームワーク及び事例研究

#### 序文

国際化学物質安全性計画(IPCS)の(動物における)<sup>1</sup>「化学物質の発がん作用機序の評価に関 する概念的フレームワーク」の公表を受けて、2004年3月3日から5日に米国バージニア州アー リントンで IPCS がんワーキンググループが開催された。このワーキンググループは、規制やその 他の目的でリスク評価において作用機序に関する情報を利用するための統一された IPCS ヒト関 連性フレームワークを開発することを目標に、動物における腫瘍のヒトへの関連性の問題をさら に検討する必要性があることに合意し、この作業のための初期のガイダンスを提供した。このワ ーキンググループのメンバーは、事務局サポートと経済協力開発機構(OECD)の代表者を含めて 以下の通りである。

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2005 年 4 月 21 日から 23 日に英国のブラッドフォードで開催された IPCS 国際ワークショップ においてアーリントン会議のガイダンスを考慮し、MOA フレームワークに人間への関連性を考慮 した内容に拡張することが検討された。このワークショップでは、2001 年の MOA フレームワー クの更新を含む IPCS ヒト関連性フレームワークの草案が作成された。

<sup>&</sup>lt;sup>1</sup> Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

ワークショップの参加者は、事務局サポート、欧州食品安全局と欧州化学品局の代表者を含め、 以下の通りである。 Peter Abbott 博士(オーストラリア・ニュージーランド食品基準、科学的リスク評価局、 オーストラリア) Antero Aitio 博士(世界保健機関、国際化学物質安全性計画、スイス) Diana Anderson 博士(ブラッドフォード大学、生物医学科、イギリス) Colin Berry 教授(イギリス) Hermann Bolt 教授(生理学研究所、ドイツ) Alan R. Boobis 教授(インペリアル・カレッジ・ロンドン、健康毒性学部門、英国) Susy Brescia 博士 (イギリス健康安全局、英国) John Bucher 博士(国立環境衛生科学研究所、米国) Vincent Cogliano 博士(国際がん研究機関 発がん物質同定・評価部門、フランス) Samuel M. Cohen 博士(病理学・微生物学、ハヴリック・ウォール腫瘍学教授、ネブラ スカメディカルセンター大学、米国) Vicki Dellarco 博士(環境保護庁、農薬プログラム部、米国) Christine Dove 氏(ブラッドフォード大学 生命科学部、英国) Jun Kanno 博士(独立行政法人国立医薬品食品衛生研究所 細胞分子毒性学研究部門、日 本) Janet Kielhorn 博士(フラウンホーファー毒物実験医学研究所 化学リスク評価部、ドイ ツ Sandra Kunz 氏(世界保健機関、国際化学物質安全性計画、スイス) Christian Laurent 博士 (欧州食品安全機関、科学的専門家サービス、イタリア) Douglas McGregor 博士(毒性評価コンサルタント、英国) Bette Meek 氏(カナダ保健省、環境衛生センター、カナダ) Sharon Munn 氏(欧州化学機関、毒物・化学物質担当、イタリア) Julian Preston 博士(環境保護庁、環境発がん部門国立健康・環境影響研究所、米国) Jerry Rice 博士 (コンサルタント、米国) Hans-Bernhard Richter-Reichhelm 博士(連邦リスク評価研究所(BfR)、ドイツ) Carolyn Vickers 氏(世界保健機関、国際化学物質安全性計画、スイス) Deborah Willcocks 氏(国家工業化学品届出・評価スキーム(NICNAS)既存化学品担当、 オーストラリア) William P. Wood 博士(環境保護庁、リスクアセスメントフォーラム、米国) Zheng Yuxin 博士(労働衛生·毒物管理機関、中華人民共和国疾病管理予防局、WHO 労 働衛生協力センター、中国)

草案はパブリックコメントのためにインターネット上で公開され、ピアレビューのために多く のWHO協力機関とIPCS参加機関に送付された。2005年12月にロンドンで開催された専門家会 議では、寄せられたコメントを検討し、最終的にフレームワークが決定された。専門家会議の参 加者は以下の通りである。

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# 頭字語と略語のリスト

ANOVA         分散分析           bw         体重           CAR         恒常的活性化受容体           cDNA         相補的デオキシリボ核酸           CoA         補酵素A           CpG         シトシンとグアニンの2塩基配列           CYP         シトクロムP-450           dA         デオキシブデノシン           dG         デオキシブデノシン           dG         デオキシブデノシン           dG         デオキシブデノシン           dG         デオキシブデノシン           DMSO         ジメチルスルホキシド           DNA         デオキシブデノシン           DMA         デオキシブボク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がん研究機関           ILO         国際学働機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小書性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           NOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           OAT         O-アセチルトランスクロー <th>bw体重CAR恒常的活性化受容体cDNA相補的デオキシリボ核酸CoA補酵素 ACpGシトシンとグアニンの2塩基配列CYPシトクロム P-450dAデオキシブデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質開架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS9900g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,78・ジベンゾア・ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸</th> <th>ADH</th> <th>アルコール脱水素酵素</th>	bw体重CAR恒常的活性化受容体cDNA相補的デオキシリボ核酸CoA補酵素 ACpGシトシンとグアニンの2塩基配列CYPシトクロム P-450dAデオキシブデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質開架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS9900g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,78・ジベンゾア・ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸	ADH	アルコール脱水素酵素
CAR         恒常的活性化受容体           cDNA         相補的デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの2塩基配列           CYP         シトクロム P-450           dA         デオキシグアラシン           dG         デオキシグアラシン           dG         デオキシグアクシン           DMSO         ジメチルスルホキシド           DNA         デオキシリズ検験           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILIN         国際生命和学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカェリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           POX         型自質開架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rr3         リスク科学研究所 (ILSI)           rr3         トリヨードサイロニン <td>CAR         恒常的活性化受容体           cDNA         相補的デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの2塩基配列           CYP         シトクロム P-450           dA         デオキシグアラシン           dG         デオキシグアラシン           dG         デオキシグアクシン           DMSO         ジメチルスルホキシド           DNA         デオキシリズ検験           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILIN         国際生命和学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカェリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           POX         型自質開架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rr3         リスク科学研究所 (ILSI)           rr3         トリヨードサイロニン     <td>ANOVA</td><td>分散分析</td></td>	CAR         恒常的活性化受容体           cDNA         相補的デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの2塩基配列           CYP         シトクロム P-450           dA         デオキシグアラシン           dG         デオキシグアラシン           dG         デオキシグアクシン           DMSO         ジメチルスルホキシド           DNA         デオキシリズ検験           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILO         国際労働機関           ILIN         国際生命和学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカェリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           POX         型自質開架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rr3         リスク科学研究所 (ILSI)           rr3         トリヨードサイロニン <td>ANOVA</td> <td>分散分析</td>	ANOVA	分散分析
ロード         日補助デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの 2 塩基配列           CYP         シトクロム P 450           dA         デオキシグアノシン           dG         デオキシグアノシン           dG         デオキシグアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシリボ核酸           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がの機機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           PNA         増育開架橋           RNA         リボ々教学の空か分離したラット肝臓上清 (肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3         トリヨードサイロニン      T4         チロキシジ <td>ロード         日補助デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの 2 塩基配列           CYP         シトクロム P 450           dA         デオキシグアノシン           dG         デオキシグアノシン           dG         デオキシグアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシリボ核酸           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がの機機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           PNA         増育開架橋           RNA         リボ々教学の空か分離したラット肝臓上清 (肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3         トリヨードサイロニン      T4         チロキシジ<td>bw</td><td>体重</td></td>	ロード         日補助デオキシリボ核酸           CoA         補酵素 A           CpG         シトシンとグアニンの 2 塩基配列           CYP         シトクロム P 450           dA         デオキシグアノシン           dG         デオキシグアノシン           dG         デオキシグアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシリボ核酸           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がの機機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           PNA         増育開架橋           RNA         リボ々教学の空か分離したラット肝臓上清 (肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3         トリヨードサイロニン      T4         チロキシジ <td>bw</td> <td>体重</td>	bw	体重
CoA         補酵素 A           CpG         シトシンとグアニンの 2 塩基配列           CYP         シトクロム P-450           dA         デオキシアデノシン           dG         デオキシリアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシリボ核酸           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がん研究機関           ILO         国際学物質安全性計画           IU         国際生命科学研究機構           PPCS         国際化学物質安全性計画           IU         国際生命           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAT         N-アセチルトランスフェラーゼ           PCNA         増殖性細胞核抗原           PFX         蛋白質問架橋           RNA         リボレク科学研究所 (ILSI)           rT3         リバーストリヨードサイロニン           S	CoA         補酵素 A           CpG         シトシンとグアニンの 2 塩基配列           CYP         シトクロム P-450           dA         デオキシアデノシン           dG         デオキシリアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシリボ核酸           DPX         DNA-タンパク質架橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がん研究機関           ILO         国際学物質安全性計画           IU         国際生命科学研究機構           PPCS         国際化学物質安全性計画           IU         国際生命           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAT         N-アセチルトランスフェラーゼ           PCNA         増殖性細胞核抗原           PFX         蛋白質問架橋           RNA         リボレク科学研究所 (ILSI)           rT3         リバーストリヨードサイロニン           S	CAR	恒常的活性化受容体
CpG         シトシンとグアニンの2塩基配列           CYP         シトクロム P-450           dA         デオキシアデノシン           dG         デオキシグアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシグアグノシン           DNA         デオキシグの営業橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がん研究機関           ILO         国際労働機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           POX         蛋白質間架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rT3         リノコントリヨードサイロニン           S9         9000g で遠心分離したラット肝臓上清(肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3	CpG         シトシンとグアニンの2塩基配列           CYP         シトクロム P-450           dA         デオキシアデノシン           dG         デオキシグアノシン           DMSO         ジメチルスルホキシド           DNA         デオキシグアグノシン           DNA         デオキシグの営業橋           FAO         国連食糧農業機関           HRF         ヒト関連性フレームワーク           IARC         国際がん研究機関           ILO         国際労働機関           ILSI         国際生命科学研究機構           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action(作用モード)           NAT         N-アセチルトランスフェラーゼ           NOAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           POX         蛋白質間架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rT3         リノコントリヨードサイロニン           S9         9000g で遠心分離したラット肝臓上清(肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3	cDNA	相補的デオキシリボ核酸
CYPシトクロム P-450dAデオキシアデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-グンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際学の機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7.8-ジベング-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	CYPシトクロム P-450dAデオキシアデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-グンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際学の機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7.8-ジベング-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	CoA	補酵素A
dAデオキシアデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際営金和学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNATN-アセチルトランスフェラーゼOAEL無毒性量TP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPNA増白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTGCD2,3,7.8-ジペンゾッ・ダイオキシンTGF腫療成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDPウリジンニリン酸UDPウリジンニリン酸	dAデオキシアデノシンdGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際営金和学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNATN-アセチルトランスフェラーゼOAEL無毒性量TP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPNA増白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTGCD2,3,7.8-ジペンゾッ・ダイオキシンTGF腫療成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDPウリジンニリン酸UDPウリジンニリン酸	CpG	シトシンとグアニンの2塩基配列
dGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PX蛋白質間架橋RNAリボ校酸RSIリスク科学研究所(ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清(肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD23,7.8-ジベング-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	dGデオキシグアノシンDMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PX蛋白質間架橋RNAリボ校酸RSIリスク科学研究所(ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清(肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD23,7.8-ジベング-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	CYP	シトクロム P-450
DMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際ゲ働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所(ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清(肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	DMSOジメチルスルホキシドDNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際ゲ働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所(ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清(肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	dA	デオキシアデノシン
DNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS9900g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8・ジベンゾーp-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDS不定期 DNA 合成	DNAデオキシリボ核酸DPXDNA-タンパク質架橋FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS9900g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8・ジベンゾーp-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDS不定期 DNA 合成	dG	デオキシグアノシン
DPXDNA-タンパク質架橋FAO国連食糧農業機関FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ々科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンTGD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDPウリジン二リン酸UDPクリジン二リン酸UDPクリジン二リン酸	DPXDNA-タンパク質架橋FAO国連食糧農業機関FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ々科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンTGD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDPウリジン二リン酸UDPクリジン二リン酸UDPクリジン二リン酸	DMSO	ジメチルスルホキシド
FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDPウリジン二リン酸UDPブリジン二リン酸UDPアニ	FAO国連食糧農業機関HRFヒト関連性フレームワークIARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action(作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDPウリジン二リン酸UDPブリジン二リン酸UDPアニ	DNA	デオキシリボ核酸
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IARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDS不定期 DNA 合成	IARC国際がん研究機関ILO国際労働機関ILSI国際生命科学研究機構IPCS国際化学物質安全性計画IU国際単位JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジン二リン酸UDS不定期 DNA 合成	FAO	国連食糧農業機関
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Internet (1)         Internet (1)           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           PCNA         増殖性細胞核抗原           PYX         蛋白質間架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rT3         リバーストリヨードサイロニン           S9         9000g で遠心分離したラット肝臓上清 (肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3         トリヨードサイロニン           T4         チロキシン           TCDD         2,3,7,8-ジベンゾ・p-ダイオキシン           TGF         腫瘍成長因子           TSH         甲状腺刺激ホルモン           UDP         ウリジンニリン酸           UDS         不定期 DNA 合成	Internet (1)         Internet (1)           IPCS         国際化学物質安全性計画           IU         国際単位           JMPR         残留農薬に関する FAO/WHO 合同会議           KM         ミカエリス・メンテン定数           LOAEL         最小毒性量           MOA         Mode of Action (作用モード)           NAT         N-アセチルトランスフェラーゼ           NAAEL         無毒性量           NTP         米国国家毒性プログラム           OAT         O-アセチルトランスフェラーゼ           PCNA         増殖性細胞核抗原           PYX         蛋白質間架橋           RNA         リボ核酸           RSI         リスク科学研究所 (ILSI)           rT3         リバーストリヨードサイロニン           S9         9000g で遠心分離したラット肝臓上清 (肝ミクロソーム)           SCE         姉妹染色分体交換           SHE         シリアンハムスター胚           T3         トリヨードサイロニン           T4         チロキシン           TCDD         2,3,7,8-ジベンゾ・p-ダイオキシン           TGF         腫瘍成長因子           TSH         甲状腺刺激ホルモン           UDP         ウリジンニリン酸           UDS         不定期 DNA 合成	ILO	国際労働機関
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JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	JMPR残留農薬に関する FAO/WHO 合同会議KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	IPCS	国際化学物質安全性計画
KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	KMミカエリス・メンテン定数LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	IU	国際単位
LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	LOAEL最小毒性量MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	JMPR	残留農薬に関する FAO/WHO 合同会議
MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	MOAMode of Action (作用モード)NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	KM	ミカエリス・メンテン定数
NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	NATN-アセチルトランスフェラーゼNOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	LOAEL	最小毒性量
NOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	NOAEL無毒性量NTP米国国家毒性プログラムOATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	MOA	Mode of Action(作用モード)
NTP       米国国家毒性プログラム         OAT       O-アセチルトランスフェラーゼ         PCNA       増殖性細胞核抗原         PPX       蛋白質間架橋         RNA       リボ核酸         RSI       リスク科学研究所(ILSI)         rT3       リバーストリヨードサイロニン         S9       9000g で遠心分離したラット肝臓上清(肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	NTP       米国国家毒性プログラム         OAT       O-アセチルトランスフェラーゼ         PCNA       増殖性細胞核抗原         PPX       蛋白質間架橋         RNA       リボ核酸         RSI       リスク科学研究所(ILSI)         rT3       リバーストリヨードサイロニン         S9       9000g で遠心分離したラット肝臓上清(肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	NAT	N-アセチルトランスフェラーゼ
OATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	OATO-アセチルトランスフェラーゼPCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	NOAEL	無毒性量
PCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	PCNA増殖性細胞核抗原PPX蛋白質間架橋RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	NTP	米国国家毒性プログラム
PPX       蛋白質間架橋         RNA       リボ核酸         RSI       リスク科学研究所 (ILSI)         rT3       リバーストリヨードサイロニン         S9       9000g で遠心分離したラット肝臓上清 (肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	PPX       蛋白質間架橋         RNA       リボ核酸         RSI       リスク科学研究所 (ILSI)         rT3       リバーストリヨードサイロニン         S9       9000g で遠心分離したラット肝臓上清 (肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	OAT	O-アセチルトランスフェラーゼ
RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	RNAリボ核酸RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	PCNA	增殖性細胞核抗原
RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	RSIリスク科学研究所 (ILSI)rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	PPX	蛋白質間架橋
rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	rT3リバーストリヨードサイロニンS99000g で遠心分離したラット肝臓上清 (肝ミクロソーム)SCE姉妹染色分体交換SHEシリアンハムスター胚T3トリヨードサイロニンT4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	RNA	リボ核酸
S9       9000g で遠心分離したラット肝臓上清(肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	S9       9000g で遠心分離したラット肝臓上清(肝ミクロソーム)         SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	RSI	リスク科学研究所(ILSI)
SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	SCE       姉妹染色分体交換         SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	rT3	リバーストリヨードサイロニン
SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	SHE       シリアンハムスター胚         T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	S9	9000g で遠心分離したラット肝臓上清(肝ミクロソーム)
T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	T3       トリヨードサイロニン         T4       チロキシン         TCDD       2,3,7,8-ジベンゾ-p-ダイオキシン         TGF       腫瘍成長因子         TSH       甲状腺刺激ホルモン         UDP       ウリジンニリン酸         UDS       不定期 DNA 合成	SCE	姉妹染色分体交換
T4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	T4チロキシンTCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	SHE	シリアンハムスター胚
TCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	TCDD2,3,7,8-ジベンゾ-p-ダイオキシンTGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	T3	トリヨードサイロニン
TGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	TGF腫瘍成長因子TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	T4	チロキシン
TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	TSH甲状腺刺激ホルモンUDPウリジンニリン酸UDS不定期 DNA 合成	TCDD	2,3,7,8-ジベンゾ-p-ダイオキシン
UDPウリジンニリン酸UDS不定期 DNA 合成	UDPウリジンニリン酸UDS不定期 DNA 合成	TGF	
UDS 不定期 DNA 合成	UDS 不定期 DNA 合成	TSH	
		UDP	
	UGT ウリジンニリン酸グルクロン酸転移酵素	UDS	
UGI リリンンニリン酸クルクロン酸転移酵素		UGT	ウリジン二リン酸グルクロン酸転移酵素

ULLI単位長及び標識指数UNEP国際連合環境計画USAアメリカ合衆国USEPA米国環境保護庁WHO世界保健機関

# 発がん MOA のヒトへの関連性を解析するための

# IPCS フレームワーク

## Alan R. Boobis, Samuel M. Cohen, Vicki Dellarco, Douglas McGregor, M.E. (Bette) Meek, Carolyn Vickers, Deborah Willcocks, & William Farland

体系化されたフレームワークの利用は、化学物質のリスク評価における調和を促進する上で非常 に重要である。したがって、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)は、実験的研究で観察された発がん性反応のヒトへの関連性の問題に対処するために、発が ん Mode of Actioin (MOA) フレームワークを更新し、拡張した。第一段階は、MOA を確立する ことが可能かどうかを判断することである。MOA は、Bradford Hill 基準に基づいたエビデンスの 重み付けアプローチを用いて特定された発がんの原因経路に沿った一連の key events で構成され ている。次に、実験動物とヒトの間で key events を定性的に、次に定量的に比較する。最後に、信 頼性、解析的検討及び意義についての明確な報告書が作成される。IPCS 発がん性ヒト関連性フレ ームワークは、データの透明性のある評価、重要なデータギャップの特定及び関連性が排除できな い場合であっても、化合物のさらなるリスク評価において価値のある情報の体系的な提示を可能 にする解析ツールを提供するものである。この価値ある情報には、用量反応曲線の形状に関するデ ータ、閾値の特定及び遺伝的またはライフステージの違いなどに基づいた潜在的に感受性の高い 集団を認知することが含まれる。

過去 30 年間のがんリスク評価の発展の基礎となったのは、がんの生物学的理解の深まりと発が んにおける key events の特定である。1980 年代半ばまでは、ヒトにおけるがんのハザードとリス クに関する国内及び国際的な評価は、主に発がん性のある物質のげっ歯類を用いた生涯試験に依 存していた。後ろ向きがん研究の基礎となるに十分なヒトにおけるエビデンスが存在する薬剤は ほとんどなく、職場や環境における一般的なばく露に対する現代の規制を考えれば、将来的に発 がん性が検出される薬剤はさらに少ないと予想される。げっ歯類を用いた評価において、実験動 物で観察された腫瘍は、潜在的にヒトに対して発がん性を有する物質を特定するために意味のあ る外挿が可能であり、数学的モデルを用いて規制上重要となるヒトにおけるリスクの上限推定値 を提供することができるという前提があった。同時期に、発がんにおいて変異原性が重要な意義 を有する可能性があるということが科学界に受け入れられつつあった。その後、かなりの数の化 学物質が、DNA との直接的な相互作用を伴わないプロセスによって実験動物にがんを引き起こす ことが次第に明らかになってきた。実験動物とヒトの両方における発がんの生物学的基盤に関す る我々の理解を深めることは、発がん性が疑われる薬剤の薬物動態及び薬理作用に関するより多 くのデータを提供することにより、リスク評価の過程を有益なものとした。特定の化合物の発が んに関与する生物学的プロセスを考察することは、Mode of action(以下、MOA)の概念につなが っている。

<sup>&</sup>lt;sup>1</sup> この論文は、WHO が著作権を有するものであり、元々は 2006 年に Critical Reviews in Toxicology, Volume 36, pages 781-792 に掲載されたものです。この論文は WHO の出版物のために編集されたもので、正誤表が含まれています。

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

推定される発がん MOA とは、試験で観察された影響につながる生物学的に妥当な key events の 一連の流れであり、堅牢な実験観察とメカニズムデータに裏付けられ、重要な細胞学的及び生化 学的事象、すなわち、観察された発がん性に必要かつ測定可能な事象を論理的なフレームワーク で記述している。MOA は作用機序 (mechanism of action)とは対照的であり、作用騎乗では一般的 に、効果の分子的基盤を十分に理解し、その詳細を記述することで、分子的観点から因果関係を 確立することができる。

2001 年に、リスク評価手法の調和の一環として、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)(WHO/ILO/UNEP)は、因果関係に関する Bradford Hill 基準 に基づいた実験動物の発がん MOA(動物 MOA)評価のためのフレームワークを発表した。本文 書書で紹介している IPCS ヒト関連性フレームワーク(HRF)は、この MOA フレームワークを更 新し、ヒトとの関連性を考慮するために拡張したものである。これは、化学物質の特定の発がん 性について利用可能なデータを透明性のある方法で体系的に評価する手段を提供する解析ツール である。フレームワークは、規制当局の内外のリスク評価者にとって価値のあるものであると同 時に、研究者にとっても貴重なツールとなることを想定している。フレームワークを利用する理 由には、以下のようなものがある。

- データ解析の一般的なアプローチを提供し、調和の推進に貢献する。
- 利用可能なデータの検討と使用及び導き出された結論の論拠の透明性を促進する。
- データの提示におけるガイダンスを提供する。
- 重要なデータの不足と必要な追加データを特定する。
- ヒトに対する発がん性リスクの定量的評価において情報を提供する。

これら及びその他のトピックについて、以下でより詳細に議論する。

# 発がん MOA のヒトへの関連性を解析するためのフレームワークを開発する上での IPCS の役割

IPCS は、化学物質へのばく露によるリスク評価のアプローチの調和に関するより大きなプロジェクトの一環として、発がんリスク評価への手法を調和させる取り組みを主導してきた。この作業の第一段階では、実験動物における化学物質の発がん MOA を評価するための「IPCS の概念的なフレームワーク」の公表につながった(Sonich-Mullin ら, 2001 年)。この出版物に記載されているように、エビデンスの重み付けを検討する際にみられる調和への障害は、主に動物 MOA の評価であった。Sonich-Mullin ら(2001 年)は、動物における化学物質の発がん MOA を評価するためのフレームワークを提供し、ヒトにおけるがんのハザードとリスクの総合的な判定の次のステップ、すなわち、動物における発がん MOA のヒトへの関連性の評価に進むことの重要性を認識した。MOA フレームワークの概念は、米国環境保護庁(USEPA)発がん物質リスク評価ガイドライン改訂版(USEPA、1999 年、2005 年)に盛り込まれており、MOA フレームワークは現在、他の規制当局や国際機関でも一般的に用いられている。英国では、このフレームワークが農薬や工業用化学物質の評価に利用されている。

英国発がん性委員会(2004年)は、機関間の調和と最新のガイドラインにおける内部の整合性の 両方の点でフレームワークの価値を指摘している。このフレームワークは、オーストラリアの機 関でも採用され、カナダではカナダ環境保護法の下での既存化学物質の評価に用いられている。 欧州連合(EU)は、新規及び既存の工業用化学物質及び殺生物剤のの評価について、発がん性を 含む技術ガイダンス文書に本フレームワークを組み込んでいる。国際機関では、FAO/WHO 合同 残留農薬専門家会議(JMPR)が、例えばピレスリン抽出物の評価やその結果として得られた研究 論文に、このフレームワークを組み入んでいることが。特筆される。

MOA フレームワークを拡張し、ヒトへの関連性の考慮を含めるためのステップは、IPCS が国際的なパートナーと協力して実施されてきた。これは 2005 年 4 月 21 日から 23 日まで英国ブラッドフォードで開催された IPCS の国際ワークショップの議題であった。このワークショップでは、2001 年の MOA フレームワークの更新を含む IPCS HRF の草案が作成された。この草案はパブリックコメントのためにインターネット上で公開され、また、ピアレビューのために多くの WHO 協力機関と PCS 参加機関に送付された。2005 年 12 月にロンドンで開催された専門家会議では、受け取ったコメントが検討され、フレームワークが最終決定された。フレームワークの内容とその発展へとつながるステップについては、以下のセクションで詳述する。

#### 2001年の動物における発がん性評価のための IPCS 概念的 MOA フレームワーク

#### フレームワークの目的

動物における発がん性評価のための IPCS MOA フレームワーク (Sonich-Mullin ら, 2001 年) は 今もなお発がん MOA のヒトとの関連性を解析するための IPCS フレームワークの基本原理とな っている。動物 MOA フレームワークは、発がん性化学物質による動物の腫瘍誘発に対して、想 定される MOA を評価する際に一般的に適用される原則への汎用的アプローチを提供するもので ある。したがって、このフレームワークは想定される MOA に対する総合的なエビデンスの重み 付けの評価に際して体系的なアプローチを提供するツールである。すなわち、この文脈では支持 された MOA は、生物学的に妥当な説明を確立するためにしっかりとした実験的観察とメカニズ ムデータのエビデンスを持つことになる。

このフレームワークは、推定される MOA の解析に透明性をもたらし、その結果、明確にされ た手法(解析と推論を裏付ける明確で一貫性のある文書化を促し、利用可能なデータの矛盾と不 確実性を明らかにする手法)を用いることにより到達した結論の信頼性が増すように設計されて いる。フレームワークの目的は、特定の状況における MOA のエビデンスの重み付けを検討する 体系的な手段を提供することであり、情報が十分か否かに関して絶対的な回答を与えるようには 設計されていない。なぜなら情報は状況によって異なるためである。このフレームワークは基準 のチェックリストではなく、むしろ解析的なアプローチである。しかし、key events 及び腫瘍の発 生に関する比較データの表形式の要約はこのプロセスの大きな助けとなる。

動物 MOA フレームワーク解析は、ハザードの特性評価の重要なステップである。動物 MOA フレームワークは、すべての分野(医薬品、工業用化学物質、農薬、食品添加物など)にわたる発がん化学物質のリスク評価に貢献することが想定されている。

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

結果として得られるリスク評価文書では、フレームワーク解析はハザードの特性評価のセクショ ン内に適切に配置されるであろう。適切な疫学データがない場合には、ヒトとの関連性、用量反 応関係、リスクの特性を議論する上で、フレームワーク解析は不可欠な要素とみなされるかもし れない。また、このフレームワークは、データギャップや矛盾の明確な定義に基づいて研究の必 要性を特定する上で規制当局と研究者の双方にとって有用であると考えられる。

MOA 解析は、化合物が以前に記述されている MOA を有すること、または新規の MOA を有す ることを確認するために用いることができる。したがって、MOA 解析の結果は、特定の化合物の 評価に役立つこともあれば、新規の MOA の確立に貢献することもある。前者において、化学物 質に特有のデータが、ヒトへの関連性を考慮した一致解析において重要な役割を果たす。後者に おいては、どのような事象が MOA を表す生物学的プロセスの鍵となるのかを特定することが重 要になる。

このように、同様の一連の key events で構成された MOA は、多くの異なる化合物にも適用でき る可能性がある。ある MOA が特定の発がん作用の原因であることを立証するために必要なエビ デンスは、そのような MOA が初めて提示される場合には重要である。後発の化合物がこの MOA を共有することが発見される場合に、受け入れに対する「障壁」は低くなるが、MOA を構成する key events が発生すること、また、以下に説明する基準を満たしていることを厳密に立証する必要 がある。また、他の可能性のある MOA を除外することも重要である。

科学的な専門家の関与は、新規に想定される MOA の確立と承認のための必要条件である。「専 門家の関与」には、MOA の確立への専門家の関与と、MOA の研究プロセスから独立した科学者 によるピアレビューの両方が含まれる。科学雑誌への発表、科学会議やワークショップでの発表 や議論も、科学界が MOA を受け入れるための「専門家の関与」を意味する。

容認は必ずしも科学者全員が合意したことを意味するわけではないが、容認されたということ は、MOA をレビューした科学者の大部分が、提示された MOA に関して関連する科学的情報が特 定され適切に解析されていること、「key events」が特定され提示された情報によって支持されてい ること、発がんとの関係が仮説の MOA において明確に説明されていること及びその代替 MOA が 検討され、それらが除外されていることに合意したということである。

知識の向上につれて MOA は変化するだろう。追加の key events が特定され、他の事象は見直されるか、あるいは取り除かれるかもしれない。しかしながら、key events に関する大きな変更は、 上記のようなピアレビューを通してある程度一般に認められることも必要である。

#### フレームワークガイドラインの更新

IPCS HRF の発展において、2001 年の動物 MOA フレームワークの内容が更新されたので、その 改訂版を以下に提示する。

## 序文

このセクションでは、観察された発がんエンドポイントまたはその他エンドポイントについて 記述し、これらのうちのどれが解析で扱われているかを特定する。フレームワーク解析に着手す る前に、実験動物における発がん性反応のエビデンスの重み付けを慎重に評価する必要がある。 フレームワークの性質上、一度に1つの MOA のみが解析される。したがって、例えば、化学物 質の投与に関連した異なる種類の腫瘍は、かりに同じ動物で記録されていても、各腫瘍の MOA を 識別するために別々のフレームワーク解析が必要となる。しかしながら、あるひとつの腫瘍の病 態を検討する際には、化学物質が複数の MOA によってそのタイプの腫瘍を誘発する可能性があ ることを認識しておくべきである。したがって、ある化学物質におけるひとつの腫瘍について複 数の MOA の解析を行う必要があるかもしれない。遺伝毒性発がん物質の場合、代謝活性化と解 毒における種や組織特異的な違いと同様に、発がん部位が異なることも多い。動物とヒトのデー タを比較する際にはこのことについて留意する必要がある。対照的に、非遺伝毒性 MOA に則っ て作用するほとんどの発がん物質は、特異的な傾向のある生理学的プロセスに影響を及ぼすとい う観察結果から、少なくとも HRF の初期の前提として、部位の一致が合理的に想定されている。

### 1. 推定される MOA (事例における仮説)

このセクションでは、被験物質において推定される MOA について、腫瘍になるまでの一連の 事象を簡単に説明している。この一連の事象の説明は、次のセクションにつながり、解析に利用 可能なデータベースから、「鍵」と考えられる事象(すなわち、必要かつ測定可能な事象)を特定 する。

#### 2. Key events

このセクションでは、「key events」一推定される MOA において仮定された腫瘍の誘発に極めて 重要な測定可能な事象について簡潔に特定し、説明する。関連性を裏付けるためには、一連の実 験で事象を定義し、それを一貫して測定する必要がある。関連する観察項目には、例えば、同じ 種類の細胞における発がん性と key events、事象に論理的に関連する作用部位、細胞増殖の増加、 特定の生化学的事象、臓器重量や組織学的変化、増殖、ホルモンや他のシグナル伝達系の変動、 受容体-リガンド相互作用、DNA や染色体への影響、細胞周期への影響などが含まれる。例えば、 再生性増殖の延長と関連していると推定される腫瘍の発生における key events は、病理組織学的 検索における細胞毒性の検出と標識指標の上昇であるかもしれない。別の例として、リン酸カル シウムを主成分とする尿固形物の形成が原因であると推定される膀胱腫瘍の誘発の key events に は、尿中の遊離カルシウム、リン酸塩、pH の上昇と尿固形物の形成、それに続く尿路上皮への刺 激及び再生性過形成が含まれる。

#### 3. 用量反応関係の一致性

このセクションでは Bradford Hill 基準の観点から、それぞれの key events と発がんの用量-効果/ 反応関係を特徴づけ、それらの相互関係について議論するべきだろう。理想的には、key events の 発生率の増加の用量依存性と、その後に発生する他の key events の発生率または重症度(病変の 進行など)の増加及び最終的な腫瘍の発生率とを相関させることができるようにすべきである。 Key events と腫瘍の発生率を表形式で比較表示することは、しばしば用量反応性を検討する上で しばしば有用である。複雑なデータセットの場合、これはほぼ必須である。 腫瘍形成に対する用量反応曲線の異なる部分において、生物学的反応(すなわち線量推移)に基本的な相違があるか否かを検討することが重要である(Slikker ら, 2004 年)。もしそうであれば、 用量反応曲線の異なる部分に関連する key events を明らかにし、フレームワーク解析に用いる必要がある。

# 4. 時間的関連性

このセクションでは、それぞれの key events と腫瘍反応の時間的関係の特徴について述べなけ ればならない。腫瘍反応に至る key events を時系列的に明らかにすべきである。key events は腫瘍 の発現前に明らかにしなければならず、互いに時間的な整合性が取れていなければならない。す なわち、時間的関連性についてはデータが想定される MOA を支持しているかどうかを判断する 上で不可欠である。腫瘍と同時に key events が観察された場合(例えば、バイオアッセイの終了時 など)は、時間的関連性の検討には役立たないが、次のセクションに述べる解析に役立てること ができる。多くの場合、時間的関連性についての基準を満たす完全なデータセットは入手できな い。

# 5. 腫瘍反応とkey events との関連性の強さ、一貫性、特異性

このセクションでは、key events、前がん病変及び腫瘍反応を結び付けるエビデンスの重み付け について議論しなければならない。Key events を遮断または低減した場合に、その後の事象または 腫瘍が発生しない、または減少することを示す中止/回復試験は、関連性を検証する上で特に重要 である。デザインが異なることによって未知のバイアスや交絡因子を減少させると考えられるた め、異なる実験デザインの研究において一貫した結果が観察されることは、key events と腫瘍の関 連性をより強く裏付けることになる。しかし、さまざまな研究で推定される MOA の key events の 再現性で示される一貫性 (consistency) は、より広範なデータベースでの結果と推定 MOA の相関 性で示される整合性 (coherence) とは区別される (6 参照)。関連する観察項目には、同じ種類の 細胞における腫瘍反応と key events、事象に論理的に関連する作用部位並びに多段階試験及び中止 /回復試験の結果が含まれる。

# 6. 生物学的妥当性及び整合性

その MOA が、一般的にがんの発生について一般的に知られていることと一致している(生物 学的に妥当である)かどうか、また、その物質について知られていることと関連している(理論 的な整合性がある)かどうかを検討しなければならない。推定される MOA とその一部である事 象が生物学的に妥当であるとするためには、それらが現在のがんについての生物学的理解と一致 している必要がある。しかしながら、我々の知識には限りがあるために生物学的妥当性について エビデンスの重み付けを評価する際の基準として用いることができる範囲は限られているであろ う。推定される MOA とより広範なデータベースの観測値との関係 ―例えば、発がん MOA と他 のエンドポイントの MOA との関連性―を扱う「整合性」は、異なる研究における推定される発 がん MOA における key events の再現性を扱う「一貫性」(ポイント5参照)とは区別される必要 がある。理論的な整合性については、構造的アナログにおける事例との類似性が参考になるかも しれない(すなわち、構造-活性解析)。推定される MOA を共有する他の化合物からの情報、たと えば、感度における性差、種差及び系統差や key events との関係に関するような情報は重要とな るだろう。さらに、このセクションでは、その薬剤に関するデータベースが内部で一貫して関連 している発がん以外の毒性を含めた MOA を支持しているかどうかを検討しなければならない。 MOA の中には、発がんにつながるホルモン障害による生殖への影響のような、発がん以外の影響 を引き起こすことが予想されるものがある。

## 7. その他のMOA

このセクションでは、事例の中で論理的に提示された代替 MOA について議論する。代替 MOA が支持されている場合は、それ自体のフレームワーク解析が必要である。これら代替 MOA は観察された影響に寄与していると考えられる推定される MOA の追加的な構成要素とは区別し、異なる独立した MOA として解析しなければならない。さもなくば、代替 MOA ではなく、元々の解析の対象であった MOA に関する解析になってしまう。

## 8. 不確実性、矛盾、データギャップ

不確実性には、腫瘍発生の生物学的知見に関連するものと、対象の化合物のデータベースに関 連するものの両方を含まなければならない。矛盾点にはフラグを立て、データギャップについて 特定しなければならない。特定されたデータギャップについては、推定される MOA の裏付けと して重要か否かを示すべきである。

## 9. 推定される MOA の評価

このセクションには、推定される MOA の信頼度を示す指標と共に(例えば、高、中、低など) 結果に関する明確な記述を含まなければならない。新規の MOA が推定されている場合は、新規 であることを明確に示すべきである。しかし、MOA が他の化合物について提示されているものと 同じである場合には、key events がこの MOA にどの程度合致しているかを明示する必要がある。 大きな違いがあれば、それを明記し、MOA に対するその違いの意味合いについて議論すべきであ る。

## ヒトへの関連性の問題

2000 年に、英国のカーシャルトンで IPCS ハーモナイゼーションプロジェクトがん計画ワーキ ンググループが招集された(IPCS、2000 年)(この最初の IPCS ワーキンググループは、ヒト関連 性プロジェクトの作業のために招集されたその後の IPCS ワーキンググループとはメンバーが異 なっていた)。この会議において、ヒトへの関連性への対応に向けて IPCS MOA フレームワークの 拡張を IPCS と国際生命科学研究機構(ILSI)が協力して並行して進めていくことが提案された。 ILSI は技術ワークショップにおいて多くの支援を提供できることが認識された。2001 年 6 月、 ILSI リスクサイエンス研究所(RSI)は、USEPA とカナダ保健省の支援を受けて、動物における 腫瘍とヒトとの関連性を判断するための MOA 情報の使用における重要な問題を検討するための ワーキンググループを結成した。これらの努力の結果、以下に記載されているいくつかの報告書 が公表された。2004 年 3 月 3 日から 5 日に米国バージニア州アーリントンで開催された IPCS が んワーキンググループは、これらの報告書を出発点として、規制目的やその他の目的で MOA 情 報をリスク評価に使用するための統一的な IPCS HRF を作成することを目標に、動物における腫 瘍のヒトへの関連性の問題を IPCS がさらに調査することに合意した(IPCS、2004 年)。

動物で決定された MOA のヒトへの関連性の問題に対処するために、ILSI/RSI は同ワーキング グループに、動物で決定された発がん MOA のヒトへの関連性の評価を含めるために IPCS MOA フレームワークを拡張することを求めた。このプロセス、事例研究及びフレームワークの詳細は、 Critical Reviews in Toxicology 誌の 2003 年 11 月号に一連の論文として発表された(Cohen ら, 2003 年)。これらの論文では ILSI/RSI HRF を説明し、その適用のための指針を提供している。さらに、 フレームワークが基づいた具体的な例への参照も含まれている。統合フレームワークを開発する ために、一般的によく知られている MOA を有する化学物質を複数回にわたって繰り返し検討し た事例研究が使用された。その意図は、動物での MOA と各 key event についてヒトへの関連性を

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

評価する規律ある透明なプロセスの指針を提供することであった。

ILSI/RSI HRF は3つの基本的な問いかけに基づいている:

- 1. 動物における Mode of action (動物 MOA) を確立するのにエビデンスの重み付けは十分か?
- 2. 動物の MOA に含まれる key events はヒトにおいて妥当であるか?
- 3. 動態的、薬力学的要因を考慮した上でも、動物の MOA における key events はヒトにおいて妥当か?

問 2 と 3 に関して、動物の MOA とその key events に関連したヒトでの情報の一致解析において、それぞれ定性的な検討と定量的な検討が必要となる。

これらに続いて、ILSI/RSI HRF は評価の信頼性、特異的なデータギャップの特定、それらのリ スク評価の意味合いを明確に記述している。ILSI/RSI では、このフレームワークの利用が全体的 なリスク評価プロセスのハザードの特性評価ステップの一部を形成することを強調した。

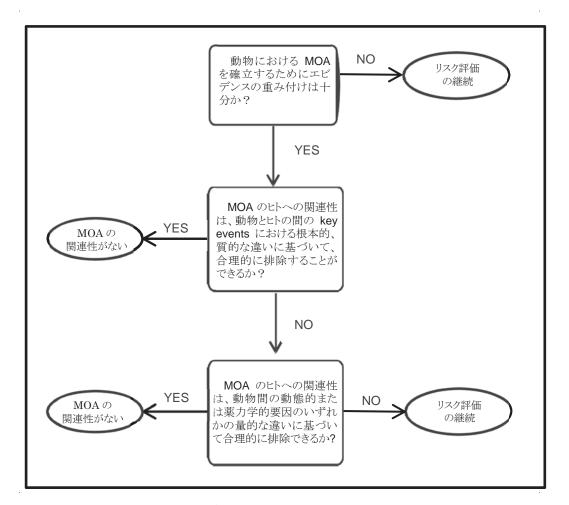
#### IPCS MOA フレームワークと ILSI/RSI HRF に基づく IPCS HRF ガイダンス文書の開発

2004年の IPCS がんワーキンググループでは、ヒトへの関連性に対処するために IPCS MOA フレームワークを拡張する作業の報告書として作成される文書のタイプについて議論された。規制 当局や他のリスク評価機関による採用と利用を促進するためには、全体を一つに統合されたガイ ダンス文書が必要であると認識された。ガイダンスは、発行論文や事例研究といったプロセスを 経て作成された他の公表資料を追加することができる。

ワーキンググループのメンバー間では、ILSI/RSI HRF の重要な構成要素として特定された問い かけは重要であり、動物で決定された MOA のヒトへの関連性に対処するのに適切であるという 点で概ね合意されていた。しかし、フレームワークの補足説明、進展、または拡大が有効と思わ れるいくつかの課題が明らかとなった。

ILSI/RSI HRF のこれらの改良は IPCS がんワーキンググループの議論と、2005 年 4 月 21 日から 23 日に英国ブラッドフォードで開催されたワークショップでの議論を経て進められた(IPCS、 2005 年)。結果として得られた IPCS HRF は、動物の腫瘍における MOA のヒトへの関連性に関す る文書化された論理的な結論へとつながる一連の 3 つの問いかけに答えるためのアプローチとし て提示されている。このガイダンスを適用すると、リスク評価のハザードの特性評価に組み入れ ることができる 4 つのセクションからなるストーリーができあがる。これらのセクションは以下 の通りである(図1を参照)。

- 1. 動物における Mode of action (動物 MOA) を立証するためにエビデンスの重み付けは十分か?
- 2. 実験動物とヒトとの間の key events の根本的、質的な違いに基づいて、MOA のヒトへの関連性 を合理的に排除できるか?
- 3. 実験動物とヒトとの間の動態的または薬力学的要因のいずれかの量的な違いに基づいて、MOA のヒトへの関連性を合理的に排除できるか?
- 4. 結論:信頼性、解析的検討及び帰結の記述。



# 図 1. IPCS の一般的なスキーム:腫瘍形成に対する動物 MOA のヒトへの関連性を評価す る際の主なステップを示す

質問は、Yes か No かを明確に答えられるように設計されているが、エビデンスの重み付けが十分 であるかどうかを判断する必要がある。図の左側に示された回答は、エビデンスに十分な重みがあ り MOA がヒトに関連するとは考えられないことを示している。図の右側の回答は、MOA がヒト に関連する可能性が高いと考えられるという十分に重みのあるエビデンスの存在を示しているか、 利用可能な情報に不確実性があるため、ヒトへの関連性の可能性についての結論を出すことができ ないことを示している。このような場合、評価はリスクの判定に進むことになる。この段階でのみ、 ヒトへのばく露評価が含まれることに留意すべきである。

このフレームワークをある化学物質に適用する場合、観察された各動物の標的臓器の腫瘍は、 異なる臓器では異なる MOA が存在しうるという仮定の下、独立して評価される。しかし、この 解析手法に基づいたとしても、異なる組織での MOA が類似している可能性はある。同様に、異 なる種とヒトにおいて、標的臓器における MOA が一致する可能性についての評価も MOA 解析 に基づいて行う必要がある。

# 動物における Mode of action (動物 MOA)を確立するのにエビデンスの重み付けは十分か

IPCS HRF のこの最初の問いかけに答えるためには、上述の(更新された) IPCS MOA フレーム ワークを適用する必要がある。因果関係を説明するための Bradford Hill 基準に基づいた MOA フ レームワークのステップは、次のとおりである:

- 1. 推定される MOA
- 2. Key events; 関連する重要なパラメータ
- 3. 用量反応関係
- 4. 時間的関連性
- 5. Key events と腫瘍反応との関連の強さ、一貫性、特異性
- 6. 生物学的妥当性と整合性
- 7. 可能性のある代替 MOA
- 8. 不確実なこと、矛盾すること及びデータギャップ
- 9. MOA に関する結論

このプロセスには、状況に応じた考え得る代替 MOA のエビデンスの重み付けの評価と、検討 中の MOA を支持するエビデンスの全体的な強度の評価が組み込まれている。最終的には、MOA を支持するエビデンスの重み付けと、それに対する信頼度について決定しなければならない。ま た、このプロセスは、ギャップを満たすことで推定される MOA の信頼性を高めるであろう決定 的に重要なデータギャップを特定する。また、推定される MOA が他の化学物質において既に記 述された MOA であり、ヒトへの関連性について既に評価されているかどうか、または、新規の MOA であり、ヒトへの関連性について新たに評価する必要があるかどうかを確認することも必要 である。

ある化学物質について、MOA を評価するための主な情報源は、腫瘍が発生した動物モデルにお けるデータである可能性が高い。当然、類似の化学構造を持つ化学物質に関するデータや、同じ または類似の MOA に関するデータ、もしくはその両方のデータといった他の情報源からのデー タも必要に応じて利用することができるし、利用すべきである。ある化学物質の MOA が新規で ある場合、その化学物質が誘発された腫瘍の発がんプロセスに関連しているという結論を裏付け るためには、同じ MOA で作用する後続の化学物質よりもかなり多くのデータが必要である。 ILSI/RSI ワーキンググループと IPCS ブラッドフォードワークショップでは、対象の化学物質の MOA を支持するのに十分なデータの数はどのくらいかという問題に関して、事例研究の中で例を 示し、新しい MOA を受け入れるには科学的コンセンサスが必要であるとの認識(上述)を示し てはいるが、それら以外についての方針等は何ら表明していない。この段階における動物とヒト との間の潜在的な違いについての MOA 解析の検討は、フレームワークにおける次のステップの 対応を容易にする。

# 実験動物ととトとの間の key events の根本的、質的な違いに基づいて、MOA のとトとの関連性を合理的に排除することができるか

この質問の文言は、IPCS ワークショップにおける質問に対する回答の意味合いについての議論 を受けて、ILSI/RSI HRF に記載されていた文言から変更された。元の質問への回答では、動物 MOA がヒトへの関連性を持たないという結論を出すには、はっきりとした「No」しか許容されていな かった。また、「plausible」という単語が他の言語に翻訳する際に問題になる可能性があることも 認識された。そのため、質問は Yes/No の回答を可能にするように書き換えられたが、エビデンスの重み付けの妥当性に関する決定は絶対的なものではなく、利用可能なデータの透明性のある解析に基づいた科学的判断が必要であるという認識に基づき、「合理的に」という語句が付け加えられた。

このステップでは、ヒトにおける発がんの可能性と MOA の関連性を定性的に評価することで ある。動物 MOA で発生する重要な特定の key events をリストアップし、それぞれの key events が ヒトで発生するか否かを直接評価することで、関連する情報の検討と透明性のある提示が容易に なる。一致表 (concordance table) と呼ばれる表形式での提示は、関連情報を明確にするのに役立 つ (例として、事例研究 6:クロロホルムばく露に伴う腎臓及び肝臓の腫瘍、Meek ら、2003 年、表 7;本書、ホルムアルデヒドに関する事例研究、McGregor ら、表 3 を参照)。腫瘍が観察された動 物におけるこれらの事象に関する情報とともに、key events (及び重要と思われるいくつかの関連 プロセス) がリストアップされる。これらの表の情報は簡潔にすることが意図されており、表に は説明文が添付されることが予想される。右側の列では、各 key events についてヒトへの影響を 評価している。追加の列として、腫瘍が発生しない別の系統、種、性、または投与経路での結果 についての情報は、腫瘍が発生するモデルとの比較情報が得られる場合に有用である。さらに、 それ自体は key events ではないが、key events を変化させ、種差または個体差に寄与する因子が同 定されることがある。そのような因子には、代謝経路の遺伝的差異、競合する代謝経路及び同時 進行する病態によって誘導される細胞増殖が含まれる。特定されたそのような要因はすべて、一 致表の脚注に記載されるべきである。

ヒトにおけるある化合物の MOA の key events の一致性の評価は、その特定の化学物質の評価と いうよりは、ヒトにおける MOA の評価である。一般的に、初期の key events の詳細は化学物質に 特異的である可能性が高い。例えば、げっ歯類の肝臓におけるフェノバルビタールによる酵素誘 導反応や、特定のチトクローム P-450 酵素によるクロロホルムからの細胞毒性代謝物の生成など である。その後の事象は MOA においてより一般的なものであり、例えば、多面的な肝増殖刺激 や再生性過形成などである。ヒトにおける key events の評価に利用できる情報は、化学物質の in vitro 及び in vivo 試験に由来するもののほか、解剖学、生理学、内分泌学、遺伝学、疫学、その他 ヒトの key events に関する既知の情報などの基本的な情報が含まれる。特定の化学物質に直接ば く露されたヒトにおける key events の評価に関する情報は、入手できないことが多い。

がんの発生・進行に関する知見が蓄積されるにつれて、関与するプロセスの基本的な生物学的 知見に基づいていくつかの MOA を組み合わせることが可能になるかもしれない。それにより、 MOA のヒトへの関連性について結論付ける際に、その化学物質の特異的な情報への依存度が低く なるかもしれない。

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ヒトの情報と動物の情報との一致性を評価する際には、エビデンスの重み付けを説明し、ヒト の情報の信頼度の評価をする必要がある。有用な情報の具体的な種類としては、以下のようなも のがある。

- 1. 対象の解剖学的部位及び細胞の種類におけるがんの発生率:年齢、性別、人種及び化学物質や その他の環境因子などのリスク因子を含む。
- 2. 標的部位の性質と機能に関する知見:生理的、細胞的、生化学的レベルでの発生、構造(肉眼 的、顕微鏡的)、制御機構を含む。
- 3. ヒト及び動物の疾患状態:標的臓器の調節及び応答性に関する洞察を提供する。
- 短期、中期、または長期ばく露後の、対象となる化学物質または類似物質に対するヒト及び動物の反応:標的臓器及び影響を含む。

当然、対象の MOA がヒトに関連していないと結論づけるには、相当量の情報が必要である。 そのような結論がデータによって強く支持されているならば、その MOA によってのみ動物の腫 瘍を誘発される化学物質は、ヒトに対するがんに関するハザードを有さず、このエンドポイント に対するリスク判定を追加で行う必要はない。がんに関するハザードがないため、検討中の腫瘍 に対する発がんリスクはない。

関連性の質問は、すべての集団とライフステージを考慮している。MOA が作用する状況は、例 えばすでにウイルスに感染しているヒト、ホルモンバランスの不均衡のあるヒト、または病を患 っているヒトなど、主に感受性の高い集団またはライフステージで起こる可能性がある。ライフ ステージの早期における発達に関する様々な動態的及び薬力学的側面を考慮して、腫瘍がライフ ステージの早期におけるばく露から発生するかどうかに特別な注意が払われている。感受性の量 的な違いを示唆するいかなる情報も、リスク判定に用いるために特定される。

# 実験動物ととトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、 MOAのとトへの関連性を合理的に排除することができるか

この質問の文言は、IPCS ワークショップにおける質問に対する回答の意味合いについての議論 を受けて、ILSI/RSI HRF に記載されていた文言から変更された。元の質問への回答では、動物 MOA がヒトへの関連性を持たないという結論を出すには、はっきりとした「No」しか許容されていな かった。そのため、質問は Yes/No の回答を可能にするように書き換えられたが、エビデンスの重 み付けの妥当性に関する決定は絶対的なものではなく、利用可能なデータの透明性のある解析に 基づいた科学的判断が必要であるという認識に基づき、「合理的に」という語句が付け加えられた。

ヒトへの関連性解析の目的では、実験動物の MOA がヒトに定性的に関連性があると判断され る場合には、実験動物とヒトの両方から得られるあらゆる動態的及び薬力学的情報を考慮して、 より定量的な評価が必要である。このようなデータは、必然的に化学物質及び MOA に特有のも のであり、腫瘍形成を起こしうる薬力学的反応を引き起こすために必要な生物学的影響を及ぼす 用量を含むものである。動態的考察には、化学物質の取り込み、分布、代謝及び排泄の性質及び 経時的変化が含まれ、一方、薬力学的考察には、化学物質と細胞、組織及び器官との相互作用の 結果が含まれる。 時折、これらの条件を作り出すために必要とされる生物学的に影響を及ぼす用量が、ヒトにおい て不可能であることがある。また、ホルモンのクリアランスなど key events に関与する生物学的 プロセスにおける定量的な違いが大きすぎるため、動物の MOA がヒトには関連しないというこ ともあり得る。しかし、量的な差異に基づいて MOA のヒトへの関連性を否定することが可能に なるのは、ごくまれであると IPCS ワークショップでは認識されている。定性評価と同様に、実験 動物とヒトの定量データを表形式で比較することで、評価を容易にすることができる(例えば、 事例研究 5、フェノバルビタールばく露に伴う甲状腺腫瘍、Meek ら、2003 年、表 6;チアゾピル に関する事例研究、Dellarco ら、本書、表 4 参照)。同様の MOA によって効果を誘導すると考え られている他の化合物の研究から同定された key events との比較も有用である。例えば、チアゾ ピルの場合、ヒトにおけるフェノバルビタールの効果に関する情報は、MOA の関連性を評価する 上で特に有用であった。分子学的及び動態学的アプローチが進化し続けるにつれ、動物及びヒト における反応の類似性及び相違点の理解が向上するであろう。動物モデルとヒトとの間の key events における定性的な違いが、特定の定量的な違いによるものであることが明らかになるかも しれない。その場合、第 2 の問いかけ(上述)への回答を「No」に変更する。

問2と同様に、この問いかけへの結論が「Yes」であれば、その MOA によってのみ動物に腫瘍 を誘発させる化学物質はヒトにがんに関するハザードをもたらさず、このエンドポイントにおけ る追加のリスク判定は必要ない。

#### 信頼性、解析及び帰結の記述

3つの問いかけのそれぞれの総合評価に続いて、解析の基礎となるデータの質と量、フレームワ ーク内での解析の一貫性、データベースの一貫性、一致解析の性質と程度についての信頼性につ いての記述が必要である。また、同等の解析法や厳密な評価を用いた代替的な MOAs の評価も不 可欠である。全体的な MOA と質的・量的一致のエビデンスの重み付けを十分に考慮し、その結 果として、MOA の信頼性を高めることができる将来の研究で実験的に特異的なデータギャップを 特定することが極めて重要である。

まれに、検討中の化合物と共通する MOA を有する化学物質の発がんリスクに関する決定的な 疫学的データ、すなわち、その化合物がヒトに発がん性を有するか否かのデータが存在すること がある。当然、そのようなデータは、ヒトへの関連性評価の結論にかなりの重みを与えるであろ う。しかし、動物では MOA を確立することが可能であるにもかかわらず、ヒトでの key events に 関する情報が不十分であり、ヒトへの関連性について明確な結論を出すことができない場合があ る。そのような場合には、疫学データを利用することで、このデータギャップを埋めることがで きるかもしれない。例えば、構成的活性化受容体(CAR)の活性化を介してフェノバルビタール と同様の作用をして肝腫瘍を誘発する化合物のヒトでの key events に関するデータベースは不完 全である。しかし、比較的高用量のフェノバルビタールに長期間ばく露しても、ヒトでは発がん しないことを示す確固たる疫学的データがある。したがって、1 つの可能性としては、これらのフ ェノバルビタールに関する知見から、げっ歯類に肝腫瘍を誘導する動物 MOA を共有する他の化 合物について「類推」し、そのような化合物によって引き起こされる腫瘍は、ヒトにおける化合 物のリスク評価には関係ないと結論づけることである(Holsapple ら、2006 年)。

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このような結論は、疫学的データの信頼性及び試験対象の化学物質の MOA と疫学的データが利用可能な化合物の MOA との類似性に極めて依存している。

このフレームワークを事例研究に適用すると、現在の研究の多くは、動物 MOA の解析及びヒトとの関連性の解析を容易にするような重要な問いに対処していないことは明らかである。多くの場合、このことは、ここで紹介したような解析フレームワークでのデータの検討に基づいて、主要なデータギャップを透明性をもって記述していないことが原因である。このように、HRF を用いることは試験設計の初期段階から研究者にとって非常に有益である。

統一された形式のヒト関連性解析の結果は、動物のエンドポイントがヒトに関連するかどうか を判断するだけでなく、それ以上に有用な情報を提供してくれる。むしろ、透明性のある解析的 フレームワークの中で関連情報を検討することで、関連する影響のリスク判定における次の段階 で多くの極めて重要な追加情報が提供される。推定される関連影響に関する MOA のヒト関連性 解析に基づくことで、例えば、動物で観察された腫瘍がヒトにおいて発生部位が一致するかどう かを予測することが可能である。また、HRF を適用することで、B型肝炎やアフラトキシン B<sub>1</sub>な どのように、リスクに影響を与える可能性のある関連する調節因子に関する情報が得られること が多い (4- Aminobiphenyl に関する事例研究、Cohen ら、本書を参照)。解析はまた、推定される MOA の構成要素のうち、一定の用量以上でのみ作用する可能性のあるものについての指標を提供 することが多い。MOA に必須なステップを引き起こすために対象の化合物の実験的高用量のばく 露が必要とされる場合、ヒトのリスクとの関連性はばく露の問題となる。このように、その後の リスク判定におけるばく露評価ステップは、ヒトでの発がん性を適切に評価するために重要であ る。さらに、フレームワーク解析で特定された情報は、MOA の key events に基づくハザードの定 量化において非常に貴重なものとなりうる。

重要なことだが、ヒト関連性解析は、リスクが増大している特別な集団(遺伝的素因を持つ集団など)を特定することにも寄与し、しばしば、様々なライフステージにおける相対リスクの検討に関連する情報を提供してくれる。場合によっては、これは化学物質固有の情報に基づくのではなく、特定の年齢層のリスクが増加しているか減少しているかについて、MOAの知識に基づいている場合もある。

フレームワークを用いたデータ及びその解析は、透明性のある方法で報告され、他の者が key events、他の MOA の除外及びヒトとの関連性の解析に関して到達した結論の根拠を判断できるよ うにすべきである。具体的な提示形式は利用可能なデータの種類によって異なるため、情報がど のように報告されるべきかを規定することに意味はない。しかし、到達した結論の透明性を確保 するために、文脈や考察のプロセスに関して十分な詳細情報が含まれていなければならない。実 験動物とヒトにおける key events の比較解析など、特定のデータを提示する際には、適切な表を 使用することが有用である。

## フレームワークの普及

IPCS HRF の普及と適用を支援するために、一般的に受け入れられている MOA と有益な事例の データベースを構築し、維持する必要がある。これは、類似の MOAs で作用する可能性のある化 合物のフレームワーク解析を進めている人が参照できるよう一連の MOAs とそれに関連した key events で構成されている。事例研究は、実際の事例で構成されており、それらは発がん性反応を引 き起こす推定 MOA の受入れを支持するために必要な解析の詳細の関連度とエビデンスの重み付 けの性質を示すために、フレームワークを用いて解析されている。そのような事例研究は、新し い MOA を開発する初期段階では特に貴重であろう。

#### DNA 反応性発がん物質への IPCS HRF の適用

げっ歯類とヒトの発がんプロセスに類似性があり、DNA 反応性発がん物質による DNA との初 期相互作用が類似しているため、一般的に DNA 反応性発がん物質は、エチレンオキサイドの場合 (Meek ら、2003 年)のように ILSI/RSI HRF の「動物の MOA の key events はヒトで起こりうる」 という質問に対し「Yes」のステップに進むと評価され、IPCS HRF における上記質問に相当する 「実験動物とヒトとの間の key evants の根本的、質的な違いに基づいて、MOA のヒトとの関連性 を合理的に排除することができるか」の質問に対し 4-Aminobiphenyl の場合(Cohen ら、本書) 「No」のステップへと進むと評価されると予想される。最近の論文では、Preston & Williams (2005 年)は、DNA 反応性発がん物質に対する ILSI/RSI HRF の使用の指針となる腫瘍発生の key events のセットを提示した。この指針は、ほとんどの DNA 反応性化学物質について、動物 MOA はヒト に関連すると予測されるという見解を支持した。しかし、例外がある可能性があり、ILSI/RSI HRF はこれらを特定するための貴重なツールになるとも指摘された。ILSI/RSI HRF と IPCS HRF を使 用することは、ヒトへのリスクを外挿する際に価値があるであろうげっ歯類とヒトの間の key eventsの違いを定量化するのにも役立つ。国際がん研究機関(IARC)のレビュープロセスで判断 されたように、すべてのげっ歯類における DNA 反応性発がん物質がヒトの発がん物質であると 確立されているわけではない。これらの例外のいくつかについては、発がん性におけるこのヒト-げっ歯類間の違いは、ヒトがその化合物へのばく露量が少ないこと、または低ばく露レベルでの 発がん性を検出する疫学研究の相対的な感度の低さに起因している。しかしながら、このような 違いの理由は他にも生物学的考察に基づくものがある。例えば、DNA 反応性発がん物質が種特異 的な器官でのみ腫瘍を誘発する場合、MOA に関する利用可能なデータを考慮する必要があるが、 この key events に基づく動物 MOA はヒトには関連がない可能性もある。同様に、ヒトではげっ歯 類と比較して一般的に DNA 修復プロセスが発達している (Cortopassi & Wang、1996 年; Hanawalt、 2001 年)、あるいはげっ歯類に特有の生体内活性化の経路があることから、IPCS HRF における「実 験動物とヒトの間の key events の根本的、質的な違いに基づいて、MOA のヒトとの関連性は合理 的に排除できるか」及び/または「実験動物とヒトの間の動態的または薬力学的な要因のいずれ かの量的な違いに基づいて、MOA のヒトとの関連性は合理的に排除できるか」という問いに対し 「Yes」のステップへと進む可能性がある。あるいは、IPCS HRF はこれらのプロセスに関するそ の後のリスク判定の段階で利用可能な定量的な情報を提供しうる。

DNA 反応性発がん物質に IPCS HRF を適用する際に必要なことは、発がん過程を明確に説明する一連の key events を開発し、検討中の DNA 反応性発がん物質に対するげっ歯類における発がん MOA のヒトへの関連性を確立するための指針としてこれらを使用することである。

#### **IPCS HRF** 及びリスク評価

このフレームワークの強みは、柔軟性、あらゆる MOA によって作用する発がん物質への広範 な適用性及び発がん性反応における各 key event の影響を探索する能力といった点である。これに は、用量反応曲線の性質の決定、個々の key event の閾値の存在の特定やその値の特定及びそれら の腫瘍の反応曲線全体への影響の特定が含まれる。さらに、各 key event に関与する動態的及び薬 力学的な因子を考慮することで、特定の集団、例えば、幼若な人々、特定の疾患を持つ人々、ま たは特定の遺伝子多型を持つ人々に対する発がん性反応の関連性の有無に関する結論に到達する ことが可能になるかもしれない。あるいは、フレームワークを適用することで、そのようなグル ープ間の違いに関する定量的な情報を得ることができる。フレームワークの適用はまた、発がん 性反応自体がヒトには関連性がないと結論づけられた場合でも、化学物質のリスク評価に概ね有 益なものになりうる。

冒頭で述べたように、MOA 解析とヒトへの関連性の解析は、リスク評価におけるハザードの特定と特性評価の段階の一側面である(National Research Council、1983 年; Meek ら、2003 年)。このフレームワークに沿って、本文書で言及しているヒトへの関連性に関する事例研究は、完全なものではないが、研究対象の化学物質のリスク評価に貢献する。これは、完全なリスク評価を行うためには、実験的研究や職業衛生研究で設定された範囲の用量を評価するだけでなく、日常生活や生涯の活動における対象化学物質のヒトへのばく露レベルへの外挿を必要とするからである。

ハザードの特性評価及び関連する MOA 解析は、一般的な用語で危害の可能性を扱うのに対し、 完全なリスク評価は、意思決定者に対してこの潜在的なハザードをばく露に関する文脈で説明す るものである。リスク評価は、一般的に低用量レベルで被ばくしているヒトにおける影響の性質 と発生する可能性を理解し、推定するために、これらの影響とヒトへのばく露量との関係を記述 しようとするものである。

用量反応関係を理解することは、ハザードの特性評価に大きな影響を及ぼす可能性があるため、 MOA 解析の重要な要素であり、生物学的に非線形的なプロセスや種間における閾値の差が内在し ている場合には特に重要な要素である。同様に、用量に関連したハザードを定量化することは、 生物学的理解と整合性のある外部モデルを提示することで、リスク評価のプロセスに役立つ。

これらの一般的に低いヒトばく露レベルを推定することは、リスク評価におけるばく露解析の 課題である。これには通常、環境媒体、動植物組織、薬物動態モデルから得られたデータの広範 な解析が含まれる。このプロセスはまた、ヒトの活動パターン、ライフステージや生活様式とい ったばく露をもたらす可能性のある要因の解析にも依存する。 理想は、この情報に基づいて懸念される集団を特定するために、異なる集団(男性、女性、子供、 乳幼児、民族性や職業などに基づく特別な集団)を対象としたそれぞれのばく露量の範囲に関す るシナリオが作成されることである。その大部分がフレームワーク解析に含まれているハザード の特性評価には定量化(用量反応解析)が含まれているが、外部ばく露量の推定及びこれらの推 定ばく露量におけるハザードの説明は、リスク評価に含まれるその次のステップにおいて行われ る。例えば、メラミンの場合(事例研究7、Meek ら、2003年)では、動物の MOA はヒトとの関 連性が高いと結論づけられた。しかし、膀胱がんの形成は非常に高用量でしか起こらないという 認識により、リスク評価、ばく露評価、リスク判定の次の段階へと解析が進められた。リスク評 価全体として、メラミンのヒトへのばく露は膀胱がんの発生に必要なレベルを大きく下回るとい う評価が下された。

## 結論

この IPCS HRF は、2001 年の IPCS MOA フレームワークから得られた経験と 2003 年の ILSI/RSI ヒト発がん性関連フレームワークの検討に基づいて作成された。これらのフレームワークにおけ る概念の多くが採用されているが、明確性を向上させ、これまで考慮されていなかった要素(例 えば、感受性の高い集団)を導入するために、多くの変更が加えられている。ハザードの特性評 価及び全体的なリスク評価/判定のフレームワークにおける解析ツールとしてのフレームワークの 有用性と役割、すなわちヒトへの関連性と用量反応性の外挿に役立つことが強調されている。こ のフレームワークの開発から得られた、多くの要点と結論は以下のとおりである。

- 1. フレームワーク解析に着手する前に、実験動物における発がん性反応のエビデンスの重み付け を慎重に評価する必要がある。
- 2. 専門家の関与及び第三者によるレビューは、新しい MOA が一般に受け入れられるため、また、 科学的な正当性を得るための必須条件である。
- 3. フレームワークは、DNA 反応性を含むすべての発がん MOA に適用できる。
- ほとんどの DNA 反応性発がん物質についてはヒトへの関連性が想定されると思われるが、ヒ ト関連性解析は、理解を深め、ハザードとリスクの特性評価/判定を改善し、例外を特定するた めの重要なアプローチである。
- 5. 新規の MOA を介して作用する可能性のある化学物質を扱う場合、解析はその化学物質に焦点 を当て、HRF を介して詳細な評価を行うことが必要となる。しかし、ある化学物質が他の化学 物質において既に確立され、専門家のピアレビューを経た MOA と一致した腫瘍反応をもたら す場合、解析は確立されている MOA と、その化学物質がその MOA の一連の流れにおいて確 立されている key events と同じ key events を介して発がん作用を生み出すか否かに焦点を当て て行われる。
- 6. 実験動物で発見された腫瘍反応のヒトへの関連性を評価する場合、key events の一致解析は MOA に対するものであり、必ずしも化学物質に焦点を当てた評価ではない。発がんプロセス に関連する化学物質特有の情報や化学物質一般の情報は、解析において貴重なものとなり得る。 知識の進歩に伴い、MOA は化学物質特有のものではなくなり、関連する重要な生物学的プロ セスにより一層依拠することとなり、それにより、ある化合物におけるヒトとの関連性を別の 化合物へと一般化することが可能になるであろう。

- 7. Key events の生物学的理解と生体における重要性は、発がんリスクの用量反応性の外挿性に関する解解析に情報を提供することができる。そのため MOA の理解はハザードとリスクの判定に大きな影響を与えうる。特に非線形性のプロセスや種間の閾値の差が、関連する生物学的性質に内在する場合に顕著である。
- 8. 一般的に受け入れられている MOA と有益な事例研究のデータベースを構築し、維持すること が推奨される。このデータベースは、ILSI/RSI 及び IPCS によって行われた既存の事例研究に 加え、フレームワーク解析の適用において有益な事例を提供するものでなければならない。こ のデータベースは、発がん物質の MOA の研究において知見が蓄積し続ける中で特に重要であ る。
- 9. 解析において、潜在的に感受性の高い集団やさまざまなライフステージを考慮することが重要 である。

結論として、IPCS HRF は、推定される化学物質の発がん MOA をデータが裏付けるかどうかを 判断し、ヒトへの関連性を評価するための厳格で透明性のあるアプローチを提供している。科学 界において発がんリスク評価における作用機序に関する情報の利用を増やす手段として、このア プローチを利用し、フィードバックを行うことが奨励されており、このことが将来的な更なる改 良につながるだろう。このフレームワークは、発がんプロセスの理解を深め、重要なデータギャ ップを特定し、MOA に関連した研究の設計に有益な情報をもたらすという点で、リスク評価と研 究の両方のコミュニティにとって価値がある。発がん反応がヒトに関連する可能性があると考え られる場合、解析中に key events について得られた情報は、その後の化合物のハザードの定量化 において極めて貴重なものとなりうる。このフレームワークは発がん以外のエンドポイントにま で拡張できるようにするべきであり、このことに関する更なる研究が推奨される。したがって、 IPCS HRF の適用は、エンドポイント間の調和のための貴重なツールとなるであろう。

## 謝辞

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# 参考文献

Cohen M, Meek ME, Klaunig JE, Patton DE, Fenner-Crisp PA (2003) The human relevance of information on carcinogenic modes of action: An overview. Critical Reviews in Toxicology, 33:581–589.

Committee on Carcinogenicity (2004) Guidance on a strategy for the risk assessment of chemical carcinogens. London, Department of Health.

Cortopassi GA, Wang E (1996) There is substantial agreement among interspecies estimates of DNA repair activity. Mechanisms of Ageing and Development, 91:211–218.

Hanawalt PC (2001) Revisiting the rodent repairadox. Environmental and Molecular Mutagenesis, 38:89–96.

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP (2006) Mode of action in relevance of rodent livers to human cancer risk. Toxicological Sciences, 89:51–56.

IPCS (2000) Scoping meeting to address the human relevance of animal modes of action in assessing<br/>cancer risk, Carshalton, United Kingdom, 8–10 November 2000. Geneva, World Health Organization,<br/>International Programme on Chemical Safety<br/>(http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2004) Report of the first meeting of the Cancer Working Group, Arlington, Virginia, USA, 3– 5 March 2004. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2005) Record of the Cancer Framework Workshop, Bradford, United Kingdom, 21–23 April 2005. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. Critical Reviews in Toxicology, 33:591–653.

National Research Council (1983) Risk assessment in the federal government. Managing the process. Washington, DC, National Academy Press.

Preston JR, Williams GM (2005) DNA-reactive carcinogens: Mode of action and human cancer hazard. Critical Reviews in Toxicology, 35:673–683.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dosedependent transitions in mechanisms of toxicity: Case studies. Toxicology and Applied Pharmacology, 20:226–294.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regulatory Toxicology and Pharmacology, 34:146–152.

USEPA (1999) Guidelines for carcinogen risk assessment (review draft). Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (NCEA-F-0644).

USEPA (2005) Guidelines for carcinogen risk assessment. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (EPA/639/P-03/001F).

# チアゾピルと甲状腺障害:

# 発がん MOA のヒトへの関連性を解析するための

# IPCS フレームワークを用いた事例研究2

#### Vicki L. Dellarco, Douglas McGregor, Sir Colin Berry, Samuel M. Cohen, & Alan R. Boobis

チアゾピルは雄ラットの甲状腺濾胞細胞腫瘍の発生率を増加させるが、マウスに対して発がん性 はないとされている。チアゾピルに遺伝毒性はない。チアゾピルは、チロキシンの代謝亢進により ホルモンバランスが崩れることにより二次的にラット甲状腺に発がん作用を及ぼす。これらのラ ットにおける腫瘍とヒトの健康への関連性は、2006年の国際化学物質安全性計画ヒト関連性フレ ームワーク(IPCS HRF)を用いて評価された。推定されたげっ歯類における発がん MOA を Bradford Hill 基準に照らして検証したところ、用量と時間の一致、生物学的妥当性、整合性、強度、一貫性、 特異性の条件を満たしており、甲状腺濾胞細胞腫瘍について確立されている MOA に適合している ことがわかった。推定された MOA はヒトでも理論的には作用する可能性はあるが、甲状腺ホルモ ンの不均衡に対するラットの発がん性の感受性がヒトとは量的に顕著に異なることから、チアゾ ピルはヒトに対する発がんに関するハザードを有さないという結論が導き出された。

多くの化学物質が、循環甲状腺ホルモンの減少を介した甲状腺ホルモンのホメオスタシスのか く乱を伴う作用機序(MOA)を介してラットの甲状腺濾胞細胞腫瘍を誘発することが示されてい る(Hurley ら、1998年; Capen ら、1999年; IARC、2001年)。甲状腺ホルモンの低下に対しホメ オスタシスを保とうとする反応は、下垂体からの甲状腺刺激ホルモン(TSH)の放出の代償的な増 加をもたらし、その結果、甲状腺を刺激して甲状腺ホルモンの合成及び放出を増加させる。TSH レベルの持続的な上昇は、甲状腺濾胞細胞の肥大及び過形成へとつながり、(化合物への持続的な ばく露の結果)高いTSH レベルが維持された場合、最終的には腫瘍形成へとつながる。ラットに おけるこの腫瘍形成 MOA は、科学界では十分に受け入れられており、国際がん研究機関(Capen ら、1999年; IARC、2001年)及び米国環境保護庁(USEPA、1998年)は、げっ歯類における甲 状腺濾胞細胞腫瘍のヒトへの関連性を評価するための独自のガイダンスまたは方針を確立してい る。

除草剤であるチアゾピルは、甲状腺ホルモンのホメオスタシスへ影響することによりラット甲 状腺濾胞細胞腫瘍を誘発するが、これは発がん MOA 解析のための 2001 年の国際化学物質安全性 計画(International Programme on Chemical Safety: IPCS)のオリジナルフレームワークを示すため に用いられた事例である(Sonich-Mullin ら、2001 年)。ここではチアゾピルの MOA を、事例研究 としてここで再検討し、2006 年の IPCS ヒト関連性フレームワーク(HRF)で提供されたヒトの 腫瘍形成 MOA 評価のための追加ガイダンスを説明した。この更新された事例研究では、ある特 定の MOA での経験の蓄積が、その後の解析の難易度をどの程度下げられるのかについて強調し ている。この事例研究は、key events が十分に定義されている確立された MOA に基づいているた め、この解析では、チアゾピルがこの経路で期待されている生物学的効果をもたらすか否かに焦

<sup>&</sup>lt;sup>2</sup> この論文は、WHO が著作権を有するものであり、元々は 2006 年に Critical Reviews in Toxicology, Volume 36, pages 793-801 に掲載されたものです。この論文は、WHO の出版物のために編集されており、正誤表が含まれています。

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

点を当てている。この事例研究はまた、動物及びヒトにおける毒性発現の経路の基礎となる生理 学的プロセスを理解することの重要性を強調している。いくつかの化合物では、化学物質特有の データがヒトでの key events を評価する上で重要な場合があるかもしれないが、その他の化合物 については、基礎となる生物学的知見があれば、発がん MOA のヒトへの関連性を定性的にも定 量的にも解釈できる場合がある。チアゾピルは後者の例である。甲状腺ホルモンのかく乱とラッ ト甲状腺濾胞細胞腫瘍のヒトへの関連性に関する他の MOA 事例研究としてフェノバルビタール についての事例研究がある(Lehman-McKeeman & Hill, in Meek ら、2003 年)。

本 MOA 解析は、チアゾピルの発がん性に関する利用可能な情報の簡単な要約から始まり、続いて、この甲状腺ホルモンかく乱 MOA で考察された生化学的及び病理組織学的な実験データの 議論が行われる。本解析は化学物質そのものの包括的な評価を意図したものではない。

## 発がん性データ

チアゾピルの発がん性に関するヒトの疫学的データは得られていない。チアゾピルは、マウス、 ラット及びイヌを含む様々な実験動物種において、肝臓及び甲状腺に影響を及ぼす。チアゾピル は雄ラットにおいてのみ甲状腺腫瘍を誘発することが確認されており、甲状腺ホルモンの肝代謝 及び排泄を増加させることによってこれを引き起こすようである。

チアゾピルはマウス及びラットへの慢性的な混餌投与により、主に雄ラットに甲状腺濾胞細胞 腫瘍を誘発したが、雌ラットでは誘発しなかった(Naylor & McDonald、1992 年; Naylor & Raju、 1992 年)。チアゾピルを最大 800 mg/kg まで混餌投与したマウスの慢性試験(雄では 1 日あたり 128.4 mg/kg 体重[bw]、雌では 1 日あたり 215.9 mg/kg bw)では、いずれの性においても腫瘍の発 生率に有意な増加は認められなかった(Naylor & Raju、1992 年)。ラット発がん性試験では、チア ゾピル(原体、純度 94.8%)を雄及び雌の Sprague-Dawley ラット(SD ラット)(各群雌雄 60 匹ず つ)に 0、1、10、100、1000 または 3000 mg/kg の濃度で混餌投与し、雄では 1 日あたり 0、0.04、 0.4、4.4、44.2、136.4 mg/kg bw、雌では 1 日あたり 0、0.06、0.6、5.6、56.3 または 177.1 mg/kg bw の用量を与えた(Naylor & McDonald、1992)。甲状腺濾胞細胞腺腫及びがんの発生率は、1000 mg/kg (1 日あたり 44.2 mg/kg bw)及び 3000 mg/kg(1 日あたり 136.4 mg/kg bw)投与群の雄ラットで増 加した(表 1)。なお、雄ラットにおける腫瘍発生率の増加は主に良性腫瘍によるものであったこ とに留意すべきである。

## ラットにおける甲状腺濾胞細胞腫瘍の誘発において推定される MOA

チアゾビル誘発性甲状腺濾胞細胞腫瘍において推定される MOA は、甲状腺外における機序に よる下垂体-甲状腺軸のホメオスタシスの乱れを含むものであった。具体的な流れを以下に述べる。 チアゾビルは肝臓におけるチロキシン(T4)-ウリジン二リン酸(UDP)グルクロン酸転移酵素(UGT) 活性を誘導し、抱合による T4 の代謝を促進し、ホルモンの抱合体の胆汁排泄を増加させる。この 肝臓代謝の促進の結果として、血清 T4 及び時にはトリヨードサイロニン(T3)の半減期が減少す る。下垂体は、TSH の放出及び血清レベルを高めることにより、T4 の循環血清レベルの低下に対 応する。循環 TSH レベルの長期的な上昇は、甲状腺を刺激して甲状腺ホルモンの貯蔵を枯渇させ、 ホルモン産生を誘導し続ける。 その結果、甲状腺濾胞細胞は大きくなり(肥大)、高い増殖率で細胞数を増やす(過形成)ように 誘導される。慢性的なチアゾピルのばく露により、甲状腺過形成は最終的には腫瘍へと進行する。

吃大 /						
		Dose $(mg/kg bw per day)^a$				
	0	0.04	0.4	4.4	44.2	136.4 <sup>b</sup>
腺腫	1/50	2/47	0/49	2/47	8/49	12/48
がん腫	1/50	1/47	0/49	0/47	1/49	4/48
合計	2/50	3/47	0/49	2/47	9/49	14/48
%	(2)	(6)	(0)	(4)	(18)	(29)
Р	$0.000^{c}$	0.470	0.253	0.668	0.024*	0.001**

# 表 1. Sprague-Dawley 雄ラットにおける甲状腺濾胞細胞腫瘍の発生率(2年間の慢性試験)

注:腫瘍の発生率は、USEPA Office of Pesticide Programs (Naylor & McDonald、1992 年)に提出されたデータから 抽出した:有意差 \* P < 0.05; \*\* P < 0.01 (フィッシャーの正確確率検定に基づく統計解析) \*1日あたりの投与量を mg/kg bw で推定した。

\*1 日めにりの投与重を mg/kg bw で推進した。 <sup>b</sup> 136.4mg/kg bw/日(3000mg/kg)混餌投与群の2匹の動物には、良性腫瘍と悪性腫瘍の両方が認められた。

・投与量に従ってP値が低下した。

# 実験動物における key events

チアゾピルの発がん MOA における一連の key events には、以下のものが含まれる。

- 肝 UGT 活性の誘導
- T4の肝臓での代謝及び胆汁排泄の増加
- 血清 T4 の半減期及び濃度の低下
- 循環 TSH 濃度の上昇
- 甲状腺濾胞細胞肥大及び濾胞細胞過形成

次に、チアゾピルが、循環甲状腺ホルモンの肝クリアランスを増加させて甲状腺-下垂体軸を乱 すことによって作用するかどうかを評価する。上記の key events に基づき、チアゾピルの MOA の 生物学的指標は、肝臓代謝の変化、ホルモンレベルの変化、甲状腺の成長の増加及び甲状腺の病 変の進行が挙げられることになる。これらの影響は、短期及び亜慢性試験さらに慢性試験におけ る中間及び計画殺において、雄ラットで観察され、測定されている(Hotz ら、1997 年)。Key events 及び腫瘍反応の用量反応性を以下に示す。

# 用量反応関係及び一致性

チアゾピルの MOA における主要な影響について、無毒性量(NOAEL)及び最小毒性量(LOAEL) の概要を表2に示す。Hotzら(1997)による56日間の試験では、雄SD ラット(各群20匹)に、 チアゾピルを0、10、30、100、300、1000、または3000 mg/kgの濃度で(測定されていないが、 チアゾピルの摂取量は1日あたり0、0.5、1.5、5、15、50及び150 mg/kg bwと推定される)を56 日間混餌投与し、肝臓(重量、肝T4-UGT活性、T4胆汁排泄)、甲状腺(重量、肥大/過形成)及 びホルモン(T4、T3、リバースT3(rT3)及びTSHの血清レベル)に対する影響を評価した。こ の試験では、チアゾピルの主要作用部位である肝臓への影響が、下垂体-甲状腺のホメオスタシス のかく乱の最も敏感な指標であると考えられる。50 mg/kg bw/day 投与群及び150 mg/kg bw/day 投

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与群では、肝 T4-UGT 活性が 56 日間の投与期間終了時に統計学的に有意に増加した(肝重量で標準化したところ、対照群と比較してそれぞれ約3倍と6倍に活性が増加した)。T4-UGT 活性の増加と一致して、血中からの T4 のクリアランス及び胆汁中への排泄(125I 標識 T4 の排泄量)は、 チアゾピルを150 mg/kg bw/day 投与後に40%増加した(150 mg/kg bw/day が評価された唯一の投 与量)。Hotz ら(1997年)による雄ラットを対象とした56日間の試験では、チアゾピルを1日あたり15、50及び150 mg/kg bw 投与した場合に、肝重量の統計学的に有意な増加が認められた。 ラット2年間の試験(Naylor & McDonald、1992年)では、絶対肝重量は対照群と比較して、44.2 mg/kg bw/day 投与群で122%、136.4 mg/kg bw/day 投与群で肝肥大の発生率が統計的に有意に増加した (対照群の0/60に対してそれぞれ47/61及び52/60)。

影響	NOAEL/LOAEL
肝臓	
UGT の誘導	15/50 mg/kg bw/day (56 日間試験)
T4 の胆汁排泄量の増加	<150/150 mg/kg bw/day (56 日間試験において 150 mg/kg bw/day のみ
	実施された)
肝臓重量の増加	5/15 mg/kg bw/day (56 日間試験)
	44.2/136.4 mg/kg bw/day (2 年間試験)
肝細胞肥大	4.4/44.2 mg/kg bw/day (2 年間試験)
ホルモン	
血清 T4 の減少	50/150 mg/kg bw/day (56 日間試験)
血清 TSH の上昇	50/150 mg/kg bw/day (56 日間試験)
甲状腺	
甲状腺重量の増加	15/50 mg/kg bw/day (56 日間試験)
	44.2/136.4 mg/kg bw/day (2 年間試験)
甲状腺過形成の増加	44.2/136.4 mg/kg bw/day (2 年間試験)
甲状腺腫瘍の増加	4.4/44.2 mg/kg bw/day (2 年間試験)

# 表 2. 雄ラットを対象とした 56 日間の試験(Hotz ら、1997)及び 2 年間の慢性試験(Naylor & McDonald、1992)から得られた肝臓、ホルモン及び甲状腺に対する影響の概要

Hotz ら(1997) が雄 SD ラットにチアゾピルを投与したとき、上述した T4 の肝クリアランスの 亢進と同様に、最高用量の投与 56 日後に血清 T4 濃度の統計学的に有意な (P $\leq 0.05$ ) 減少 (30%減 少)と TSH の増加(60%増加)が認められた(表 3)。T3 の血清レベルは、1.5 mg/kg bw/day では 56 日投与後に統計学的に有意ではないものの低下し、150 mg/kg bw/day では有意に上昇した。一 般に、肝ミクロソーム酵素誘導剤による影響は T4 よりも T3 に影響を与えにくいようである。し たがって、T4 及び TSH は、下垂体-甲状腺のホメオスタシスの変化の指標としてはより信頼性の 高い傾向がある (Liu ら、1995 年、Hurley ら、1998 年、Hood ら、1999 年)。

チアゾピルの場合、T4 及び TSH に影響を及ぼす用量と、甲状腺濾胞細胞腫瘍の発生率の増加を 引き起こす用量との間には、相関関係が乏しいようである。雄 SD ラットにおいて甲状腺濾胞細 胞腫瘍の統計学的に有意(P<0.05)な増加をもたらしたチアゾピルの最低用量は、2年間の試験で は1日当たり44.2 mg/kg bw であったが、T4 及び TSH に対する影響の NOAEL は56 日間の試験 では1日当たり50mg/kg bwであった(表2)。一般的に、肝酵素/肝重量及び下垂体-甲状腺ホル モン濃度への影響は、この甲状腺ホルモンかく乱 MOA が閾値を有する現象であることを考える と、少なくとも甲状腺の重量変化及び甲状腺腫瘍発生率の増加をもたらす用量と同程度の低用量 で起こると予想される。引用された投与量のどちらも正確ではないので、この見かけの不一致は おそらく真ではない。2年間の試験における体重1kg当たりの投与量は、試験期間全体の平均的 な推定値であったのに対し、比較に用いられた 56 日間のメカニズム試験での投与量は、12~20 週 齢のラットにおけるものである。これらの投与量は、容易に算出可能な投与量よりも少なくとも 2 倍高かったと考えられる(したがって、腫瘍に対する実際の LOAEL は1日あたり約 90 mg/kg bw であったはずである)。また、ホルモンのかく乱(例えば TSH の長期上昇)が病理学的変化を もたらし始める臨界期は、2年間の試験において後期ではなく早期であったため、これらの投与 量は腫瘍に対する関連性も高かったと考えられる。56日間の試験で計算された投与量は公表され た論文からは摂餌量の情報が入手できなかったため、摂餌量の推定値に基づいて計算された。そ のためこれについても不正確である可能性が高い。この不確実性を認識した上で、1日あたり 50 mg/kg bw で甲状腺重量が有意に増加し、1日あたり15 mg/kg bw で肝臓重量の増加が認められた が、これはチアゾピルの MOA では最初のターゲットが肝臓であることと一致している。

	Dose (mg/kg bw per day) <sup>a</sup>						
	0	0.5	1.5	5	15	50	150
T4 (μg/dL)	$4.1\pm0.2$	$4.3\pm0.3$	$3.9\pm 0.2$	$4.1\pm0.2$	$4.0\pm0.2$	$4.0\pm0.2$	$2.9\pm0.1^a$
T3 (ng/dL)	$84\pm3$	$82\pm4$	$68 \pm 2$	$84\pm3$	$82\pm3$	$91\pm 4$	$110 \pm 6^a$
TSH (ng/mL)	$2.7\pm0.2$	$3.5\pm 0.4$	$2.7\pm0.1$	$3.1\pm 0.4$	$2.9\pm0.3$	$3.1\pm 0.2$	$4.3\pm0.4^a$

表 3. 雄ラットにおける 56 日間の試験:ホルモン作用(Hotz ら、1997 年)

注: 投与量 mg/kg bw/day は推定値である。値は平均値±標準誤差で表している;1 群あたり19 または20 匹。 <sup>a</sup> 分散分析 (ANOVA) 後の Dunnett の検定で対照群と統計学的に有意に異なる (*P*≦0.05)。

上述のように、長期の TSH 刺激は甲状腺の肥大と過形成の両方を引き起こす。ラットの2年間の試験では、甲状腺肥大のみと腫瘍発生率との間には用量相関性は見られなかった。腫瘍発生率は44.2 mg/kg bw/day 投与群で増加したが、過形成の発生率の統計学的に有意な増加(対照群 1/60 に対し 8/58)は136.4 mg/kg bw/day 投与群でのみ認められた。さらに、ラットの56日間の試験では、組織学的に甲状腺濾胞細胞肥大と過形成が併発しており、150 mg/kg bw/day 投与群での発生率は統計学的に有意に増加したが、それ以下の用量では増加しなかったと報告されている(Hotz ら、1997年)。しかし、56日間の試験での甲状腺重量の増加と2年間の試験での腫瘍発生率との間には良好な用量相関性があった。150 mg/kg bw/day 投与群で 46%、50 mg/kg bw/day 投与群で 25%の統計学的に有意な甲状腺重量の増加が認められた(Hotz ら、1997年)。

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## 時間的関連性

ある事象が腫瘍形成の必須要素である場合、それは腫瘍の発生に先行して発現しなければなら ない。雄 SD ラットに 3000 mg/kg (1日あたり 150 mg/kg bw)のチアゾピルを混餌投与した7、14、 28、56 及び 90 日後といった複数のタイミングにおけるデータが利用可能である(Hotz ら、1997 年)。肝重量増加及び肝 T4-UGT 活性上昇は、最も早く測定した7日目から全ての測定時期におい て認められた。この実験では抱合化 T4 の胆汁排泄は測定されなかったが、血清 T4 はすべての観 察時期において減少した。循環 TSH の増加は、投与開始後 14 日目においては有意ではなかった が、すべてのサンプリング時期で観察された。甲状腺重量の増加もすべてのサンプリング時期で 観察された。組織学的には、14 日目から肥大/過形成の経時的な増加が認められた。2 年間のラッ ト試験では、1 日あたり 136.4 mg/kg bw を投与した 69 週目に最初の甲状腺腺腫が観察された。こ のように、チアゾピル誘発性甲状腺濾胞細胞腫瘍形成においては、すべての key events が腫瘍形 成に先行するという論理的な経時的反応が認められた。

# 腫瘍反応と key events との関連性の強さ、一貫性及び特異性

この関連性の強さ、一貫性、特異性は、上述の試験から確立できる。推定される MOA の基礎と なる定量可能な前駆事象は、甲状腺濾胞細胞腫瘍の発現と比較的一致している。チアゾピルを混 餌投与されたラットにおいて、肝重量の増加及び肝 T4-UGT の誘導が観察されることから、甲状 腺外の機序による下垂体-甲状腺軸のホメオスタシスのかく乱と一致しているようである。肝 T4-UGT 活性の上昇は、他の重要な生化学的変化や甲状腺濾胞細胞の肥大及び過形成の前に起こる段 階である。チアゾピルの投与は肝臓における T4 の代謝が促進されるため、循環 T4 の減少及び TSH の増加が明らかである。さらに、亜慢性試験では、甲状腺重量の増加及び肥大・過形成の発 生は、甲状腺ホルモン濃度及び甲状腺ホルモン代謝の変化の出現や回復と同じ用量及び時間の条 件下で統計学的に有意な程度で現れることが示された。中止/回復試験(Hotz ら、1997 年)では、 チアゾピルの投与中止後、ホルモン値がコントロール値に戻り、肝臓と甲状腺の重量が減少し、 甲状腺濾胞細胞の過形成が回復することが示された。早期に投与を中止することで、この非遺伝 毒性 MOA における甲状腺ホルモンの低下と病変の進行が回復することが期待された。唯一回復 が遅くなったのは、最も長い投与期間の後の甲状腺重量の増加であった。

# 生物学的妥当性及び整合性

実験用のげっ歯類を用いた研究から、視床下部-下垂体-甲状腺軸の持続的なかく乱、TSH による甲状腺への長期的な刺激及び甲状腺濾胞細胞の肥大、過形成、最終的な腫瘍への進行との関係を示すデータが数多く存在する(McClain、1995年; Hard、1998年; Hurley ら、1998年; Capen ら、1999年; IARC、2001年)。チアゾピルの場合と同様に、TSH の分泌増加は、T4 の肝クリアランスの増加を含むいくつかのメカニズムを介して生じる可能性がある。

T4の循環レベルは、TSH の合成を担う下垂体の甲状腺刺激細胞によってモニターされている。 下垂体では、T4 は、5-dried-deio-dinase (ヨウ素ペルオキシダーゼ) II 型により T3 に代謝され、T3 は細胞核内の特定の受容体に結合する。T3 受容体の占有率が低下すると、TSH 合成と分泌が刺激 される。ラットへの TSH の注射が、非増殖細胞特異的核抗原である甲状腺濾胞細胞核スタチンが 減少し、これらの細胞が非分裂状態を脱して細胞周期を再開していることが生体内での研究で示 されている (Bayer ら、1992 年)。この試験では、低用量の TSH を反復投与(ラット1 匹あたり 0.25 IU を1日2回) すると、核内スタチンレベルが 10 日間にわたって累積反応が生じ、TSH の 注射を中止してから5 日以内に正常な安静時のレベルに戻ることが示された。核内スタチンの減 少もまた、TSH に対する最も早い反応として知られているピノサイトーシスと類似した初期の事 象である。これらのデータは、TSH 濃度の増加だけでラットの甲状腺濾胞細胞を増殖前の状態に なることと矛盾しない。したがって、チアゾピルが最初に肝 T4-UGT を誘導することでラットの 甲状腺濾胞細胞腫瘍を引き起こすという指摘は、少なくとも肥大と過形成の段階までは視床下部-下垂体-甲状腺の動的な制御システムに関する既知の生理学的知見と一致している。

最後に、チアゾピルにより誘発される腫瘍反応はげっ歯類の甲状腺発がん物質の典型的なもの であり、甲状腺濾胞細胞腫瘍は雄ラットにはみられるが、雌のラットやマウスには認められない。 ラットはマウスよりも甲状腺発がん物質に対し感受性が高い傾向があり、さらに、雄ラットは雌 ラットよりも甲状腺腫瘍を誘発する化学物質の割合が大きいことがよくみられている (Hurley ら、 1998 年)。このことと一致して、TSH レベルは通常雄ラットの方が雌よりも高い (Hill ら、1989 年)。さらに、雄ラットは時に同系統の雌よりも肝酵素が誘導されやすいことがあるが、これは酵 素の種類、酵素誘導を起こす化合物の用量や動物の年齢に依存する (Sundseth & Waxman、1992 年; Agrawal & Shapiro、1996 年; Oropeza-Hernandez ら、2003 年)。

# その他の MOA

変異原性は常に考慮すべき可能性のある MOA の 1 つであるが、以下の試験においてチアゾピルの遺伝毒性は示されていない。

- ネズミチフス菌 (Salmonella typhimurium) の4つの菌株を用いた突然変異試験 (Bakke、1989 年 a)。
- チャイニーズハムスター卵巣細胞を用いたの突然変異試験(hgpt 座位)(Li & Myers、1989年)。
- マウスの骨髄細胞を用いた In vivo 小核試験(Flowers、1990年)。
- ラットの肝細胞を用いた In vivo 不定期 DNA 合成試験(Backke、1989 年 b)。

したがって、利用可能なエビデンスは、突然変異誘発がチアゾピルの代替 MOA ではないこと を示している。

その他の視床下部-下垂体-甲状腺軸への影響及び甲状腺ホルモン代謝における他の経路のかく 乱は、甲状腺のホメオスタシスを変化させる可能性がある。しかし、これらの変化は、チアゾピ ルについて推定されているものと根本的には変わらず、すべて継続的なばく露による TSH 刺激の 長期化につながっているであろう。

# 不確実性、矛盾、データギャップ

甲状腺腫瘍とホルモンの変化には用量の一致がみられないが、これは比較に用いられた体重 1 kg 当たりのミリグラム投与量(摂餌量と体重値から算出された)が不正確であること、比較した 投与量が一方は若齢期のラットにおける平均値であり、もう一方が試験全体の平均値であったこ と、また、試験間のばらつきなどに起因している可能性がある。

# 推定される MOA の評価

提示されたデータは、中程度に高い信頼度で、チアゾピルの混餌投与による慢性ばく露後の雄 ラットにおける甲状腺濾胞細胞腫瘍の発生を説明するのに十分であると判断される。チアゾピル は、腫瘍を引き起こす用量よりも低い用量で明らかに肝臓重量を増加させ(すなわち、肝臓が最 初の標的臓器である)、腫瘍を誘発させる中で最も低い用量で甲状腺の成長(すなわち、甲状腺重 量の増加)を促進させた。

## 推定される MOA のヒトへの適用可能性

IPCS HRF はリスクサイエンス研究所/国際生命科学研究機構の「ヒト関連性フレームワーク」 (Meek ら、2003 年)をもとに開発され、「IPCS がんワーキンググループ」の議論をもとに修正さ れたものであり(Boobis ら,本書)、一連の3つの問いかけに対処し、動物の腫瘍における MOA のヒトへの関連性に関する文書化された論理的な結論を導くための4つの部分からなるアプロー チを提示している。

1. 動物 MOA を確立するのにエビデンスの重み付けは十分か?上で詳述したように、チアゾピルは UGT 誘導により血清 T4 レベルを低下させ、その結果として血清 TSH を上昇させることで、 甲状腺ホルモンのホメオスタシスを変化させるという明確なエビデンスがある。

実験動物とヒトとの間のkey events の根本的、質的な違いに基づいて、MOA のヒトとの関連 2. <u>性を合理的に排除することができるか?ヒトにおける甲状腺ホルモンのホメオスタシスの調節に</u> 関する現在の理解と、(甲状腺ホルモンのホメオスタシスの変化の結果としての) TSH レベルの上 昇が甲状腺がんのリスク因子として果たす役割について、チアゾピルの動物における発がん MOA の key events のヒトへの関連性を評価するために検討がなされた。ラットにおける視床下部-下垂 体-甲状腺軸の機能と調節に関与する基本的なメカニズムは、量的な薬力学的差異(後述)はある が、定性的にはヒトのそれと類似している(Bianco ら、2002 年)。したがって、ラットで T4 レ ベルを低下させる薬剤は、ヒトでも同様に T4 を低下させる可能性があり、その結果、TSH レベル の上昇につながる可能性がある。げっ歯類とヒトは、下垂体-甲状腺機能のかく乱に対して同様の 反応を示すデータがある。例えば、甲状腺ホルモンのレベルを容易に低下させるヨウ素欠乏症は、 ヒトでは甲状腺細胞の増殖を刺激して甲状腺腫を引き起こすことが知られている。ヨウ素欠乏症 を放置すると、まれではあるが腫瘍形成につながることがある(Thomas & Williams、1999 年)。甲 状腺がんになりやすいというエビデンスはないが、甲状腺に直接作用して(例えば、ホルモン合 成や放出を阻害する、T4からT3への変換を阻害する)甲状腺ホルモンのホメオスタシスを乱す 多くの医薬品(プロピルチオウラシル、リチウム、アミオダロン、イオパノ酸など)は、ヒトでは 甲状腺ホルモンの低下及び TSH の上昇につながることが知られている(Ron ら、1987 年)。

ラットとは対照的に、ヒトでは、肝ミクロソーム酵素が誘導され、循環 T4 レベルを低下させる 薬剤にばく露されても TSH レベルの上昇は認められていない(Lehman-McKeeman & Hill, in Meek ら、2003 年)。例えば、医薬品のフェニトイン、リファンピン、カルバマゼピンは、UGT を含む 肝ミクロソーム酵素を誘導し、血中の T4 レベルを低下させるが、TSH レベルは変化しない(Curran & DeGroot、1991 年)。同様に、実験的に高用量で T4 のグルクロン酸抱合及び胆汁排泄を増加さ せることによりラットに甲状腺腫瘍を発生させる薬剤(例えば、オメプラゾール、ランソプラゾ ール、パントプラゾール)は、ヒトにおいて臨床用量では甲状腺ホルモンに変化をもたらさない (Masu-buchi ら、1997 年)。このように、ラットにおける下垂体-甲状腺軸のホメオスタシスの変 化の用量反応関係には、ヒトと比較してかなりの違いがあるように思われる。後述するように、 この現象は、ラットとヒトでは下垂体-甲状腺機能の基礎となる生理学的プロセスの量的な薬力学 的差異に起因している。

3. 実験動物とヒトとの間の薬力学的または動態的要因のいずれかの量的差異に基づいて、MOA のヒトへの関連性を合理的に排除することができるか?チアゾピルは甲状腺を直接標的とするものではない。むしろ、その主な作用は肝代謝酵素に対するものであり、代謝活性の亢進は間接的にT4の全身クリアランスを増加させ、ラットでみられるような甲状腺機能低下状態とTSHの代償性の増加をもたらす。チアゾピルがヒトにおける甲状腺ホルモンの恒常性を乱す可能性については、チアゾピル特有のデータはないが、フェノバルビタール(Lehman-McKeeman & Hill, in Meekら、2003 年)のような他の多くのミクロソーム酵素誘導剤で広く研究されている。上述したように、T4の肝クリアランスを変化させることで甲状腺機能減退症下を引き起こす薬剤は、ヒトではTSHレベルの上昇をもたらさないようである。おそらく、T4の減少が閾値まで達していないので、TSH は上昇しないのではないかと考えられる。

甲状腺機能に関連して、ラットとヒトの間にはいくつかの重要な生理学的及び生化学的相違が ある。ラットはヒトに比べて甲状腺ホルモンの予備容量が小さい。ラットはヒトに比べて甲状腺 ホルモンの半減期が非常に短く、T4の半減期は、ヒトの5~9日に対し、ラットでは約12時間で ある(Dohler ら、1979年)。ラットの半減期が短いのは、ヒトに存在するT4の高親和性結合グロ ブリンが存在しないことに関係していると考えられる(Hill ら、1989年)。ラットでは、クリアラ ンスの増加によって正常なT4レベルを維持するために、より高い体重あたりのT4産生率を必要 とする。対照的に、ヒトではこのグロブリンへ甲状腺ホルモンが結合することによって、ラット と比較して代謝的分解及びクリアランスが遅く、その結果、甲状腺はラットよりも活性が低い。 恒常的なTSHレベルは、ラットではヒトよりも約25倍高く、ラットでは下垂体-甲状腺軸の活性 が高いことを反映している(Dohler ら、1979年; McClain、1992年)。したがって、ヒトは、T4を 減少させTSHの上昇をもたらす薬剤に対して、ラットよりも定量的に感受性が低い。TSH が上昇 していなければ、甲状腺腫瘍発生のリスクが高まることはない。

ヒトとの比較においてラットのもう一つの違いは、甲状腺の組織像にある。この組織学的な相 違は、ラットでは安定した一定の血清 T4 濃度を維持するための T4 のより高い産生速度と関連し ており、これによりラットの甲状腺は、人間を含む霊長類のそれよりも"機能的に活性化"した状 態となっている(McClain、1995 年)。

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ラットの濾胞上皮の多くは、刺激を受けてサイログロブリンを合成するため、濾胞細胞の多くは 背の高い立方体状をしており、活発にホルモン合成しているように見える。対照的に、ヒトの濾 胞細胞の多くは、短い立方体状またはほぼ扁平な外観を呈し、静止状態のように見える傾向があ る。ラットの濾胞細胞はすでに全体的に活性化しているので、TSHによる刺激を受けると、ヒト の濾胞細胞よりも容易に反応し過形成を生じる。ヒトの甲状腺は貯蔵能力が高く、休止状態にあ る細胞の数が多い。そのため、ヒトの甲状腺濾胞細胞は恒常性を回復するための過形成反応を必 要とせずに、休止状態から覚醒して追加の甲状腺ホルモンを合成・分泌する。したがって、ヒト 甲状腺における主要な反応は、過形成ではなくサイログロブリンの再吸収と細胞の肥大であろう。 要するに、ラット甲状腺よりもヒト甲状腺の方が、生化学的な面においてははるかに大きな緩衝 能力を持っているということである。

ある薬剤がヒトにおいて甲状腺ホルモンレベルの低下を引き起こす可能性があるとしても、こ れらの薬剤が甲状腺がんの感受性を高めるという明確なエビデンスはない(Ron ら、1987 年)。例 えば、フェノバルビタールを用いた疫学研究では、甲状腺がんのリスクの増加は示されていない (Olsen ら、1993 年)。TSH が上昇するような状態にある人々(バセドウ病または甲状腺腫の患者) を対象とする研究は、このような状況においても甲状腺がんの発生はまれであることを示してい る (例えば、Mazzaferri、2000 年;Gabriele ら、2003 年)。全国規模のスウェーデンの家族がんデ ータベースを用いた 960 万人のがんの環境的及び遺伝的原因を調査した結果、環境が甲状腺がん の主な原因となる役割を果たしているようには見えないことが明らかにされた(Lichtenstein & Hemminki、2002 年)。唯一知られているヒト甲状腺の発がん物質は放射線であり、すなわち変異 原性物質のばく露である。

表4に要約されているように、甲状腺機能の生化学的及び生理学的差異に関する一般的な文献 には、ラットとヒトの間で腫瘍に対する感受性に違いがあることを示す十分なエビデンスがある。 ヒトとは対照的に、ラットは甲状腺ホルモンの低下症に続発する甲状腺腫瘍に対して非常に感受 性が高い。特に、甲状腺ホルモンの恒常性にわずかでも変化があるとラットでは腫瘍形成が促進 される。したがって、げっ歯類におけるホルモンの肝クリアランスの増加と下垂体-甲状腺軸のホ メオスタシスの変化を伴うチアゾピルによって誘発される甲状腺腫瘍は、量的な動態の違いから ヒトへの関連性はないと考えられる。

4. 結論:信頼性、解析及び帰結の記述。ラットにおけるチアゾピル誘発性甲状腺濾胞細胞腫瘍に関 して、甲状腺ホルモンかく乱 MOA を確立するのに十分な実験的エビデンスが存在する。チアゾピル はヒトにおいて甲状腺機能減退症を引き起こす可能性があるが、一般的な文献には、げっ歯類におけ る甲状腺ホルモンの肝クリアランスの増加と下垂体-甲状腺軸のホメオスタシスの変化を伴うプロセ スによって誘発される甲状腺腫瘍は、ヒトの腫瘍発生に対する感受性の増加にはつながらないと結論 づけるのに十分な基本的な生理学的プロセスに関する定量的エビデンスがある。チアゾピルに関する ヒトでのデータはないが、他の肝ミクロソーム酵素誘導剤に関する臨床データは、チアゾピルのヒト への関連性の解析には不可欠であった。一般的な文献からは、ラットとは異なりヒトでは T4 レベルの 低下が代償的に TSH レベルを上昇させることを示すエビデンスはないことを示すのに十分なエビデ ンスが得られた。また、ラットの下垂体-甲状腺軸がこのようなホルモンのかく乱に対してヒトよりも はるかに感受性が高いという細胞学的及び生化学的エビデンスもある。 この感受性の高さは、甲状腺の活動を維持するための TSH の需要が高いことと相まって、ラット における T4 の急速なターンオーバーが早いことに起因すると思われる。

Key events	ラットにおけるエビデンス	ヒトにおけるエビデンス
T4 の肝クリアラ	ラットの短期及び慢性試験で、肝臓が	チアゾピルについてのデータはない
ンスの増加	最も感受性の高い標的であることが	が、ミクロソーム酵素の誘導が起こっ
	判明しており、T4 肝 UGT 活性、T4 半	ていると思われる。
	減期、T4 胆汁排泄、肝重量及び肥大に	
	関する研究により、T4 肝クリアランス	
	の増加のエビデンスが得られている。	
血清 T4 の減少	直接的な実験的エビデンス。	チアゾピルのデータはないが、他のミ
		クロソーム酵素誘導剤がヒトで T4 を
		減少させることが示されていること
		を考えると、チアゾピルについてもそ
		うであると思われる。
TSH レベルの上昇	直接的な実験的エビデンス。	チアゾピルのデータはないが、他のミ
		クロソーム酵素誘導剤において T4 が
		減少しても TSH レベルが上昇するこ
		とは示されていない。
TSH の増加は甲状	直接的な実験的エビデンス。	甲状腺ホルモンの低下に続発する甲
腺細胞の増殖と腫		状腺濾胞細胞腫瘍の誘発は、甲状腺機
瘍形成を促進させ		能/ホメオスタシスの齧歯類との量的
る。		な違いを考えると、ヒトでは起こりそ
		うにない。
		甲状腺ホルモンレベルがかなり低い
		ヒトにおいても甲状腺がんの発生は
		まれである。

表 4. ラットとヒトにおける key events の比較

## IPCS HRF の結果

チアゾピルの例は、過去に明確にされ、確立された MOA と一致する誘発された腫瘍反応について示したものである。したがって、フレームワーク解析の最初の質問である「動物 MOA を確立するのにエビデンスの重み付けは十分か」に対応することは、その化学物質に関するデータセットが対象となる経路に対して定義にされた同じ key events に適合しているか否かを判断する必要があった。この例は、MOA に関与する生物学的プロセスの基本的な理解に関するデータが、どのようにげっ歯類とヒトの key events を比較するための重要な手段となるかを示している。このように、実験動物とヒトの間の質的及び量的な差異を評価し、ヒトに対する発がん MOA の妥当性(すなわち、HRF の問 2 と 3)評価するためには、このヒトに関する一般的な情報は、不可欠であった。

# 参考文献

Agrawal AK, Shapiro BH (1996) Phenobarbital induction of hepatic CYP2B1 and CYP2B2: Pretranscriptional and post-transcriptional effects of gender, adult age, and phenobarbital dose. *Molecular Pharmacology*, **49**(3):523–531.

Bakke JP (1989a) *Ames/*Salmonella *mutagenicity assay with MON 13200: Study No. ML-88- 191/EHL No. 88124.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275535).

Bakke JP (1989b) Evaluation of MON 13200 to induce unscheduledDNAsynthesis in the in vitro hepatocyteDNArepair assay in the male F-344 rat: Study No. SR-88-204/SRI No. LSC 6327. Testing facility: SRI International, Menlo Park, CA. Submitted by Monsanto, St. Louis, MO (MRID No. 42275538).

Bayer I, Mitmaker B, Gordon PH, Wang E (1992) Modulation of nuclear statin expression in rat thyroid follicle cell following administration of thyroid stimulating hormone. *Journal of Cellular Physiology*, **150**:276–282.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, **23**(1):38–89.

Capen CC, Dybing E, Rice JM, Wilbourn JD, eds (1999) *Species differences in thyroid, kidney and urinary bladder carcinogenesis*. Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 147).

Curran PG, DeGroot LJ (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Reviews*, **12**:135–150.

Dohler KD, Wong CC, Von Zur Muhlen A (1979) The rat as a model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacology and Therapeutics*, **5**:305–318.

Flowers LJ (1990) *Micronucleus assay with MON 13200: ML-88-390/EHL Study No. 88230.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275537).

Gabriele R, Letizia C, Borghese M, De Toma G, Celia M, Izzo L, Cavalla A (2003) Thyroid cancer in patients with hyperthyroidism. *Hormone Research*, **60**(2):79–83.

Hard GC (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. *Environmental Health Perspectives*, **106**(8):1–21.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF (1989) Thyroid follicular cell carcinogenesis. *Fundamental and Applied Toxicology*, **12**(4):629–697.

Hood A, Liu YP, Gattone VH 2nd, Klaassen CD (1999) Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicological Sciences*, **49**:263–271.

Hood A らの文献が欠落

Hotz KJ, Wilson AG, Thake DC, Roloff MV, Capen CC, Kronenberg JM, Brewster DW (1997) Mechanism of thiazopyr-induced effects on thyroid hormone homeostasis in male Sprague-Dawley rats. *Toxicology and Applied Pharmacology*, **142**:133–142.

Hurley PM, Hill RN, Whiting RJ (1998) Mode of carcinogenic action of pesticides inducing thyroid follicular-cell tumors in rodents. *Environmental Health Perspectives*, **106**(8):437–445.

IARC (2001) *Some thyrotropic agents*. Lyon, International Agency for Research on Cancer, 763 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 79).

Li AP, Myers CA (1989) *CHO/HGPRST gene mutation assay with MON 13200: Study No. ML-88-382/EHL No. 88071.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275536).

Lichtenstein CK, Hemminki K (2002) Environmental and heritable cause of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. *International Journal of Cancer*, **1099**(2):260–266.

Liu J, Liu Y, Barter RA, Klaassen CD (1995) Alteration of thyroid homeostasis by UDPglucuronosyltransferase inducers in rats: A dose–response study. *Journal of Pharmacology and Experimental Therapeutics*, **273**:977–985.

Masubuchi N, Hakusui H, Okazaki O (1997) Effects of proton pump inhibitors on thyroid hormone metabolism in rats: A comparison of UDP-glucuronyltransferase induction. *Biochemical Pharmacology*, **54**(11):1225–1231.

Mazzaferri EL (2000) Thyroid cancer and Graves' disease: The controversy ten years later. *Endocrine Practice*, **6**:221–225.

McClain RM (1992) Thyroid gland neoplasia: Non-genotoxic mechanisms. *Toxicology Letters*, **64/65**:397–408.

McClain RM (1995) Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research*, **333**(1–2):131–142.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**(6):591–654.

Naylor MW, McDonald MM (1992) Chronic study of MON 13200 administered in feed to albino rats. Project No. ML-88-247/EHL 88148.

Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-24).

Naylor MW, Raju NR (1992) Chronic study of MON 13200 administered in feed to albino mice. Project No. ML-88-248/EHL 88147.

Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-23).

Olsen JH, Wallin H, Boice JD, Rask K, Schulgen G, Fraumaen FF Jr (1993) Phenobarbital, drug metabolism and human cancer. *Cancer Epidemiology, Biomarkers and Prevention*, **5**:449–452.

Oropeza-Hernandez LF, Lopez-Romero R, Albores A (2003) Hepatic CYP1A, 2B, 2C, 2E and 3A regulation by methoxychlor in male and female rats. *Toxicology Letters*, **144**(1):93–103.

Ron E, Kleinerman RA, Boice JD, LiVolsi VA, Flannery JT, Fraumeni JF Jr (1987) A population-based case–control study of thyroid cancer. *Journal of the National Cancer Institute*, **79**:1–12.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Sundseth SS, Waxman DJ (1992) Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid omega-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. *Journal of Biological Chemistry*, **267**(6):3915–3921.

Thomas GA, Williams ED (1999) Thyroid stimulating hormone (TSH)-associated follicular hypertrophy and hyperplasia as a mechanism of thyroid carcinogenesis in mice and rats. In: Capen CC, Dybing E, Rice JM, Wilbourn JD, eds. *Species differences in thyroid gland, kidney and urinary bladder carcinogenesis.* Lyon, International Agency for Research on Cancer, pp. 45–59 (IARC Scientific Publications No. 147).

USEPA (1998) *Assessment of thyroid follicular cell tumors*. Washington, DC, United States Environmental Protection Agency, Office of Research and Development, Risk Assessment Forum (EPA/630/R-97/002; http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=13102; accessed 22 November 2004).

# 4-AminobiphenylとDNAの反応性: 発がん MOA のヒトへの関連性を解析するための IPCS フレームワークを用いた事例研究1

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DNA 反応性(遺伝毒性)発がん物質である 4-Aminobiphenyl を例に、動物とヒトにおける豊富なデ ータに基づいて国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒト 関連性フレームワーク(Human Relevance Framework: HRF)を評価した。この Mode of action (MOA) は、N-水酸化による代謝的活性化、N-エステル化による反応性親電子物質の生成を経て、この親電 子物質が DNA(主にデオキシグアノシン)と共有結合し、DNAの突然変異を促進させ、最終的に はがんの発生につながると考えられている。ヒトとイヌでは、膀胱が標的臓器であるのに対し、マ ウスでは膀胱と肝臓が標的臓器であり、他の種では他の組織が関与することもある。臓器特異性の 違いは、代謝的活性化と不活性化の違いによるものと考えられている。定性的及び定量的な考察か ら、ヒトの MOA が可能である。毒性や再生性増殖などの他の生物学的プロセスは、4-Aminobiphenyl 誘発性腫瘍の用量反応に大きく影響する可能性がある。IPCS HRF によれば、4-Aminobiphenyl はヒ トにおいて発がん物質であると予測され、これは広範な疫学的エビデンスによって裏付けられて いる。IPCS HRF は DNA 反応性発がん物質の評価に有用である。

4-Aminobiphenyl は、様々な経路で複数の種において発がん性が確認されている(IARC、1972 年、1986年、1987年)。4-Aminobiphenyl は、代謝的活性化、DNA 反応性、遺伝毒性、発がん性を 評価した多くのデータに基づき、動物モデルにおける MOA が確立されていることから、代表的 な DNA 反応性発がん物質として、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒト関連性フレームワーク(Human Relevance Framework: HRF)の事例研究対象 化学物質に選定された。また、芳香族アミンに属する既知の動物及びヒト発がん物質と類似して おり(構造活性相関)、ヒトにおける疫学的、代謝学的、生化学的データが豊富に存在している。 この事例研究は、DNA 反応性発がん物質の MOA を明確にするのに役立つデータの性質を示して いる。MOA 解析における調節因子と key events の区別についても示されている。

動物における有力なエビデンスと広範な疫学的データに基づいて、国際がん研究機関(IARC) は 4-Aminobiphenyl を既知のヒト発がん物質として分類した(IARC、1972 年、1987 年)。4-Aminobiphenyl は最初、職業的に高レベルにばく露されたヒトの膀胱発がん物質として同定された。 その後、タバコの煙の主成分として実証され、喫煙者の膀胱がんのリスク増加につながるといわ れている(Del Santo ら、1991 年; Curigliano ら、1996 年)。追加研究では、窒素を含む有機物が燃 焼する際に自然に発生し、至る所に存在する環境化学物質であることが示されている。

<sup>&</sup>lt;sup>1</sup> この論文は、WHO が著作権を有するものであり、元々は 2006 年に Critical Reviews in Toxicology, Volume 36, pages 803-819 に掲載 されたものである。この論文は WHO の出版物のために編集されたもので、正誤表が含まれている。

# 動物における 4-Aminobiphenyl の発がん性

実験的研究では、標的組織と感受性には有意な差が認められているものの、4-Aminobiphenylは マウス、ラット、ウサギ、イヌにおいて発がん性があることが示されている(IARC、1972年)。4-Aminobiphenylは、ほとんどのばく露経路において、マウスでは主に肝臓と、低頻度で膀胱に対し て発がん性が認められるが、イヌ(及びヒト)では膀胱が標的臓器であると考えられる。これら の研究の多くが数年前に行われたものであり、詳細な情報は限られたものしか公表されていない。 さらに、試験途中における潜在的な前駆病変はほとんど記録されておらず、どの研究にも MOA で 参考となる中止/回復などの研究計画書は含まれていなかった。それにもかかわらず、研究結果に は競合する代謝的活性化及び不活性化プロセスが競合する MOA に特徴的な反応における影響の 種及び個体差(例えば、ブロックら、1978年)が示されている(表1)。

動物種	投与経路/用量	発生率	コメント	参考文献
マウス	経口投与;1mg/週を38 週間投与	90 週まで生存した 2/12 匹に膀胱がん		Clayson et al. (1965)
マウス	経口投与;0または 1.5 mg/週を 52 週間投与	対照群 0/19 匹に対し、 雄 1/21 匹に膀胱がん; 雌雄とも肝臓がんの発 生率が増加		Clayson et al. (1967)
マウス	200 µg を最大 52 週間皮 下注射	48~52 週後の雄 19/20 匹、雌 6/23 匹に肝臓が ん		Gorrod et al. (1968)
マウス (BALB /cStCrl fC3Hf/ Nctr)	飲料水中 0-220 mg/L (雄)、0-300 mg/L (雌)を最大 96 週間投 与	膀胱がん(雄のみ)、肝 細胞がん(雌のみ)、血 管肉腫(雄雌)の有意 な増加	75 mgL (雌) 及び 55 mg/L (雄) 以上の投与 では、雌雄ともほとん どのマウスで膀胱の過 形成が認められたが、 対照マウスでは認めら れなかった。	Schieferste in et al. (1985)
マウス (新生 児 B6C3F 1)	異なる投与用法;離乳 前に注射された	肝臓腫瘍		Dooley et al.(1988, 1992); Von Tungeln et al. (1996); Parsons et al. (2005)
ラット	総量 3.6~5.8 g/kg bw の ピーナッツオイルを皮 下注射	乳腺腫瘍・腸管腫瘍		al. (2003) Walpole et al. (1952)

# 表1. (続き)

動物種	投与経路/用量	発生率	コメント	参考文献		
ウサギ	不定量の経口投与	1 匹に膀胱乳頭腫、3 匹 に膀胱がん	投与開始から4年後に 観察された最も早いが ん	Bonser (1962)		
イヌ(2)	ゼラチンカプセルを週6 回終身投与、合計30ま たは34g	膀胱がんが 33 ヶ月で出 現		Walpole et al. (1954)		
イヌ	ゼラチンカプセル 0.3 g を週 3 回(総投与量: イヌ 1 匹あたり 94.5~ 144 g)	21~34ヶ月後に膀胱が ん		Deichmann et al. (1958)		
イヌ(6)	1.0 mg/kg bw を週 5 回、 34 ヵ月または 37 ヵ月間 (イヌ 1 頭あたりの総 投与量 5.5~7.0 g)。	膀胱乳頭腫3匹、膀胱 がん(移行上皮型)3 匹		Deichmann et al. (1965)		
イヌ	単回投与	5年間にわたり膀胱腫 瘍は誘発されなかった		Deichmann & MacDonald (1968)		
イヌ (24、ビ ーグル)	経口投与週5回、3年間	4 匹のイヌでは陰性ま たは軽症(2 匹で腫瘍 なし) 11 匹では腫瘍はゆっく りと進行したが、残り の9 匹では急速に進行		Block et al. (1978)		
hw 休重						

#### bw, 体重

経口投与(マウス1週あたり1 mg、38週間)の後、90週まで生存した2/12のマウスに膀胱がんが発生した(Clayson ら、1965年)。別の実験では、マウスに1.5 mgの4-Aminobiphenylを52週間投与したところ、対照群では0/19匹であったのに対し、1/21匹の雄マウスで膀胱がんが発生した。この実験では、雄マウスと雌マウスの両方で肝臓がんの頻度が対照群よりも有意に高かった(Clayson ら、1967年)。4-Aminobiphenyl 200 µgをマウスに3回皮下注射したところ、48~52週後に19/20匹の雄及び6/23匹の雌で肝臓がんが生じた(Gorrod ら、1968年)。4-Aminobiphenylを、BALB/cStCrlfC3Hf/Netrマウスの雄と雌に、それぞれ220 mg/Lと300 mg/Lまでの濃度で飲水投与すると、投与量に相関して、血管肉腫(雄と雌)、膀胱がん(雄のみ)、肝細胞がん(雌のみ)の有意な増加が認められた。膀胱の過形成は、75 mg/L(雌)及び55 mg/L(雄)以上の濃度の投与を受けた約118匹の群では、両性のマウスのほとんどで観察されたが、対照群では報告されていない(Schieferstein ら、1985年)。多くの実験において、新生児B6C3F1マウスは、4-Aminobiphenyl投与後に、主に肝発がんに感受性があった(Dooley ら、1988年、1992年; Von Tungeln ら、1996年; Parsons ら、2005年)。

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ピーナッツオイル中に 4-Aminobiphenyl を混入して合計 3.6~5.8 g/kg 体重(bw)の用量をラットに毎日皮下注射すると、乳腺及び腸の腫瘍の発生率が有意に増加した(Walpole ら、1952 年)。

市販の4-Aminobiphenylを経口投与(用量は記載なし)した7匹のウサギのうち、1匹に膀胱乳 頭腫が、3匹に膀胱がんが認められた。最も早い発がんは投与開始から4年後に観察された(Bonser、 1962年)。

4-Aminobiphenyl をゼラチンカプセルで週6回投与し続けた2匹のイヌ(1匹あたりの総投与量: 30、34g)は、33ヶ月で膀胱がんを発症した(Walpole ら、1954年)。同様に4-Aminobiphenylを含むカプセル(イヌ1匹あたり0.3g)を週3回与えても、21~34ヶ月後に膀胱がんが観察された(総投与量:イヌ1匹あたり94.5~144.0g)(Deichmann ら、1958年)。4-Aminobiphenylを1.0 mg/kg bwに減量し、6匹のイヌに週5回、34ヶ月または37ヶ月間投与したところ(総投与量:1匹当たり5.5~7.0g)、それぞれ3例で膀胱乳頭腫及び膀胱がん(移行上皮型)が観察された(Deichmann ら、1965年)。単回投与では、5年間で膀胱腫瘍は誘発されなかった(Deichmann & MacDonald、1968年)。4-Aminobiphenylを週5日、3年間経口投与した24匹のビーグル犬では、膀胱発がん物質の反応の基本的な3つのパターンが認められた。陰性または最小限の病変は4匹にみられ、そのうちの2匹には腫瘍が発生しなかった。腫瘍は11匹のイヌでゆっくりと進行したが、残りの9匹では急速に進行するパターンが観察された(Block ら、1978年)。

## 動物 MOA を立証するのにエビデンスの重み付けは十分か

IPCS HRF の最初の問いかけは、動物 MOA そのものの評価である。これは、疫学研究における 因果関係を説明するための Bradford Hill 基準 (Hill、1965 年)から発展し、IPCS が開発した、2001 年発表の MOA フレームワーク (Sonich-Mullin ら、2001 年)に示されている手順に基づいている。

## A. 推定される MOA

4-Aminobiphenyl は、肝酵素によって *N*-hydroxy-4-aminobiphenyl に代謝され、肝及び他の組織に おいて *N*-エステル化 (*N*-アセチル化、*N*-グルクロン化、または *N*-硫酸化) される (Miller ら、1961; Kadlubar ら、1977 年、1991 年; Miller & Miller、1977 年; Delclos ら、1987 年; Chou ら、1995 年) (図 1)。*O*-エステル化及び環状水酸化は、無毒化につながる競合する酵素反応である。これらの 反応の活性における組織差や種差により、少なくとも部分的には、4-Aminobiphenyl の発がん作用 に対する感受性の違いや、腫瘍の発生における臓器特異性に違いを生じさせる。最終的には、*N*-エステル化の後、反応性親電子ニトレニウムイオンが標的組織に形成され、これが DNA 付加体を 形成しうる。主な DNA 付加体は、*N*- (deoxyguanosin-8-yl)-4-aminobiphenyl である (Talaska ら、1990 年; Kadlubar ら、1991 年; Flammang ら、1992 年; Hatcher & Swaminather、1995 年、2002 年)。 これらの反応が重要な遺伝子の重要な部位で起こることにより突然変異がおこり、その結果、最終的に腫瘍細胞が発生する。

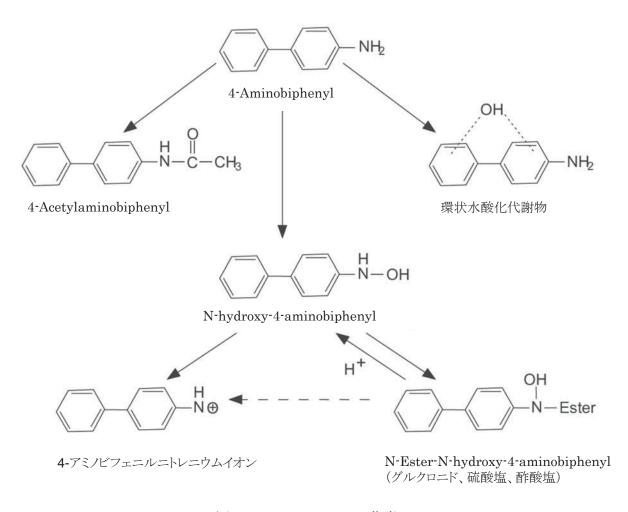


図 1.4-Aminobiphenyl の代謝

#### **B.** Key events

4-Aminobiphenylの肝活性化の主要経路は、少なくともラットとヒトでは CYP1A2 によって触媒 される 4-Aminobiphenylの *N*-水酸化から始まる(Butler ら、1989 年 b)。マウスでは、CYP1A2 や チトクローム P-450 が唯一もしくは主要に関与しているわけではないという報告がある(Kimura ら、1999 年)。また、*N*-hydroxylamine はシクロオキシゲナーゼのプロスタグランジン合成酵素 (Kadlubar ら、1982 年)のような様々なオキシダーゼ及びペルオキシダーゼとの反応によって生 成される。これらの非チトクローム P-450 反応のいずれかが生体内で起こるが、毒性学的に意義 があるかは不明なままである。*N*-hydroxylamine は、*N*-アセチルトランスフェラーゼ 1(NAT1) (Flammang & Kadlubar、1986 年; Oda、2004 年)による *N*-アセチル化を受け、酸性条件下では不 安定な *N*-アセトキシエステルを生じ、DNA と直接反応し得るアリルニトレニウムイオンを形成 し、グアニンの C-8 位に DNA 付加体を形成する(Hammons ら、1985 年; Flammang & Kadlubar、 1986 年; Hatcher & Swaminathan、2002 年)。さらに、肝臓で生成された N-hydroxylamine は、ウリ

ジンニリン酸(UDP)グルクロン酸転移酵素(UGT)の基質として機能し、N-グルクロニド抱合体を生じ、これが膀胱に運ばれる(Kadlubarら、1977年)。グルクロニドは尿中に排泄されるか、酸性条件下において、加水分解後に膀胱内の N-hydroxylamineの追加供給源として機能する。N-ア セチルトランスフェラーゼ-2(NAT2)による 4-Aminobiphenylの N-アセチル化など、この反応と

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競合する反応が多数存在するが、得られるアリルアセトアミドは、CYP1A2 にとって貧弱な基質 であり、主に解毒反応であると考えられている。その結果、親アミンの N-アセチル化は不活性化 反応と考えられている。アセチル化の速度は、活性化と不活性化のバランスに影響を与える。ヒ トの表現型には、rapid acetylators と slow acetylators が存在する(Lower ら、1979 年)。ヒトのそれ ぞれのアセチレーターに類似したマウスの系統が存在し、C57BL/6 は rapid acetylators 系統であり、 A/J は slow acetylators 系統である(Hein、1988)。これらの違いに対する研究は、種間、系統間、 個体間での反応の違いを説明しうる。形成された DNA 付加体により、突然変異が生じる可能性が ある。key events を表 2 にまとめた。

## 表 2. 動物における 4-Aminobiphenyl の発がん性における key events

- 1. 代謝的活性化
  - a) N-ヒドロキシル化
  - b) N-エステル化 (グルクロニド、アセチル、硫酸塩)
  - c) 加水分解してニトレニウムイオンにする
- 2. 標的職器の多能性細胞における DNA 付加体形成(dG-C8, dA-C8, dG-N2)
- 3. がんにつながる重要な遺伝子の DNA 突然変異
- 4. 発がん
- dA、デオキシアデノシン;dG、デオキシグアノシン

## C. 用量反応関係

関連する研究の多くが何年も前に実施されたという事実を考慮すると、腫瘍の前駆病変に対す る用量反応の一致に関するデータは、マウスの膀胱過形成に限定されている。イヌでは、4-Aminobiphenylの単回投与後に膀胱腫瘍が発生することはなく(Deichmann & MacDonald、1968 年)、 複数回投与後の用量反応関係の研究は行われていない。前駆病変の発生率に関する情報が報告さ れている唯一の研究では、雄の BALB/c マウスに 0、7、14、28、55、110、220 mg/Lの濃度の 4-Aminobiphenyl を含む飲料水を介して 96 週間まで処置したものがある (Schieferstein ら、1985 年)。 これらの処置後の膀胱がん発生率は、それぞれ 0/116、1/117、1/118、0/118、6/115、5/118 及び 23/118 であった。55 mg/L 以上の群の発生率は、対照群に比べて統計的に有意に高かった。雌マウスに 4-Aminobiphenyl を濃度 0、7、19、38、75、150 及び 300 mg/Lの飲料水を介してばく露させると、 膀胱がんの発生率は、0/118、0/118、0/119、1/118、0/118、5/117及び1/117であった。重症度は示 されていなかったが、過形成の発生率ははるかに高かった。雄では、0、7、14、28、55、110及び 220 mg/L の投与量で、過形成の発生率は 0/116、4/117、9/118、71/118、108/115、107/118 及び 102/118 であった。雌では、それぞれ 0、7、19、38、75、150 及び 300 mg/L の用量で 0/118、0/118、3/119、 53/119、106/118、97/117 及び 83/117 であった。このように、腫瘍及び過形成の用量反応曲線は、 シグモイド型またはホッケースティック型であった。対照的に、尿路上皮細胞のC-8 グアニンDNA 付加体の平衡レベルは直線的な用量反応関係を示した(Poirier ら、1995年)。

同研究(Schieferstein ら、1985年)では、雄では肝腫瘍の発生率の増加は認められなかったが、 雌では、それぞれ0、7、19、38、75、150及び300mg/Lの投与量で、肝腫瘍(腺腫及びがんを合 わせたもの)の発生率は0/117、0/120、2/120、4/119、11/119、17/118及び10/117であった。様々 な組織を合わせた血管肉腫の発生率は、雄と雌の高い3用量で増加したが、発生率は雄よりも雌 の方がやや高かった。

## D. 時間的関連性

発がん過程における事象の順序を確立することは、その測定方法の感度に部分的ではあるが大 いに依存する。したがって、腫瘍は組織学的に検出できる大きさに達していなければならず、一 方で突然変異や DNA 付加体の測定には時間だけでなく十分な量の組織が必要である。その結果、 後者は膀胱よりも肝臓で研究されるのが一般的だが、特にげっ歯類では尿道の組織が少ないため、 生化学的・生物学的事象の頻度とは関係のない技術的な困難が生じている。DNA 付加体の代謝及 び形成は初期の事象であり、4-Aminobiphenyl による in vitro 処理後、数分または数時間以内及び 1 日以内に観察される(Kadlubar ら、1991年; Swaminathan & Reznikoff、1992年; al-Atrash ら、1995 年; Hatcher & Swaminathan、1995年; Doerge ら、1999年; Tsuneoka ら, 2003年)。しかしながら、 多くの in vivo 実験では、付加体を蓄積させ、平衡レベルに達し、検出を容易にするために、3~4 週間ばく露を継続する(例えば、Talaska ら、1990年; Flammang ら、1992年; Poirier & Beland、 1992 年; Poirier ら、1995 年; Underwood ら、1997 年)。また、突然変異は in vitro では短時間で検 出され得るが、生体内では数週間または数ヶ月のばく露後でないと検出されない(例えば、マウ ス肝臓における H-ras; Parsons ら、2002 年)。この期間は、突然変異が最初に生じた時期を正確に 反映しているとは限らない。ある研究では、4-Aminobiphenyl の単回投与から 14 日後に Muta™Mouse 膀胱アッセイで突然変異が検出された(Fletcher ら、1998 年)。膀胱のがんや過形成 は、マウスやイヌでは明らかに遅発性の病変であるが、時間経過の変化は体系的に評価されてい ない。ある2年間の研究では、マウスを13週から一定間隔で安楽殺し、膀胱に過形成病変を誘導 したが、異なる時期の発生率は示されていない(Schieferstein ら、1985)。膀胱の腫瘍は、一般的 に、マウスでは約2年後(Schieferstein ら、1985年)、イヌではそれより長い時間が経過するまで 発見されない(Walpole ら、1954 年; Deichmann ら、1958 年、1965 年)。しかし、ヒトの(SV40 に感染した)尿道粘膜細胞を 4-Aminobiphenyl で in vitro 処理し、6 週間 in vitro 培養した後、ヌー ドマウスに接種した場合、腫瘍性の形質転換が認められた(Bookland ら、1992 年 b)。

## E. 腫瘍反応と key events との関連性の強さ、一貫性、特異性

腫瘍反応と key events との関連性を支持するエビデンスは、膀胱に関する研究から得られたものがあるが、肝臓に関する研究からも有力なエビデンスが得られている。DNA 付加体の形成は両 組織で実証されている。

4-Aminobiphenyl が変異原性物質であることを示す研究は豊富にあり、S. typhimurium 菌の特定 のフレームシフト型突然変異及び塩基対置換感受性株(TA1538、TA98 及び TA100)に対する陽性 反応が、げっ歯類肝 S9 代謝的活性化製剤の存在下でのみ認められている。S9 による代謝的活性 化が必要であるということは、親アミンの DNA 反応性及び変異原性の欠如を示している。さら に、4-Aminobiphenylは、in vitro でラット肝細胞において不定期 DNA 合成を誘導する(米国環境 保護庁遺伝的活性プロファイル)。これらの in vitro 研究は、4-Aminobiphenyl が代謝的活性化後に 遺伝的損傷を引き起こす可能性があるというエビデンスを示している。また、細菌を用いた突然 変異試験でも代謝的活性化システムの解析が行われている (Dang & McQueen、1999)。Aroclor 1254 誘発雄 Sprague-Dawley ラット及び C57BL/6 マウスの肝臓ホモジネート及び、異なるレベルの N-及び O-アセチルトランスフェラーゼ (OAT) 活性を発現する S. typhimurium TA100 試験菌株が使 用された。TA100 は NAT/OAT 遺伝子のシングルコピーを有し、YG1029 は NAT/OAT 遺伝子のマ ルチコピーを有し、TA100/1.8DNP6 は NAT/OAT 欠損株である。マウス及びラット S9 を用いた効 果は類似していた(ただし、Aroclor 1254 処理の効果は調べなかった)。4-Aminobiphenyl または 4-Acetylaminobiphenyl を基質とした場合、YG1029 では TA100 または TA100/1,8DNP6 よりも多く突 然変異が誘発された。このことは、これらの細菌における N-hydroxylamine による突然変異誘発に は、高いアセチル化活性が関与していることを示唆している。

生体内での突然変異誘発機構におけるアリルニトレニウムイオンへの非酵素的ステップは、高 OAT-発現 *S. typhimurium* TG1024 株における *N*-hydroxy-4-aminobiphenyl の突然変異誘発が培地の pH に依存し、4.0~8.0 の範囲で突然変異体数と pH との間に逆相関の関係が観察されたことによ って支持される (Sarkar ら、2002 年)。

BALB/c マウスに 4-Aminobiphenyl を 28 日間飲水投与したところ、雌では膀胱よりも肝臓の方 が DNA 付加体の量が多かったのに対し、雄では逆の結果が得られた。このように、各性におい て、DNA 付加体のレベルは、4-Aminobiphenyl による腫瘍誘導に対する組織の感受性と相関して いた(Poirier ら、1995 年)。しかしながら、用量反応曲線の形状は、両組織の DNA 付加体では直 線的であった(雌マウスでは飽和して比較的平坦)のに対し、腫瘍の用量反応曲線はシグモイド 型であった(Poirier ら、1995 年)。

付加体の量は、50 nmol の 4-Aminobiphenyl を 21 週間にわたって局所投与した Hsd:ICR(Br)マウスの雌マウスの膀胱で最も高かった (一般的な職業的ばく露経路)。検査した全組織 (膀胱、肝臓、肺、皮膚)の主な付加体は、*N*-(deoxyguanosin-8-yl)-4-aminobiphenyl とのコクロマトグラフィーを用いて検査した (Under-wood ら、1997 年)。

雄の Muta<sup>™</sup>Mouse トランスジェニックマウス(すなわち、トランスジェニック CD2F、 [BALB/c×DBA/2]) に 10 mg/kg bw/日の 4-Aminobiphenyl を 10 日間経口投与した突然変異誘発の研 究では、膀胱、肝臓及び骨髄における突然変異頻度がそれぞれ 13.7 倍、4.8 倍及び 2.4 倍に増加し たことが報告されている(Fletcher ら、1998 年)。

新生児 B6C3F1(C57BL/6×C3H)マウスは、4-Aminobiphenyl の投与に反応して高頻度の肝腫瘍を 発症し、その多くは *H-ras* 遺伝子のコドン 61 における CAA→AAA 突然変異であった(Parsons ら, 2005 年)。in vivo では、0.3 µmol の 4-Aminobiphenyl を 24 時間前に投与した新生児マウスに おいて、主要な DNA 付加 *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl 量は、5 adducts /10<sup>6</sup> ヌクレオチ ドであった。8 ヶ月後、CAA→AAA 突然変異は、投与マウスの 67%及び対照(ジメチルスルホキ シド、または DMSO)の 50%で検出されたが、投与マウスの平均突然変異率は 45×10<sup>-5</sup> であったの に対し、対照ではわずか 2×10<sup>-5</sup> であった。12 ヵ月後には、投与マウスの 79%、対照マウスの 8% に肝腫瘍が発生していた。これらの腫瘍はヒトの標的臓器の腫瘍ではないが、本研究の結果は、 膀胱がんの発生において提示されている一般的な MOA(すなわち、DNA 付加体の形成、重要な 遺伝子の突然変異、その後の腫瘍の出現)を支持するものである。

4-Aminobiphenyl (5 mg/kg bw) を単回経口投与してから 24 時間後に死亡したイヌ (性別は明記 されていない)の DNA 付加体は、肝臓で 5.4 fmolDNA adducts/µg、膀胱で 4.8 fmolDNA adducts/µg であった。しかし、4-Aminobiphenyl を膀胱に投与したイヌの肝臓と膀胱では DNA 付加体は検出 されなかった。一方、反応性中間体である *N*-hydroxy-4-aminobiphenyl を膀胱に投与したイヌの膀 胱では、3.9 fmol DNA adducts/µg の DNA 付加体が検出され、肝臓では付加体は検出されなかった。 免疫化学的方法で定量を行った (Roberts ら、1988 年)。トリチウム標識された 4-Aminobiphenyl (経 口、静脈内、または尿道内)、*N*-hydroxy-4-aminobiphenyl (静脈内、または尿道内)、*N*-hydroxy-4aminobiphenyl *N*-glucuronide (静脈内)を投与された雌イヌの試験では以下のことが実証された。

(1) 血中赤血球中の 4-aminobiphenyl -ヘモグロビン付加体の存在(2) 4-aminobiphenyl 投与後、膀胱内腔に入る *N*-hydroxy-4-aminobiphenyl の大部分は遊離 *N*-hydroxy-4-aminobiphenyl (0.7%) であり、酸溶性 *N*-グルクロニドは低濃度(0.3%) であること(3) *N*-hydroxy-4-aminobiphenyl を尿道内に注入した後の DNA 付加体量は、4-Aminobiphenyl を尿道内に注入した後と比較して 60 倍多かったこと、(4) *N*-hydroxy-4-aminobiphenyl へのばく露及びそれに続く 4-Aminobiphenyl-DNA 付加体の形成は、排尿の頻度及び尿の pH に依存すること(Kadlubar ら、1991 年)。

イヌの尿中 pH は、食事内容(Merck、1998年)、食後からの時間、時間帯及び消費水量に依存 して、約 4.5 から 7.5 まで変化することがあり、これらは発がん性に影響を及ぼす可能性のある因 子である(Cohen、1995)。イヌの肝臓及び膀胱からのミクロソーム調製物を用いた in vitro の研究 では、両臓器においてトランスアセチル化活性が存在することが示されており、そのため、4-Acetylaminobiphenyl、*N*-hydroxy-4-acetyl-aminobiphenyl、またはアセチルコエンザイム A(CoA)が アセチル供与体として存在する場合には、*N*-hydroxy-4-aminobiphenyl の RNA 及び DNA への結合 が起こるが、結合量は肝臓ミクロソームよりも膀胱ミクロソームの方が少なかった(Hatcher & Swaminathan、1992 年)。

4- Aminobiphenyl を投与したイヌの尿に含まれる尿膜細胞を調べたところ、*N*-hydroxy-4aminobiphenyl で in vitro で修飾した DNA や、4-aminobiphenyl を投与したイヌの剖検時に単離した イヌの膀胱尿路上皮細胞の DNA と同一の DNA 付加体が検出された。4- aminobiphenyl-DNA 付加 体形成の用量依存的増加が示された(Talaska ら、1990 年)。

# F. 生物学的妥当性及び整合性

4-Aminobiphenyl が DNA と付加体を形成し、腫瘍が発生する器官で変異原性を示すという結果 は、一般的に提案されている MOA の妥当性を示している(Fletcher ら、1998 年)。さらに、*N*hydroxy-4-aminobiphenyl は、腫瘍化していない SV40 不死化ヒト尿細管細胞の腫瘍化を引き起こす ことができる(Bookland ら、1992 年 b)。また、4-Aminobiphenyl に関する知見はいくつかの関連 芳香族アミンの代謝的活性化、DNA 付加体形成、突然変異誘発及びいくつかの種(ヒトを含む) における膀胱発がんに関する膨大な文献と一致している(Kadlubar ら、1977 年; Miller & Miller、 1977 年; Delclos ら、1987 年)。代謝的活性化を伴わない様々な in vitro 系での親アミンの DNA 付 加体形成及び変異原性の欠如は、代謝的活性化の必要性を明確に示している。アミンの投与後、 または *N*-ヒドロキシル代謝物へのばく露後に組織内で同じ DNA 付加体が確認され、付加体の構 造が化学的に確認されている。特定の生物物理学的側面は 2-aminofluorene のような構造的に関連 する芳香族アミンの方がより明らかにされているが、C-8 グアニン DNA 付加体の変異原性につい ても明らかにされている、(Kriek、1992 年)。

# G. その他の MOA

既に記載されている MOA の構成要素の代替的なものが提案されている。しかし、それらは MOA の全体的な説明を損なうものではなく、限局的側面(他の活性化酵素など)、または定量的側面に 影響を与える可能性のある過程のいずれかを示唆している。4-Aminobiphenyl は、CYP1A2 以外の 肝酵素(Kimura ら、1999年)によって N-水酸化代謝物に酸化され、肝臓及び膀胱の毒性及び発が んを引き起こすが、これには酸化酵素及びペルオキシダーゼが関与している可能性がある (Kadlubar ら、1982年、1991年)。代謝的活性化に関与する酵素は異なるかもしれないが、反応 性親電子物質の生成、DNA 付加体の形成、変異原性、発がんという順序は一貫している。さらに、 MOA のこの時点から、活性化酵素にかかわらず、CYP1A2を介した活性化と同じ順序で起こると 考えるのが妥当である。

巨大な付加体に加えて、*N*-hydroxy-4-aminobiphenylは、内因性ペルオキシダーゼが関与している可能性があり、尿道粘膜DNAに酸化的損傷を引き起こすことを示唆するエビデンスがある(Burger ら、2001 年)。このことと 4-Aminobiphenyl の発がん活性との関連性は不明である。

N-hydroxy-4-aminobiphenyl 及びその活性型は、in vitro で尿道粘膜及び他の細胞に対して細胞毒性を示す(Reznikoff ら、1986年)が、これが発がん作用に果たす役割は不明である(原因ではなく、尿道粘膜発がんにおける増強的な役割については以下を参照のこと)。この過程で用量反応関係は変化するが、上述の基本的な MOA は変化しないと考えられる。

# H. 推定される MOA の評価

推定される MOA の初期段階は、利用可能なエビデンスによって十分に裏付けられており、4-Aminobiphenyl はイヌやマウスでは膀胱発がん物質であり、げっ歯類では他の組織(主に肝臓)で の発がん物質である十分なエビデンスがあると判断されている。このように、肝臓や他の標的臓 器では DNA 付加体を形成しうる生成物に代謝され、突然変異が生じることが実証されている。 他の臓器も 4-Aminobiphenyl 誘発性腫瘍の標的となりうるが、イヌやマウスの一部の系統では膀 胱が主な標的となっている。一般的な遺伝毒性と腫瘍発生との間の段階についてのエビデンスは 十分ではない。4-Aminobiphenyl の膀胱の細胞増殖への影響についての研究は著しく不足している が、関連する芳香族アミンやアミドについての情報は入手可能であり、特にメガマウス ED-01 実 験(Cairns、1979年; Gaylor、1979年; Littlefield ら、1979年)のデータを用いたマウスの膀胱に おける 2-acetylaminofluorene によって誘導される DNA 反応性(及び変異原性)と細胞増殖との間 の相互作用の解析が行われている。変異原性が細胞増殖に依存していることは、DNA 付加体の用 量反応が直線的であるにもかかわらず、腫瘍の用量反応がシグモイド型であることの説明を提供 することができる(Cohen & Ellwein、1990年)。この関連性は、4-Aminobiphenyl 誘発性膀胱がん の発生と用量反応性を評価する上で重要な意味を持つ(以下の議論を参照)。

# I. 不確実性、矛盾、データギャップ

4-Aminobiphenyl の代謝的活性化を伴う細菌を用いた突然変異試験では、ほとんどの変異がフレ ームシフトであることが示されているが、バクテリオファージ M13 クローニングベクターから得 た一本鎖 DNA 中の lacZ 遺伝子の 4-Aminobiphenyl 誘発突然変異の配列解析を行ったところ、80% 以上が G 部位で生じる塩基対置換が明らかになった。G→T トランスバージョンが優勢で、次に G→C トランスバージョン、G→A トランジションが続いた。次に、主要な DNA 付加体である *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl を M13 ゲノム内に挿入し、生体内複製後に変異頻度と特異性 を測定した。目標とする突然変異率は約 0.01%であり、一次突然変異は G→C トランスバージョ ンであった。このように、in vitro での観察結果は in vivo での観察結果と矛盾しないが、変異原性 は弱いものであった (Verghis ら、1997 年)。

in vivo での研究のほとんどはマウスで行われている。無理からぬ理由ではあるが、イヌは膀胱 発がんに対して感受性が高い種であるにもかかわらず、あまり注目されていない。反応における マウスの系統の違いは明らかである。B6C3F1及び雌のBALB/cStCrlfC3Hf/Netrマウスは、肝臓発 がんに対してより感受性が高く、一方、雄のBALB/cStCrfC3Hf/Netrマウスは、4-Aminobiphenylへ のばく露後に膀胱腫瘍を発症する(Schieferstein ら、1985 年; Dooley ら、1988 年、1992 年)。そ れにもかかわらず、利用可能な研究では、マウスの系統差による影響はあまり注目されていない。

4-Aminobiphenyl が代謝され、肝臓や膀胱で DNA と付加体を形成する基本的な酵素と考えられ ているのは CYP1A2 である(Butler ら、1989 年 a、1989 年 b)。しかし、CYP1A2(+/+)野生型マウ スと CYP1A2(-/-)ノックアウトマウスの影響を比較すると、予想に反して、CYP1A2 の発現は、4-Aminobiphenyl 誘発性酸化ストレスまたは 4-Aminobiphenyl-DNA 付加体形成とは関連していない ことが示された。さらに、他の第1相及び第2相反応の酵素の発現とともに肝 CYP1A2 タンパク 質の発現を5倍に増加させた 2,3,7,8-テトラクロロジベンゾ-p-ジオキシン(TCDD)は、肝臓にお ける付加体量を変化させないか、または実際に減少させた。このような誘導の定量的効果は、誘 導された酵素のバランスに依存するであろう。これらの結果は、CYP1A2 が 4-Aminobiphenyl の主 要な代謝的活性化因子ではないか、CYP1A2 の非存在下でもマウスの他の酵素がこの化合物を活 性化させていることを示唆している(Tsuneoka ら, 2003 年)他の芳香族アミンを用いた研究に基 づくと、活性化酵素は他にも、P-450 酵素、酸化酵素、またはペルオキシダーゼを含むかもしれな い(Lakshmi ら、1990 年; Smith ら、1991 年; Hughes ら、1992 年)。 4-Aminobiphenyl によって誘導される発がんにおいて重要であると考えられている別の反応は、 アセチル化である。アセチル化は 4-Aminobiphenyl の発がんにいくつかの役割を果たしている。*N*hydroxy-4-aminobiphenyl の *O*-アセチル化及び *N*,*O*-アセチル化は、ヒトではリスクを増加させると 予想されるが、4-Aminobiphenyl の *N*-アセチル化はリスクを減少させるはずである(Lower ら、 1979年)。アセチル化は、NAT1 または NAT2 によって触媒され、後者は集団内で顕著な多型が存 在する(Hein ら、2000年; Cascorbi ら、2001年)。親アミンである 4-Aminobiphenyl のアセチル化 はヒトでは解毒過程と考えられているため、アセチル化が遅い表現型は膀胱がんのリスクを増加 させると予測されている。それゆえ、アセチル化が速い表現型はリスクの減少と関連しているは ずである。

しかし、マウスの acetylator の表現型の研究では、相反する結果が得られている。ある研究では、 他のすべての点で明らかに同一であると思われるホモ接合性の rapid acetylator マウスまたはホモ 接合性の slow acetylator マウスの雄雌に、4-Aminobiphenyl 塩酸(55-300 mg/L)を 28 日間飲水投与 した。肝 DNA 付加体のレベルは雌雄ともに投与量の増加とともに増加したが、acetylator の表現 型とは無関係であった。膀胱では、DNA 付加体は雄マウスでは 100 mg/kg でプラトーに到達し、 acetylator の表現型とは無関係であった。雌マウスでは、DNA 付加体量は雄マウスよりも低く、最 高用量で減少したが、予想に反して rapid acetylator 表現型では DNA 付加体量が高かった (Flammang ら、1992 年)。これらの結果は、アセチルトランスフェラーゼ活性がマウスの DNA 付加体形成の 速度を決定するものではないことを示唆していると解釈された。McQueen ら(2003 年)の研究で は、C57BL/6、B6.A 及び A/J マウス株と、ヒト NAT1 トランス遺伝子を持つトランスジェニック マウス hNAT1:A/J 及び hNAT1:C57 を用いて、マウス NAT2 対立遺伝子と 4-Aminobiphenyl-DNA 付 加体量との間には相関関係がないという同様の結論に達した。しかし、マウスの NAT2 活性の違 いは軽微であり、おそらく 4-Aminobiphenyl の遺伝毒性に影響を与えるには十分ではなかった。最 近の研究では、ヒトでは N-hydroxy-4-aminobiphenyl の 0-アセチル化には NAT2 ではなく NAT1 が 関与していることが示唆されている(Oda、2004 年)。

また、説明が必要なマウスの系統特異的突然変異もある。例えば、B6C3F1 では、4-Aminobiphenyl は、*H-ras* 遺伝子のコドン 61 において主に C→A 突然変異(非コード鎖の G→T トランスバージョンを反映)を誘発するのに対し、CD-1 マウスでは、*H-ras* 遺伝子コドン 61 における主な突然変異は A→T トランスバージョンであった(Manjanatha ら、1996 年)。

腫瘍の発生には細胞増殖も必要であるが、4-Aminobiphenylの発がん過程の初期段階での細胞増 殖を調べた研究は少ない。また、先に述べた発がん性試験(Schieferstein ら、1985年)では、膀胱 がんの発生は雄のみであったが、過形成の発生率は雌雄ともに高いことが報告されている。しか しこのことについてはそれ以上調査されていない(後述)。

要約すると、4-Aminobiphenyl 誘発性膀胱発がんの MOA は、代謝的活性化、DNA 付加体形成、 遺伝子突然変異を含む一連の key events が関与していることが明らかになった。

このことは、構造的に関連のある芳香族アミンを用いた研究によってさらに強化されている。 しかし、関与する特定の酵素の詳細、種間での臓器特異性の違いの根拠や効力の詳細、ヒトにお ける用量反応曲線の形状については、データギャップが残っている。これは、競合する代謝経路 の複雑さを考えれば、想定内である。利用可能なデータは仮説 MOA を支持するのに十分である と考えられるが、これらの不確実性の影響については総合的な定量的評価を考慮する必要がある (表 3)。

## 表 3. 4-Aminobiphenyl 膀胱発がん性に影響を与える調節因子

- 1 エステル化酵素の競合的活性
- 2 酵素の活性化または不活性化に影響を与える遺伝的多型(例: slow acetylator 及び rapid acetylator)
- 3 尿の pH(主に食事の影響を受ける)と他の尿成分
- 4 尿路粘膜細胞増殖(4-アミノビフェニルの高用量投与、または尿路粘膜増殖に影響を与える 他の薬剤との併用投与により誘導される)

# 実験動物とヒトとの間の key evants の根本的、質的な違いに基づいて、MOA のヒト との関連性を合理的に排除することができるか

4-Aminobiphenyl 誘発性膀胱がんの key events それぞれを支持するヒト及びヒトの細胞系におけ る有力なエビデンスがある。N-hydroxylamine への代謝的活性化が実証されており、活性化のため にいくつかの酵素が示されているほか、N-アセチル化など N-ヒドロキシル化の効果を増強または 減少させる可能性のある他の酵素も示唆されている。遺伝的多型はこれらの酵素活性に大きく影 響し、4-Aminobiphenyl ばく露に対する膀胱発がんの感受性に影響する変異を生じさせる。マウス やイヌの DNA から検出されたものと同一の DNA 付加体がヒトの尿路上皮細胞で確認されてお り、同様の変異原性を有することが明らかになった。さらに、広範な疫学的研究から、4-Aminobiphenyl のヒトにおける膀胱発がん性が証明されている。

膀胱がんは、喫煙や職業的ばく露といった 4-Aminobiphenyl の摂取と関連している。4-Aminobiphenyl は、1935年から1955年まで米国で製造され(Melick ら、1955年)、効率のいいゴ ム酸化防止剤として使用されていたが、現在では商業的には生産されていない。市販の 4-Aminobiphenyl に職業的にばく露された労働者の1系列に限定した疫学研究では、膀胱がんの発生 率が高いことが報告されている(Melick ら、1955年、1971年; Melamed ら、1960年; Koss ら、 1965年、1969年)。503人の労働者のうち、細胞診において 59例が陽性、そのうち 35例は組織学 的に膀胱がんが確認された;公表時、7例は細胞診において陽性のまま、7例は他の原因で死亡し、 10例は追跡調査できていない(Koss ら、1969年)。たばこの煙に加えて、炭素及び窒素を含む物 質の燃焼で生じるものへのばく露や不明確な環境中の物質もあるようである(Skipper ら、2003年)。

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喫煙が原因とされているものは、米国とヨーロッパにおける膀胱がんの症例の 40%から 70%を占めている(IARC、1986年; Castelao ら、2001年)。黒タバコ(自然乾燥)は、ブロンドタバコ(熱風乾燥)に比べて 4-Aminobiphenyl をより多く含んでいる(Bryant ら、1988年)。

4-Aminobiphenylの膀胱発がんにおけるマウスやイヌで示された key events は、主にタバコの煙中の 4-Aminobiphenyl にばく露されたヒトを対象に評価されているが、ヒトの尿路上皮細胞を用いた in vitro 評価法も利用されている(表4を参照)。

表 4.4-Aminobiphenyl による尿路膀胱発がんの key events の種間一致性評価

key events	マウス	イヌ	ヒト
1. 反応性親電子物質への代謝的活性化	+	+	+
2. DNA 付加体の形成	+	+	+
3. 変異原性	+	+	+
4. 発がん	+	+	+

吸収された 4-Aminobiphenyl は、CYP1A2 によって肝臓で N-酸化される。CYP1A2 は CYP1A1 との相同性がかなり高いにもかかわらず、異なる基質特異性を有しており、肝臓でのみ見出される (Lang & Pelkonen、1999)。他の酵素は、N-hydroxylamine への代謝的活性化をサポートする。

NAT1 及び NAT2 はそれぞれ、3 種類のアセチル化を触媒する。すなわち、アリルアミンの N-ア セチル化、N-hydroxylamineのO-アセチル化及びアリルヒドロキサム酸のN, O-アセチル化である (Flammang & Kadlubar、1986 年; Mattano ら、1989 年; Fretland ら、1997 年; Hein ら、2000 年)。 N-アセチルトランスフェラーゼによる N-アセチル化は、主に、形成された 4-Aminobiphenyl のア セトアミドがアミンに比べて N-ヒドロキシル化の基質として小さいために、膀胱発がん性に対し て保護効果を有すると考えられている。NAT アイソフォームをコードする 2 つの遺伝子には、 NATIとNAT2 があり、対立遺伝子の変異はヒトにおける膀胱がんの感受性と関連している(Hein ら、2000年)。ほとんどの研究は、NAT2の slow acetylator が膀胱発がんのリスク増加を示唆して いるが、芳香族アミン膀胱発がんに対する NATI 遺伝子型の寄与は明らかではない(Cartwright ら、 1982年; Hein ら、2000年)。喫煙者の間では、slow acetylator の発現に関連する 4-Aminobiphenyl -ヘモグロビン付加体がより多く存在する (Vineis ら、1990年)。また、NAT1 と NAT2 の相互作用 が示唆されている(Cascorbiら、2001年)。ドイツの膀胱がん患者 425 人を対象とした研究で、 Cascorbi ら(2001 年)は、以下のことを明らかにした。(1) NATI\*10 遺伝子型の NAT2\*4 遺伝子 型への部分的な連鎖(2)調査した症例中の rapid NAT2 遺伝子型を有するヒトにおける、NAT1\*10 遺伝子型の発現量の低さ(3)職業ばく露歴とNAT2\* slow /NATI\*4 遺伝子型の組み合わせは、職業 ばく露によるリスクのない対照群に比べて、がん症例は 5.96 倍(2.96-12.0)の頻度であった  $(P < 0.0001)_{\odot}$ 

したがって、特に環境リスク因子にばく露される場合、NAT2\*4 及び NAT1\*10 を有する個体は、 膀胱がんのリスクが有意に低いことが示唆される。

*CYP1A2* (Oscarson ら、n.d.) 及び *NAT2* (Hein ら、2000 年)遺伝子の多型は、ヒト集団における これらの酵素活性の変動と関連しているが、CYP1A2 活性の変動が遺伝的要因によるものである かはまだ明らかにされていない (Sachse ら、2003 年)。さらに、喫煙者に誘導される *CYP1A2* 遺伝 子の発現は、CYP1A2 酵素活性を高める (Sesardic ら、1988; Eaton ら、1995 年)。4-Aminobiphenyl にばく露され、CYP1A2 と高い *slow NAT2* 活性を発現する個体は、CYP1A2 と低い *rapid NAT2* 活 性を発現する個体よりも、*N*-hydroxy-4-aminobiphenyl のレベルが高い。したがって、肝臓と膀胱に おける 4-Aminobiphenyl -ヘモグロビン付加体と 4-Aminobiphenyl-DNA 付加体の高いレベルを有す ることが予測される。

腫瘍抑制遺伝子 RB1 及び TP53 は、膀胱がん、特に低悪性度の乳頭腫瘍よりも高悪性度の尿路 上皮がんに関与しているようである。両遺伝子は細胞周期の調節に関与している。さらに、TP53 は、DNA 損傷、細胞死及び血管新生の役割を果たし(Hickman ら、2002 年)、その遺伝子産物は、 複数の遺伝子の発現を調節する(Vousden & Lu、2002 年)。RB1 の不活性化と筋肉内への浸潤に強 い関連が見出されている(Cairns ら、1991 年; Ishikawa ら、1991 年; Presti ら、1991 年; Primdahl ら、2000年)。45 例の膀胱がんを対象とした研究では、TP53 変異を有する 9 人のうち 7 人がグレ ード3の腫瘍(周囲組織への浸潤を含む)であった(Martoneら、1998年)。RB1の不活性化は、 筋肉内浸潤性膀胱がんの 30~80%で発生し(Cairns ら、1991 年; Logothetis ら、1992 年; Wright ら、1995年; Ioachim ら、2000年)、多くの場合、ヘテロ接合性 13q 欠失と残りの対立遺伝子の突 然変異の結果発生する (Cordon-Cardo & Reuter、1997 年)。少なくとも 30 の腫瘍を調査した研究 では、浸潤性膀胱がんの 40~60%で TP53 突然変異が認められた(Tiguert ら、2001 年; Lu ら、 2002年)。特定変異のホットスポットは確認されなかったが、変異の90%以上はエクソン4-9に発 生していた。TP53のエクソン 5、7 及び 8 を含む DNA 断片における N-hydroxy-4-aminobiphenyl の 結合スペクトルの研究では、非 CpG 部位である 285 コドン及び CpG 部位である 175 及び 248 コ ドンに優先的に結合することが確認されたが、C5シトシンメチル化が起こった後にのみ確認され た(Fengら、2002 年)。著者らは、膀胱がんにおける TP53 の変異スペクトルが、この腫瘍の原因 である 4-Aminobiphenyl の役割を強く示唆していると結論づけた。

4-Aminobiphenylの環境中ばく露の源であるタバコの煙へのばく露は、成人と胎児の両方で、4-Aminobiphenyl-ヘモグロビン付加体量の増加と関連している。喫煙者(*n*=14)と非喫煙者(*n*=38)の女性の研究では、4-Aminobiphenyl-ヘモグロビンレベルは、喫煙者では183±108 pg/g ヘモグロビン、非喫煙者では22±8 pg/g ヘモグロビンであったのに対し、それぞれの胎児のレベルは92±54 pg/g ヘモグロビンと17±13 pg/g ヘモグロビン (Coghlin ら、1991年)であった。この差は、腫瘍組織 DNA の研究において成人でも観察されている (Curigliano ら、1996年)。イタリア、トリノの男性における研究では、ヘモグロビン付加体量(ばく露量の代用として、また DNA 付加体の可能性の指標として使用される)は、4-Aminobiphenyl の供給源であるタバコへのばく露量と関連しており(黒タバコ>膀胱がんのリスクでも同様の傾向がみられた(Bryant ら、1988年)。主にばく露の指標である付加体の存在とがん発生とを結びつける情報には大きなギャップがある。

ヒトでは、4-Aminobiphenyl は膀胱がんとのみ関連しているが、マウスでは肝臓と膀胱の腫瘍が 誘発されている。臓器特異性におけるこれらの種差の具体的な理由は明らかにされていないが、 競合する N-エステル化酵素の活性化の違いによるものと考えられる。硫酸化は芳香族アミンによ る肝発がんと密接に関連しているようだが、N-グルクロン酸化は膀胱発がんと関連しているよう である。アセチル化には様々な効果があるが、ヒトでは主に解毒プロセスであり、アセチル化が 速いか遅いかを決定する*N*-アセチルトランスフェラーゼの型に大きく影響される。ヒトの組織が、 4-Aminobiphenyl とその代謝物の代謝に関与しているかについて研究されてきた。CYP1A2は、ヒ ト肝ミクロソーム分画による 4-aminobiphenyl から N-hydroxy-4-aminobiphenyl への代謝に関与して いる (Butler ら、1989 年 b)。*N*-hydroxy-4-aminobiphenyl は、ヒト肝臓及びそれより程度は低いが、 結腸由来の細胞質性スルホトランスフェラーゼによって、牛胸腺 DNA と共有結合する生成物に 代謝されるが、膵臓や膀胱由来のものによっては代謝されない。膀胱でのスルホトランスフェラ ーゼ活性の欠如を考慮すると、肝スルホトランスフェラーゼは肝外組織での N-hydroxy-4aminobiphenyl の生物学的利用能を低下させ、膀胱の解毒機構として機能する可能性が示唆されて いる (Chou ら、1995 年)。一方、ヒト尿路上皮細胞に存在する N-アセチルトランスフェラーゼ (Frederickson ら、1992; Swaminathan & Reznikoff、1992年)は、N-hydroxy-4-aminobiphenyl 及び そのアセチル化化合物 N-hydroxy-4-acetylaminobiphenyl 及び N-アセトキシ-4-アセチルアミノビフ エニルを DNA 反応性物質に代謝することができる。N-(deoxyguanosin-8-yl)-4-aminobiphenyl と主 要な付加体はコクロマトグラフされ、N-hydroxy-4-aminobiphenyl のサイトゾル媒介結合で得た DNA を用いた <sup>32</sup>P-ポストラベリング法により、4 つの放射性スポットが明らかになった。健康な ヒト尿路上皮細胞を用いた場合には5つの付加体が認められたが、そのうち2つはサイトゾルを 用いた場合に認められた2つの付加体と同じであった。このことから、アセチル化以外の活性化 経路の存在が示唆された。

イヌ組織を用いた実験と同様の実験により、ヒトの尿路上皮細胞ミクロソームがアセチル基転移活性を有していることが示されており、N-hydroxy-4-aminobiphenylのRNAやDNAへの結合は、 4-Acetylaminobiphenyl、N-hydroxy-4-acetylaminobiphenyl、またはアセチル CoA をアセチル基供与体とした場合に起こることが示されている(Hatcher ら、1993 年)。また、N-hydroxy-4-aminobiphenyl、 N-acetoxy-4-acetylaminobiphenyl、N-acetoxy-4-aminobiphenyl と反応させて生成した DNA 付加体の  $^{32}$ P-ポストラベリングは、類似のプロファイルを示し、N-acetoxy-4-aminobiphenyl から生じるアリ ルニトレニウムイオンが共通の反応種であることを発見した。この付加体の構造は、N-(deoxyguanosin-8-yl)-4-aminobiphenyl(dG-C8-aminobiphenyl) や N-(deoxyadenosin-8-yl)-4aminobiphenyl(dA-C8-aminobiphenyl)の 3',5'-ビスホスホ誘導体(Frederickson ら、1992 年; Hatcher & Swaminathanosin、1995 年)及び N-(deoxyguanosin-N(2)-yl)-4-azobiphenyl (Hatcher & Swaminathan、 2002 年)として同定されている。

喫煙者と非喫煙者を比較した結果は、仮説 MOA のヒトへの関連性を支持するものである。T1 膀胱がん 46 例の研究では、4-Aminobiphenyl-DNA 付加体の平均相対染色強度は、非喫煙者(113±71、n=22)よりも現在の喫煙者(275±81、n=24)の方が有意に高かった(Curigliano ら、1996 年)。同様の結果が喉頭組織(Flamini ら、1998 年)及び乳腺組織(Faraglia ら、2003 年)についても報告されている。4-Aminobiphenyl-ヘモグロビン付加体をばく露の指標として使用すると、膀胱がん患者は対照者よりも高いレベルであったのに対し(Del Santo ら、1991 年)、肺がん患者はそうではなかった(Weston ら、1991 年)。この違いの根拠は不明である。

ヒト以外の試験系で得られた遺伝毒性のエビデンスに加えて、4-Aminobiphenylは、ヒト尿路上 皮細胞のミクロソーム調製物によって、*S. typhimurium* YG1024(*O*-アセチルトランスフェラーゼ 活性の高い TA98 の派生株)の変異原性物質に代謝されることがあるが、TA98 株自体では代謝さ れない(Hatcher ら、1993 年)。この研究では、他の種または他のヒト組織は調べられていない。

6-チオグアニン耐性変異体は、4-Aminobiphenyl 自体へのばく露、または N-hydroxy-4aminobiphenyl、N-hydroxy-4-aminobiphenyl、N-acetoxy-4-acetylaminobiphenyl へのばく露によって、 非腫瘍性 SV40 不死化ヒト尿路上皮細胞株で誘導することができる(Bookland ら、1992 年 a)。観 察された活性には、外因性代謝的活性化経路は必要とされなかった。変異体分画の統計的に有意 な増加をもたらすための最小濃度は以下の通りであった。N-acetoxy-4-acetylaminobiphenyl、2 µmol/L; N-hydroxy-4-acetylaminobiphenyl、5µmol/L; N-hydroxy-4-aminobiphenyl、20µmol/L; 及び 4-Aminobiphenyl、100 µmol/L。また、これらの物質のうちの 3 つは処理した細胞をヌードマウス に皮下注射したときの発がんをエンドポイントとした in vitro-in vivo アッセイにおいて、同じヒト 不死化尿路上皮細胞を使用した腫瘍性形質転換についても検討が実施された(Bookland ら、1992 年 b)。形質転換はすべての試験で示されたが、最小濃度は以下の通りであった。N-hydroxy-4acetylaminobiphenyl、0.5 µmol/L; N-hydroxy-4-aminobiphenyl、0.5 µmol/L; 及び 4-Aminobiphenyl、0.5 µmol/L; N-hydroxy-4-aminobiphenyl、0.5 µmol/L; 海防 4-Aminobiphenyl、20 かどのように解釈すべきかは明らかではない。それは、形質転換が突然変異に依存せず、形質転換 次要素のようにするの素のと同じているか、または単にあるの感

要約すると、マウス、イヌ、ヒトにおいて、MOA の key events は定性的には同じである: *N*-hydroxylamine への代謝的活性化とそれに続く反応性親電子物質(おそらくニトレニウムイオン)の形成、グアニン付加体の形成、遺伝子突然変異及びがんの形成である。どの遺伝子が変異し、 どのようにしてがんが誘発されるのかなど、遺伝子変異とがんとの間に介在する事象は明らかに されていない。それにもかかわらず、MOA は明確に示されており、動物モデルでもヒトでも同じ である。

# 実験動物とヒトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、MOAのヒトへの関連性を合理的に排除することができるか

以上詳述したように、4-Aminobiphenylの代謝的活性化、DNA 付加体、変異原性は、マウス、イ ヌ、ヒトでは定性的に同じであり、これら3種では膀胱腫瘍を誘発し、マウス、ラット、ウサギ ではその他の腫瘍を誘発する。吸収、分布、排泄に関する詳細は報告されていないが、マウス、 イヌ、ヒトで発生する尿路上皮における DNA 付加体形成量が類似していることから、3種の間で は速度論的な差異は有意ではないことが示唆されている。

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3 種の酵素の過程は類似しているが、量的な違いは明らかである。これらの違いは、種間で見られ る標的臓器の特異性の違いの一部を説明し、DNA 付加体の生成における定量的な違いを示唆して いる可能性がある。それにもかかわらず、これらの違いは、どの種でも、あるいは異なる標的臓 器でも、全体的な MOA を否定するものではなく、代謝的活性化と不活性化のための競合する経 路の複雑さと一致している。

おそらく、異なる付加体の修復能や種間、組織間における量的な違いが存在するだろう。しか し、3種すべてで比較的多くの付加体が検出されたことから、かなり多くの安定的な付加体が産 生されていることがわかる。

マウス、イヌ、ヒトに共通する標的組織である膀胱の尿路上皮は、形態学的に類似している(Pauli ら、1983年)。尿路上皮は、腔内表面の尿に対するバリア機能の大部分を担う特徴的な非対称性単 位膜を有している。尿路上皮は、尿路上皮特異的タンパク質であるウロプラキンから構成されて おり、その配列は種間で高度に保存されている(Wuら、1994年)。さらに、尿路上皮は、3種す べての種において代謝的に活性化している。

また、尿 pH や排尿頻度など、尿路上皮 DNA 付加体の最終的な産生量に影響を与える因子も同 定されている(Cohen ら、1995年; Sarkar ら、2002年)。pH の範囲は種によって異なるが、マウ ス、イヌ及びヒトの尿 pH は、容易に酸性及びアルカリ性、また中性に変動する。繰り返しになる が、量的な違いは生じるものの、これらはヒトにおける MOA の存在を排除するものではない。

DNA 反応性以外の MOA を示唆するエビデンスはない。しかし、4-Aminobiphenyl の膀胱発がん に対する見かけの影響に関しては、種間で量的な差が存在する。4-Aminobiphenylの代謝物はDNA と同様にタンパク質(ヘモグロビンなど)とも相互作用し、4-Aminobiphenylの代謝物は細胞毒性 を有することが明らかになっている (Schieferstein ら、1985 年; Reznikoff ら、1986 年; Kadlubar ら、1991年)。4-Aminobiphenylの高用量投与によるマウス膀胱の細胞毒性と再生性増殖には、尿 路上皮細胞タンパク質との相互作用が関与している可能性がある。DNAの反応性と、相反する変 異原性と細胞増殖の相互作用は、DNA 付加体の用量反応が直線的であるにもかかわらず、腫瘍の 用量反応曲線がシグモイド型であることを説明している(Cohen & Ellwein、1990年)。尿路上皮細 胞毒性を生じる可能性があるマウスの尿中に見られる高濃度の4-Aminobiphenvlは、一般的に受動 喫煙者では達成されていない。しかし、他の(未知の)物質は、喫煙者(Auerbach & Garfinkel、 1989年)で尿路上皮の過形成を生成するように見える。この細胞増殖の増加は、標的となる DNA 複製細胞の数を大幅に増やし、正常にゆっくりと複製する少数の尿路上皮と比較して、膀胱に対 する 4-Aminobiphenyl の影響を大幅に増強する。このように、マウスと比較してヒトの方が 4-Aminobiphenyl の明らかに大きな効力は考えにくい。しかし、マウスでは 4-Aminobiphenyl 単一の 物質による DNA 反応性と細胞増殖の相乗効果がタバコの煙の複雑な混合物中の様々な物質によ って引き起こることが示されている。

4-Aminobiphenyl への職業的ばく露は、たばこの煙よりも 4-Aminobiphenyl の用量が多いと考えられる。なぜなら、膀胱がんの発生率が喫煙者に比べて職業的にばく露される集団の方がかなり高かったためである。しかし、これらの職業ばく露が発生した時点では代謝物濃度や尿路上皮細胞内の DNA 付加体レベルを定量的に測定することができず、また、個人の喫煙歴を評価することはなかった(Koss ら、1965 年、1969 年)。

要約すると、種間での量的な違いは存在するが、マウスやイヌと同じ MOA がヒトにも当ては まることを排除するものではない。

#### |結論:信頼性、解析及び帰結の記述

推定される MOA の初期段階は、代謝的活性化、DNA 付加体形成及び突然変異の key events が、 マウス、イヌ及びヒトにおいて定性的に同じであることを示す。これは利用可能なエビデンスに よって十分に支持されている。4-Aminobiphenyl がヒトの膀胱発がん物質であるという強力かつ十 分なエビデンスがある。しかし、突然変異とがんの発生との間に介在するステップについてのエ ビデンスは不足している。付加体量と TP53 突然変異との関連について説明したが、これらの特定 の遺伝的変化は腫瘍の進行から遅れて現れ、しばしば内因性の原因 (例えば、メチル化された CpG 部位での脱プリン) であるため、説得力のあるものではない。膀胱がんにおける TP53 突然変異に ついては、症例対照研究で研究されている (Schroeder ら、2003 年)。さらに、ヒトの尿路上皮腫 瘍のほとんどは低悪性度の乳頭病変であり、一般に TP53 変異を有していない。

N-hydroxy-4-acetylaminobiphenylの変異スペクトルは、Big Blue mouseの胚性線維芽細胞におい て研究されている(Bessaratinia ら、2002年)。これらの細胞を24時間処理すると、cII 遺伝子の突 然変異頻度がバックグラウンドと比較して最大 12.8 倍の用量依存的な増加をもたらした。一塩基 置換は、処理細胞では突然変異の 86%、対照細胞では突然変異の 74%を占めていた。これらの変 異のうち、63%と 36%はそれぞれ cll 遺伝子に沿ったグアニン残基で発生した。処置細胞では cll 遺伝子の変異の 47%が G→T のトランスバージョンであったのに対し、対照細胞では最も一般的 な変異は挿入であり、これは変異の19%を占めていた。誘導された付加体のマッピングにより、 5 つの DNA 付加体部位が確立された。そのうちの 4 つは N-hydroxy-4-acetylaminobiphenyl による 主な変異部位であり、特に G→T トランスバージョンを生じていた。しかしながら、ヒト膀胱が んの TP53 遺伝子では、G→A トランジションが優勢(53%) であり、その5つの変異ホットスポ ット(コドン175、248、273、280、285)のすべてで優勢で、そのうちの3つはメチル化 CpGホ ットスポット(175、248、273)にあった。cll 遺伝子では、誘導される突然変異ホットスポット も、メチル化 CpG ジヌクレオチドに偏っていない。この研究から、*cII* 遺伝子における *N*-hydroxy-4-acetylaminobiphenyl によって誘導される突然変異パターンと、ヒト膀胱がんにおける TP53 遺伝 子で観察される突然変異パターンとの間には大きなギャップがあると結論づけられる。しかし、 4-Aminobiphenyl によって誘導される突然変異スペクトルにおけるメチル化状態や転写活性の役 割はまだ明らかにされていない。また、TP53遺伝子の変異スペクトルは、腫瘍発生過程の選択を 反映したものであることにも留意しなければならない。

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以上の解析から、4-Aminobiphenyl 発がん MOA は動物モデルで知られており、その MOA は定 性的にも定量的にもヒトに関連していることが明らかになった。この評価に基づく結論は、疫学 的なエビデンスがなくても、4-Aminobiphenyl はヒトに対して発がんの危険性があるということで ある。

完全なリスク評価を行うためには、用量反応及びヒトへのばく露に関する追加情報が必要であ る。上記の情報に基づいて、職業的(過去の)ばく露と喫煙ばく露など予想されるばく露による 発がんリスクを予測している。非喫煙者の環境ばく露における潜在的リスクについては、さらな る解析が必要である。MOA 解析は、そのような評価のための基礎と基盤を提供する。4-Aminobiphenyl に関する疫学的エビデンスは、MOA の HRF によって推定される結論を支持してい る。

## 4-Aminobiphenyl とヒト関連性フレームワーク

4-Aminobiphenyl は、提唱されている IPCS HRF を用いて MOA 解析に基づいて評価した。その 結果、DNA 反応性 MOA の key events である代謝的活性化、DNA 付加体形成、変異原性、発がん は、ヒトにおいてもマウスやイヌなどの動物モデルと同様であり、4-Aminobiphenyl はヒトに対し て発がんの危険性を示すことが明らかになった。この MOA 解析の情報は、ヒトに対する完全な がんリスク評価を構築するための実質的な基礎を提供するものである。この化学物質については、 HRF 解析から導き出された結論を検証するための実質的な疫学的エビデンスも存在する。

この MOA では、どのような遺伝子が突然変異し、その遺伝子変化がどのようにして発がんに つながるのかという追加の key events は、4-Aminobiphenyl では知られていない。しかし、ここで 提示されたフレームワーク解析に基づいて MOA のエビデンスの信頼性を考えると、このことは 結論を損なうものではない。

ある化学物質が DNA 反応性 MOA によって発がんすると結論づけるには、どのようなデータが 必要なのか?我々が提案するのは、少なくとも DNA 付加体が、標的組織で生成され、その化学物 質に変異原性(代謝的活性化の有無にかかわらず)があることを実証することである。変異原性 は、ここでは遺伝毒性という広い意味よりも、より具体的で限定的な意味で用いられている。in vivo ばく露後の標的組織における DNA 付加体及び変異原性が証明されれば、推定された MOA の 信頼性が向上する。特定の代謝経路と誘導される特定の DNA 付加体を同定することにより、動物 モデルをヒトに外挿するための優れた根拠が得られる。

この事例は、MOA 解析における代替化合物に関するデータの潜在的な有用性を示している。しかし、関連化合物に関するデータの妥当性は、in vivo であれ in vitro であれ、十分に正当化される必要がある。また、DNA 反応性や変異原性については、これまでに十分に開発されてきた構造活性相関に関するエビデンスの重み付け解析も、フレームワーク解析に貢献すべきである。

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# 参考文献

al-Atrash J, Zhang YJ, Lin D, Kadlubar FF, Santella RM (1995) Quantitative immunohistochemical analysis of 4-aminobiphenyl–DNA cultured cells and mice: Comparison to gas chromatography/mass spectroscopy analysis. *Chemical Research in Toxicology*, **8**:747–752.

Auerbach O, Garfinkel L (1989) Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer*, **64**:983–987.

Besaratinia A, Bates SE, Pfeifer GP (2002) Mutational signature of the proximate bladder carcinogen N-hydroxy-4-acetylaminobiphenyl: Inconsistency with the p53 mutational spectrum in bladder cancer. *Cancer Research*, **62**:4331–4338.

Block NL, Sigel MM, Lynne CM, Ng AB, Grosberg RA (1978) The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. *Investigative Urology*, **16**:50–54.

Bonser GM (1962) Precancerous changes in the urinary bladder. In: Severi L, ed. *The morphological precursor of cancer*. Perugia, University of Perugia, p. 435.

Bookland EA, Reznikoff CA, Lindstrom M, Swaminathan S (1992a) Induction of thioguanine-resistant mutations in human uroepithelial cells by 4-aminobiphenyl and its *N*- hydroxy derivatives. *Cancer Research*, **52**:1615–1621.

Bookland EA, Swaminathan S, Oyasu R, Gilchrist KW, Lindstrom M, Reznikoff CA (1992b) Tumorigenic transformation and neoplastic progression of human uroepithelial cells after exposure in vitro to 4-aminobiphenyl or its metabolites. *Cancer Research*, **52**:1606–1614.

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR (1988) Hemoglobin adducts of aromatic amines: Associations with smoking status and type of tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, **85**:9788–9791.

Burger MS, Torino JL, Swaminathan S (2001)DNAdamage in human transitional cell carcinoma cells after exposure to the proximate metabolite of the bladder carcinogen 4- aminobiphenyl. *Environmental and Molecular Mutagenesis*, **38**:1–11.

Butler MA, Guengerich FP, Kadlubar FF (1989a) Metabolic oxidation of the carcinogens 4aminobiphenyl and 4,4q-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Research*, **49**:25–31.

## IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989b) Human cytochrome P-450PA (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3- demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proceedings of the National Academy of Sciences of the United States of America*, **86**:7696–7700.

Cairns P, Proctor AJ, Knowles MA (1991) Loss of heterozygosity at the *RB* locus is frequent and correlates with muscle invasion in bladder carcinoma. *Oncogene*, **6**:2305–2309.

Cairns T (1979) The ED<sub>01</sub> study: Introduction, objectives, and experimental design. *Journal of Environmental Pathology and Toxicology*, **3**:1–7.

Cartwright RA, Rogers HJ, Barham-Hall D, Glashan RW, Ahmad RA, Higgins E, Kahn MA (1982) Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **16**:842–846.

Cascorbi I, Roots I, Brockmoller J (2001) Association of *NAT1* and *NAT2* polymorphisms to urinary bladder cancer: Significantly reduced risk in subjects with *NAT1\*10*. *Cancer Research*, **61**:5051–5056.

Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, Ross RK, Yu MC (2001) Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute*, **93**:538–545.

Chou HC, Lang NP, Kadlubar FF (1995) Metabolic activation of the *N*-hydroxy derivative of the carcinogen 4-aminobiphenyl by human tissue sulfotransferases. *Carcinogenesis*, **16**:413–417.

Clayson DB, Lawson TA, Santana S, Bonser GM (1965) Correlation between the chemical induction of hyperplasia and of malignancy in the bladder epithelium. *British Journal of Cancer*, **19**:297–310.

Clayson DB, Lawson TA, Pringle JAS (1967) The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of C57  $\times$  IF mouse. *British Journal of Cancer*, 1:755–762.

Coghlin J, Gann PH, Hammond SK, Skipper PL, Taghizadeh K, Paul M, Tannenbaum SR (1991) 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, **83**:274–280.

Cohen SM (1995) The role of urinary physiology and chemistry in bladder carcinogenesis. *Food and Chemical Toxicology*, **33**:715–730.

Cohen SM, Ellwein LB (1990) Proliferative and genotoxic cellular effects in 2- acetylaminofluorene bladder and liver carcinogenesis: Biological modeling of the  $ED_{01}$  study. *Toxicology and Applied Pharmacology*, **104**:79–93

Cordon-Cardo C, Reuter VE (1997) Alterations of tumor suppressor genes in bladder cancer. *Seminars in Diagnostic Pathology*, **14**:123–132.

Curigliano G, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A, Santella RM (1996) Immunohistochemical quantitation of 4-aminobiphenyl–DNAadducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis*, **17**:911–916.

Dang LN, McQueen CA (1999) Mutagenicity of 4-aminobiphenyl and 4-acetylbiphenyl in *Salmonella typhimurium* strains expressing different levels of *N*-acetyltransferase. *Toxicology and Applied Pharmacology*, **159**:77–82.

Deichmann WB, MacDonald WE (1968) The non-carcinogenicity of a single dose of 4-aminobiphenyl in the dog. *Food and Cosmetics Toxicology*, **6**:143–146.

Deichmann WB, Radomski JL, Anderson WAD, Coplan MM, Woods FM (1958) The carcinogenic action of *p*-aminobiphenyl in the dog; final report. *Industrial Medicine and Surgery*, **27**:25–26.

Deichmann WB, Radomski JL, Glass E, Anderson WAD, Coplan M, Woods FM (1965) Synergism among oral carcinogens. Simultaneous feeding of four bladder carcinogens to dogs. *Industrial Medicine and Surgery*, **34**:640–649.

Delclos KB, Miller DW, Lay JO Jr, Casciano DA, Walker RP, Fu PP, Kadlubar FF (1987) Identification of C8-modified deoxyinosine and N2- and C8-modified deoxyguanosine as major products of the in vitro reaction of *N*-hydroxy-6-aminochrysene withDNA and the formation of these adducts in isolated rat hepatocytes treated with 6-nitrochrysene and 6- aminochrysene. *Carcinogenesis*, **8**:1703–1709.

Del Santo P, Moneti G, Salvadori M, Saltutti C, Delle RA, Dolara P (1991) Levels of the adducts of 4aminobiphenyl to hemoglobin in control subjects and bladder carcinoma patients. *Cancer Letters*, **60**:245–251.

Doerge DR, Churchwell MI, Marques MM, Beland FA (1999) Quantitative analyses of 4aminobiphenyl–C8-deoxyguanosylDNAadducts produced in vitro and in vivo using HPLC- ES-MS. *Carcinogenesis*, **6**:1055–1061.

Dooley KL, Stavenuiter JF, Westra JG, Kadlubar FF (1988) Comparative carcinogenicity of the food pyrolysis product, 2-amino-5-phenylpyridine, and the known human carcinogen, 4- aminobiphenyl, in the neonatal B6C3F1 mouse. *Cancer Letters*, **41**:99–103.

Dooley KL, Von Tungeln LS, Bucci T, Fu PP, Kadlubar FF (1992) Comparative carcinogenicity of 4aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1 male mouse. *Cancer Letters*, **62**:205–209. Eaton DL, Gallagher EP, Bammler TK, Kunze KL (1995) Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. *Pharmacogenetics*, **5**:259–274.

Faraglia B, Chen SY, Gammon MD, Zhang Y, Teitelbaum SL, Neugut AI, Ahsan H, Garbowski GC, Hibshoosh H, Lin D, Kadlubar FF, Santella RM (2003) Evaluation of 4- aminobiphenyl–DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis*, **24**:719–725.

Feng Z, Hu W, Rom WN, Beland FA, Tang MS (2002) *N*-Hydroxy-4-aminobiphenyl–DNA binding in human *p53* gene: Sequence preference and the effect of C5 cytosine methylation. *Biochemistry*, **41**:6414–6421.

Flamini G, Romano G, Curigliano G, Chiominto A, Capelli G, Boninsegna A, Signorelli C, Ventura L, Santella RM, Sgambato A, Cittadini A (1998) 4-Aminobiphenyl–DNA adducts in laryngeal tissue and smoking habits: An immunohistochemical study. *Carcinogenesis*, **19**:353–357.

Flammang TJ, Kadlubar FF (1986) Acetyl coenzyme A-dependent metabolic activation of *N*- hydroxy- $3,2_{0}$ -dimethyl-4-aminobiphenyl and several carcinogenic *N*-hydroxy arylamines in relation to tissue and species differences, other acyl donors, and arylhydroxamic acid- dependent acyltransferases. *Carcinogenesis*, **7**:919–926.

Flammang TJ, Couch LH, Levy GN, Weber WW, Wise CK (1992)DNAadduct levels in congenic rapid and slow acetylator mouse strains following chronic administration of 4- aminobiphenyl. *Carcinogenesis*, **13**:1887–1891.

Fletcher K, Tinwell H, Ashby J (1998) Mutagenicity of the human bladder carcinogen 4- aminobiphenyl to the bladder of Muta Mouse transgenic mice. *Mutation Research*, **400**:245–250.

Frederickson SM, Hatcher JF, Reznikoff CA, Swaminathan S (1992) Acetyl transferase- mediated metabolic activation of *N*-hydroxy-4-aminobiphenyl by human uroepithelial cells. *Carcinogenesis*, **13**:955–961.

Fretland AJ, Doll MA, Gray K, Feng Y, Hein DW (1997) Cloning, sequencing, and recombinant expression of NAT1, NAT2, and NAT3 derived from the C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: Functional characterization of the activation and deactivation of aromatic amine carcinogens. *Toxicology and Applied Pharmacology*, **142**:360–366.

Gaylor DW (1979) The  $ED_{01}$  study: Summary and conclusions. *Journal of Environmental Pathology and Toxicology*, **3**:179–183.

Gorrod JW, Carter RL, Roe FJ (1968) Induction of hepatomas by 4-aminobiphenyl and three of its hydroxylated derivatives administered to newborn mice. *Journal of the National Cancer Institute*, **41**:403–410.

Hammons GJ, Guengerich FP, Weis CC, Beland FA, Kadlubar FF (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. *Cancer Research*, **45**:3578–3585.

Hatcher JF, Swaminathan S (1992) Microsome-mediated transacetylation and binding of *N*- hydroxy-4aminobiphenyl to nucleic acids by hepatic and bladder tissues from dog. *Carcinogenesis*, **13**:1705–1711.

Hatcher JF, Swaminathan S (1995) Detection of deoxyadenosine-4-aminobiphenyl adduct inDNAof human uroepithelial cells treated with *N*-hydroxy-4-aminobiphenyl following nuclease P1 enrichment and <sup>32</sup>P-postlabeling analysis. *Carcinogenesis*, **16**:295–301.

Hatcher JF, Swaminathan S (2002) Identification of *N*-(deoxyguanosin-8-yl)-4-azobiphenyl by <sup>32</sup>P-postlabeling analyses of DNAin human uroepithelial cells exposed to proximate metabolites of the environmental carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **39**:314–322.

Hatcher JF, Rao KP, Swaminathan S (1993) Mutagenic activation of 4-aminobiphenyl and its *N*-hydroxy derivatives by microsomes from cultured human uroepithelial cells. *Mutagenesis*, **8**:113–120.

Hein DW (1988) Acetylator genotype and arylamine-induced carcinogenesis. *Biochimica et Biophysica Acta*, **948**:37–66.

Hein DW, Grant DM, Sim E (2000) *Arylamine* N-*acetyltransferase* (NAT) nomenclature (http://louisville.edu/medschool/pharmacology/NAT.html).

Hickman ES, Moroni MC, Helin K (2002) The role of p53 and pRB in apoptosis and cancer. *Current Opinion in Genetics and Development*, **12**:60–66.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Hughes MF, Smith BJ, Eling TE (1992) The oxidation of 4-aminobiphenyl by horseradish peroxidase. *Chemical Research in Toxicology*, **5**:340–345.

IARC (1972) 4-Aminobiphenyl. In: *Some inorganic substances, chlorinated hydrocarbons, aromatic amines,* N-*nitroso compounds, and natural products*. Lyon, International Agency for Research on Cancer, pp. 74–79 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 1).

### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

IARC (1986) *Tobacco smoking*. Lyon, International Agency for Research on Cancer, 421 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 38).

IARC (1987) 4-Aminobiphenyl (Group 1). In: *Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42*. Lyon, International Agency for Research on Cancer, pp. 91–92 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

Ioachim E, Charchanti A, Stavropoulos NE, Skopelitou A, Athanassiou ED, Agnantis NJ (2000) Immunohistochemical expression of retinoblastoma gene product (Rb), p53 protein, MDM2, c-erbB-2, HLA-DR and proliferation indices in human urinary bladder carcinoma. *Histology and Histopathology*, **15**:721–727.

Ishikawa J, Xu HJ, Hu SX, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R (1991) Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Research*, **51**:5736–5743.

Kadlubar FF, Miller JA, Miller EC (1977) Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Research*, **37**:805–814.

Kadlubar FF, Frederick CB, Weis CD, Zenser TV (1982) Prostaglandin endoperoxide synthetase-mediated metabolism of carcinogenic aromatic amines and their binding toDNA protein. *Biochemical and Biophysical Research Communications*, **108**:253–258.

Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, Bailey JR, Young JF, Skipper PW, Tannenbaum SR (1991) Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladderDNAadduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Research*, **51**:4371–4377.

Kimura S, Kawabe M, Ward JM, Morishima H, Kadlubar FF, Hammons GJ, Fernandez- Salguero P, Gonzalez FJ (1999) CYP1A2 is not the primary enzyme responsible for 4- aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis*, **20**:1825–1830.

Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE (1965) Carcinogenesis in the human urinary bladder. Observations after exposure to *para*-aminodiphenyl. *New England Journal of Medicine*, **272**:767–770.

Koss LG, Melamed MR, Kelly RE (1969) Further cytologic and histologic studies of bladder lesions in workers exposed to *para*-aminodiphenyl: Progress report. *Journal of the National Cancer Institute*, **43**:233–243.

Kriek E (1992) Fifty years of research on *N*-acetyl-2-aminofluorene, one of the most versatile compounds in experimental research. *Journal of Cancer Research and Clinical Oncology*, **118**:481–489.

Lakshmi VM, Mattammal MB, Zenser TV, Davis BB (1990) Mechanism of peroxidative activation of the bladder carcinogen 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT): Comparison with benzidine. *Carcinogenesis*, **11**:1965–1970.

Lang M, Pelkonen O (1999) Metabolism of xenobiotics and chemical carcinogenesis. *IARC Scientific Publications*, **148**:13–22.

Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG (1979) Effects of dose and time in a long-term, low-dose carcinogenic study. *Journal of Environmental Pathology and Toxicology*, **3**:17–34.

Logothetis CJ, Xu HJ, Ro JY, Hu SX, Sahin A, Ordonez N, Benedict WF (1992) Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. *Journal of the National Cancer Institute*, **84**:1256–1261.

Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT (1979) *N*- Acetyltransferase phenotype and risk in urinary bladder cancer: Approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environmental Health Perspectives*, **29**:71–79.

Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C (2002) Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. *Clinical Cancer Research*, **8**:171–179.

Manjanatha MG, Li EE, Fu PP, Heflich RH (1996) H- and K-*ras* mutational profiles in chemically induced liver tumours from B6C3F1 and CD-1 mice. *Journal of Toxicology and Environmental Health*, **47**:195–208.

Martone T, Airoldi L, Magagnotti C, Coda R, Randone D, Malaveille C, Avanzi G, Merletti F, Hautefeuille A, Vineis P (1998) 4-Aminobiphenyl–DNA adducts and *p53* mutations in bladder cancer. *International Journal of Cancer*, **75**:512–516.

Mattano SS, Land S, King CM, Weber WW (1989) Purification and biochemical characterization of hepatic arylamine *N*-acetyltransferase from rapid and slow acetylator mice: Identity with arylhydroxamic acid *N*,*O*-acyltransferase and *N*-hydroxyarylamine *O*- acetyltransferase. *Molecular Pharmacology*, **68**:599–609.

McQueen CA, Chau B, Erickson RP, Tjalkens RB, Philbert MA (2003) The effects of genetic variation in *N*-acetyltransferases on 4-aminobiphenyl genotoxicity in mouse liver. *Chemico- Biological Interactions*, **146**:51–60.

Melamed MR, Koss LG, Ricci A, Whitmore WF Jr (1960) Cytohistological observations on developing carcinoma of urinary bladder in man. *Cancer (Philadelphia)*, **13**:67–74.

### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Melick WF, Escue HM, Naryka JJ, Mezera RA, Wheeler EP (1955) The first reported cases of human bladder tumors due to a new carcinogen—Xenylamine. *Journal of Urology (Baltimore)*, **74**:760–766.

Melick WF, Naryka JJ, Kelly RE (1971) Bladder cancer due to exposure to *para-* aminobiphenyl: A 17year follow-up. *Journal of Urology (Baltimore)*, **106**:220–226.

Merck (1998) Merck veterinary manual. Whitehouse Station, NJ, Merck & Co., Inc.

Miller JA, Miller EC (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of human cancer*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 605–627.

Miller JA, Wyatt CS, Miller EC, Hartmann HA (1961) The *N*-hydroxylation of 4- acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of *N*-hydroxy-4- acetylaminobiphenyl in the rat. *Cancer Research*, **21**:1465–1473.

Oda Y (2004) Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. *Mutation Research*, **554**:399–406.

Oscarson M, Ingelman-Sundberg M, Daly AK, Nebert DW (n.d.) *Human Cytochrome P450 (CYP) Allele Nomenclature Committee* (http://www.cypalleles.ki.se/).

Parsons BL, Culp SJ, Manjanatha MG, Heflich RH (2002) Occurrence of H-*ras* codon 61 CAA to AAA mutation during mouse liver tumor progression. *Carcinogenesis*, **23**:943–948.

Parsons BL, Beland FA, Von Tungeln LS, Delongchamp RR, Fu P, Heflich RH (2005) Levels of 4aminobiphenyl-induced somatic H-*ras* mutation in mouse liver correlate with potential for liver tumor development. *Molecular Carcinogenesis*, **42**:193–201.

Pauli BU, Alroy J, Weinstein RS (1983) The ultrastructure and pathobiology of urinary bladder cancer. In: Bryan GT, Cohen SM, eds. *The pathology of bladder cancer, Vol. II*. Boca Raton, FL, CRC Press, pp. 41–140.

Poirier MC, Beland FA (1992)DNAadduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications forDNAadduct-based human cancer risk assessment. *Chemical Research in Toxicology*, **5**:749–755.

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995)DNAadduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis*, **16**:2917–2921.

Presti JCJ, Reuter VE, Galan T, Fair WR, Cordon-Cardo C (1991) Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Research*, **51**:5405–5409.

Primdahl H, von der Maase H, Christensen M, Wolf H, Orntoft TF (2000) Allelic deletions of cell growth regulators during progression of bladder cancer. *Cancer Research*, **60**:6623–6629.

Reznikoff CA, Loretz LJ, Johnson MD, Swaminathan S (1986) Quantitative assessments of the cytotoxicity of bladder carcinogens towards cultured normal human uroepithelial cells. *Carcinogenesis*, 7:1625–1632.

Roberts DW, Benson RW, Groopman JD, Flammang TJ, Nagle WA, Moss AJ, Kadlubar FF (1988) Immunochemical quantitation of DNA adducts derived from the human bladder carcinogen 4aminobiphenyl. *Cancer Research*, **48**:6336–6342.

Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ, Colorectal Cancer Study Group (2003) Polymorphisms in the cytochrome P450 CYP1A2 gene (*CYP1A2*) in colorectal cancer patients and controls: Allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *British Journal of Clinical Pharmacology*, **55**:68–76.

Sarkar MA, Nseyo UO, Zhong B-Z (2002) Mutagenic outcome of the urinary carcinogen 4aminobiphenyl is increased in acidic pH. *Environmental Toxicology and Pharmacology*, **11**:23–26.

Schieferstein GJ, Littlefield NA, Gaylor DW, Sheldon WG, Burgers GT (1985) Carcinogenesis of 4aminobiphenyl in BALB/cStCrlfC3Hf/Nctr mice. *European Journal of Cancer and Clinical Oncology*, **21**:865–873.

Schroeder JC, Conway K, Li Y, Mistry K, Bell DA, Taylor JA (2003) *p53* mutations in bladder cancer: Evidence for exogenous versus endogenous risk factors. *Cancer Research*, **63**:7530–7538.

Sesardic D, Boobis AR, Edwards RJ, Davies DS (1988) A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *British Journal of Clinical Pharmacology*, **26**:363–372.

Skipper PL, Tannenbaum SR, Ross RK, Yu MC (2003) Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **12**:503–507.

Smith BJ, Curtis JF, Eling TE (1991) Bioactivation of xenobiotics by prostaglandin H synthase. *Chemico-Biological Interactions*, **79**:245–264.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Krose D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Swaminathan S, Reznikoff CA (1992) Metabolism and nucleic acid binding of *N*-hydroxy-4-acetylaminobiphenyl and *N*-acetoxy-4-acetylaminobiphenyl by cultured human uroepithelial cells. *Cancer Research*, **52**:3286–3294.

Talaska G, Dooley KL, Kadlubar FF (1990) Detection and characterization of carcinogen–DNAadducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by <sup>32</sup>P- postlabelling and subsequent thin-layer and high-pressure liquid chromatography. *Carcinogenesis*, **11**:639–646.

Tiguert R, Bianco FJJ, Oskanian P, Li Y, Grignon DJ, Wood DPJ, Pontes JE, Sarkar FH (2001) Structural alteration of p53 protein in patients with muscle invasive bladder transitional cell carcinoma. *Journal of Urology*, **166**:2155–2160.

Tsuneoka Y, Dalton TP, Miller ML, Clay CD, Shertzer HG, Talaska G, Medvedovic M, Nebert DW (2003) 4-Aminobiphenyl-induced liver and urinary bladderDNAadduct formation in Cyp1a2( $\mathbf{i}/\mathbf{i}$ ) and Cyp1a2( $\mathbf{+/+}$ ) mice. *Journal of the National Cancer Institute*, **95**:1227–1237.

Underwood PM, Zhou Q, Jaeger M, Reilman R, Pinney S, Warshawsky D, Talaska G (1997) Chronic, topical administration of 4-aminobiphenyl induces tissue-specificDNAadducts in mice. *Toxicology and Applied Pharmacology*, **144**:325–331.

Verghis SBM, Essigmann JM, Kadlubar FF, Morningstar ML, Lasko DD (1997) Specificity of mutagenesis by 4-aminobiphenyl: Mutations at G residues in bacteriophage M13DNAand G  $\mathbf{1}$  C transversions at a unique dG<sup>8-ABP</sup> lesion in single-stranded DNA. *Carcinogenesis*, **18**:2403–2414.

Vineis P, Caporaso N, Tannenbaum SR, Skipper PL, Glogowski J, Bartsch H, Coda M, Talaska G, Kadlubar FF (1990) Acetylation phenotype, carcinogen–hemoglobin adducts, and cigarette smoking. *Cancer Research*, **50**:3002–3004.

Von Tungeln LS, Bucci TJ, Hart RW, Kadlubar FF, Fu PP (1996) Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6- phenylimidazo-[4,5-b]pyridine (PhIP) in the CD1 newborn mouse bioassay. *Cancer Letters*, **104**:133–136.

Vousden KH, Lu X (2002) Live or let die: The cell's response to p53. *Nature Reviews. Cancer*, **2**:594–604.

Walpole AL, Williams MHC, Roberts DC (1952) The carcinogenic action of 4- aminodiphenyl and 3:2<sub>9</sub>-dimethyl-4-aminodiphenyl. *British Journal of Industrial Medicine*, **9**:255–263.

Walpole AL, Williams MHC, Roberts DC (1954) Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl. *British Journal of Industrial Medicine*, **11**:105–109.

Weston A, Caporaso NE, Taghizadeh K, Hoover RN, Tannenbaum SR, Skipper PL, Resau JH, Trump BF, Harris CC (1991) Measurement of 4-aminobiphenyl–hemoglobin adducts in lung cancer cases and controls. *Cancer Research*, **51**:5219–5223.

Wright C, Thomas D, Mellon K, Neal DE, Horne CH (1995) Expression of retinoblastoma gene product and p53 protein in bladder carcinoma: Correlation with Ki67 index. *British Journal of Urology*, **75**:173–179.

Wu XR, Lin JH, Walz T, Haner M, Yu J, Aebi U, Sun TT (1994) Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. *Journal of Biological Chemistsy*, **269**:13716–13724.

# ホルムアルデヒド及びグルタルアルデヒドの鼻腔内細胞毒性: 発がん MOA のヒトへの関連性を解析するための IPCS フレームワークを用いた事例研究<sup>1</sup>

#### Douglas McGregor, Hermann Bolt, Vincent Cogliano, & Hans-Bernhard Richter-Reichhelm

ホルムアルデヒド及びグルタルアルデヒドは、吸入によりラット及びマウスの鼻腔上皮に毒性を 示す。また、一定濃度以上のホルムアルデヒドはラットとマウスの鼻腔腫瘍を用量依存的に増加さ せるが、グルタルアルデヒドにそのような作用はない。発がん MOA 解析のための 2006 年国際化 学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒトフレームワークを用い て、ホルムアルデヒドの MOA を策定し、非発がん性のグルタルアルデヒドの特性との関連性につ いて検討した。これらの化合物は、病理組織学的検査及び遺伝毒性試験において同様の反応パター ンを示した (ホルムアルデヒドの方が広範囲に研究されているものの)。 MOA は、 持続的な細胞毒 性と再生細胞増殖の誘発に基づいており、長期ばく露によりホルムアルデヒドが鼻腔内腫瘍を誘 発する濃度で生じるというものである。key eventsの用量依存性と時間的関係に関するデータは、 この MOA と一致している。少なくとも in vitro 評価で遺伝毒性が明らかな化合物については、遺 伝毒性 MOA を除外することはできない。しかし、提示されている MOA の基本となる非遺伝毒性 は、鼻の腫瘍性病変を説明することができ、リスク評価において遺伝毒性よりも有益な情報を提供 するかもしれない。グルタルアルデヒドを吸入しても発がん性を示さない理由はまだ完全には解 明されていないが、固有の毒性が重要な要因である可能性がある。グルタルアルデヒドの二つのア ルデヒド官能基は、ホルムアルデヒドに比べて、(特に分化制御に関与するタンパク質の場合)運 動性の低下や、(核酸の)修復機能の低下へとつながる損傷を引き起こす可能性が高い。グルタル アルデヒドに関連した発がん性に関する研究はほとんどない。一方、ホルムアルデヒドがヒトの発 がん物質であるというエビデンスは、副鼻腔がんでは少ないが、鼻咽頭がんについては多い。ホル ムアルデヒドの吸入ばく露がはるかに少ない副鼻腔の腫瘍を鼻腔腫瘍と区別せず分類しているこ とが一因となり、見かけの不一致を生み出していると考えられる。提示されているげっ歯類におけ るホルムアルデヒドの MOA はヒトへの関連性評価については、key events は妥当ではあるが、ヒ トのデータが限られているため限定的である。げっ歯類におけるホルムアルデヒドの MOA のヒト への関連性は、動態的または薬力学的な理由から除外できないことは明らかである。

# 序文

ホルムアルデヒド、グルタルアルデヒドはそれぞれ脂肪族のモノアルデヒド、ジアルデヒドで あり、アルデヒドに典型的な反応を経てアセタール、シアノヒドリン、オキシム、ヒドラゾン、 重亜硫酸塩錯体を形成する。これらは反応性の高い化学物質であり、DNA やタンパク質と共有結 合で架橋した錯体を生成する。これらの代謝には、アルデヒド脱水素酵素によって酸化されると いう共通点がある。いくつかの研究では、ホルムアルデヒドの吸入ばく露はラットに鼻腔腫瘍を 引き起こすことが示されているが、グルタルアルデヒドをラットに2年間吸入ばく露させた試験 では鼻腔腫瘍は観察されなかった。

<sup>&</sup>lt;sup>1</sup> WHO が著作権を有するこの論文は、Critical Reviews in Toxicology, Volume 36, pages 821-835 に 2006 年に掲載されたもの である。この論文は、WHO の出版物のために編集されたものであり、コリジェンダが含まれている。

#### ホルムアルデヒド

ホルムアルデヒドの発がん性試験は、マウス、ラット、シリアンハムスターを対象とした吸入 試験、ラットを対象とした経口投与(飲料水)試験、マウスを対象とした皮膚貼付試験、ラット を対象とした皮下投与試験が行われている。ラットではホルムアルデヒドが発がん物質であると いう決定的なエビデンスは吸入試験の結果から得られている。

ホルムアルデヒドへの長期吸入ばく露は、ラットの前方及び後方の側鼻道腫瘍の発生率を、非 線形性用量相関的に増加させるというエビデンスがある(Morgan ら、1986; Feron ら、1988年; Woutersen ら、1989年; Monticello ら、1996年; Kamata ら、1997年; CIIT、1999年)。ホルムア ルデヒド濃度が 7.2 mg/m<sup>3</sup>以上になると、腫瘍発生率が急激に増加する。2.4 mg/m<sup>3</sup>以下の濃度で はばく露しても、悪性の鼻腔腫瘍は誘発されなかった。表1は、同じ研究室で実施された2つの ラットの試験データ(Kerns ら、1983年a; Monticello ら、1996年)と、これらの研究のうちの1 つで、公表時には調査されていなかった数匹のラットについての追加情報を組み合わせたもので ある(Schlosser ら、2003年)。ホルムアルデヒド誘発性腫瘍の多くは扁平上皮がんであった。

ホルムアルデヒド濃度	リスクを抱えるラットの数。	実際の腫瘍数り		
$(mg/m^3)$				
0	122	0		
0.84	27	0		
2.4	126	0		
7.2	113	3		
12	34	22		
18	182	157		

表 1. ホルムアルデヒドにばく露されたラットにおける鼻腔扁平上皮がんの複合発生率

注: Schlosser ら (2003) からの引用

<sup>a</sup> リスクを抱えるラットとは、2年まで生存し、その時点で検査を受けたラットと、腫瘍が見つかった2年以内に死亡したラットのことである。

<sup>b</sup>2年以内に腫瘍が認められたラット

対照的に、シリアンハムスターを対象とした吸入試験では、12.3 mg/m<sup>3</sup>の単回投与で発がん性 を示さなかった(Dalbey、1982年)。マウスを用いた2つの吸入試験のうち1つは、17.6 mg/m<sup>3</sup>の 高用量濃度で雌には変化がなく、2年の時点でと殺した雄17匹のうち2匹には扁平上皮がんが認 められた(Kerns ら、1983年 a、1983年 b)。もう1つの試験は評価が不十分であった(Horton ら、 1963年)。

ラットを対象とした他のばく露経路を用いた試験では、4 つの飲水投与試験のうち 2 つの試験 (Takahashi ら、1986 年; Tobe ら、1989 年)では有意な結果は得られなかった。もう 1 つの試験 (Til ら、1989 年)では前胃乳頭腫、さらにもう一つの試験(Soffritti ら、1989 年)では白血病及 び消化管腫瘍を認めた。しかし、最後に挙げた試験の解釈は疑問視されている(Feron ら、1990)。 マウスの皮膚貼付試験及び皮下投与試験は評価に適さなかった。げっ歯類を対象とした研究では、 ラットを対象とした 5 つの吸入ばく露試験以外に、鼻腔内腫瘍の有意な増加が認められた研究は なかった。

### グルタルアルデヒド

グルタルアルデヒドの発がん性については、マウス及びラットでは吸入試験、ラットでは経口 投与(飲水投与)試験が行われている。吸入試験では、B6C3F1マウスに400µg/m<sup>3</sup>の単濃度で78 週間ばく露させた場合(Zissu ら、1998年)、または1000µg/m<sup>3</sup>までの複数濃度で2年間ばく露さ せた場合(NTP、1999年)、またはF344 ラットに3000µg/m<sup>3</sup>までの濃度で2年間ばく露させた場 合(NTP、1999年)のいずれにおいても発がん性は認められなかった。雌雄のF344 ラットを最大 4000 mg/m<sup>3</sup>のグルタルアルデヒド濃度に2年間ばく露した飲水試験では、すべてのばく露濃度で 雌の脾臓に大顆粒リンパ球性白血病の発生率増加が認められた(Ballantyne、1995; Van Miller ら、 1995年)。

### 1. 動物における作用機序(MOA)を立証するのにエビデンスの重み付けは十分か

#### A. 推定される MOA

一定濃度以上のホルムアルデヒドを長時間ばく露させると、持続的な細胞毒性と細胞増殖が誘発される。この増殖細胞塊内における遺伝的変化の結果、腫瘍が発生する。このような遺伝的変化は、ホルムアルデヒドによって明らかに誘導される細胞毒性、化生、過形成の二次的なものであると推測されている。ホルムアルデヒドは in vitro では遺伝毒性物質であり、DNA-タンパク質架橋(DPX)を形成する。DPX はホルムアルデヒドばく露の指標として確立されているが、それらが腫瘍発生に必要な突然変異前病変(DNA 複製エラーを起こし、突然変異をもたらす)であるかは明らかではない。ラットで DPX が豊富であることを除けば、ホルムアルデヒドが生体内で哺乳類の細胞に変異原性を示すというエビデンスはほとんどない。

この MOA は、主に3 つの key events (持続的な細胞増殖、DPX、腫瘍) すべてについて一貫した非線形の用量反応関係が観察されたこと及び鼻腔全体でこれらの影響の発生率が一致していることに基づいて推測される。

## **B** Key events

#### ホルムアルデヒド

ホルムアルデヒドばく露後の障害が侵入口に限定されることは明らかに重要であり、その過程 では代謝が重要な役割を果たしている。ホルムアルデヒド誘発性鼻腔腫瘍における侵入口の重要 性は、ホルムアルデヒド溶液を経口ばく露させたラットにおいて、主要な非腫瘍性の影響が前胃 及び腺胃の組織学的変化の進展であったことから裏付けられる(Til ら、1989 年; Tobe ら、1989 年)。

ホルムアルデヒドは、細胞内の N-、O-及び S-脱メチル化反応の内因性代謝産物であり(Hardman ら, 2001 年)、ばく露を受けていない哺乳類の正常な血中濃度は約 2.0~2.6 µg/g である(Heck ら、1982 年、1985 年; Casanova ら、1988 年)。外因性ホルムアルデヒドは吸収されると急速に解毒される。ホルムアルデヒドを静脈内投与されたラットにおける血漿中の半減期は約 1 分で(Rietbrock、1965 年)、還元されたグルタチオンと容易かつ自然に結合する。そして、アルコール脱水素酵素 3

(ADH3,グルタチオン依存性ホルムアルデヒド脱水素酵素としても知られている)の基質である S-ヒドロキシメチルグルタチオンを形成し、S-ホルミルグルタチオンを産生する(Uotila & Koivusalo、1974年; Koivusaloら、1989年)。 これは S-ホルミルグルタチオンヒドロラーゼ(Uotila & Koivusalo、1997年)によってさらにギ酸に代謝され、グルタチオンを減少させる。ADH3 とヒドロキシ-メチルグルタチオンの初期結合のための K<sub>M</sub> 値は約 0.004 mmol/L であり、遊離ホルムアルデヒドの濃度はさらに低い可能性が高い(Uotila & Koivusalo、1997年; Hedberg ら、1998年)。また、ホルムアルデヒドがシステイン及びシステイニルグリシンのようなチオール類と結合することは、毒性学的に重要であるかもしれない(Holmquist & Vallee、1991年)。この効率的な解毒代謝メカニズムに加えて、絨毛粘膜はガスや蒸気から下層の上皮を保護する。したがって、標的組織において細胞毒性が発生する遊離ホルムアルデヒドを高濃度で得るためには、これらの保護機構を克服することが必要であり、比較的高濃度のホルムアルデヒド蒸気を標的部位に到達させる必要がある。発がん性にとって明確で意義のある機序的な事象は、ラットにおけるホルムアルデヒドの解毒機構が飽和している用量で発現することである(Casanova & Heck、1987年)。

ホルムアルデヒド吸入ばく露後に測定された鼻のがん形成と関連している主な非腫瘍性及び前 腫瘍性徴候には、細胞毒性、DPX 形成、鼻腔上皮細胞の再生性増殖、扁平上皮化生及び炎症が含 まれる。これらは部位特異的で高度に非線形な反応過程であり、鼻腔がんの発生率と一致してい る。

相対的な細胞増殖の亢進の程度は、鼻腔内の特定領域内の標的細胞集団の大きさに依存し、ば く露期間または累積ばく露の総量と常に相関するわけではない(Swenberg ら、1983 年、1986 年; Monticello ら、1991 年、1996 年; Monticello & Morgan、1994 年)。これらの因子は、ラット、サル 及びヒト上皮細胞を対象とした多くの研究で明らかにされ、測定されている。3、6、12 及び 18 ヶ 月の途中計画殺を伴う 24 ヶ月の発がん性試験では、7.2、12 及び 18 mg/m<sup>3</sup>の濃度でばく露された ラットはすべての時点で細胞増殖活性が実証された(Monticello ら、1991、1996 年)。

免疫組織化学的手法を用いて、同一腫瘍の病理組織切片中の細胞増殖マーカーである p53 タン パク質(増殖細胞核抗原、または PCNA)及び腫瘍成長因子(TGF)を評価した。正常な鼻粘膜は 陰性であったが、扁平上皮がんでは p53 陽性となり、特に前腫瘍性病変である角化亢進性プラー ク内において角化した細胞での p53 陽性が観察された。PCNA の免疫染色の分布と p53 の免疫染 色の分布との間に相関関係が認められた(Wolf ら、1995 年)。

ラットにおける DPX の形成は、濃度の非線形関数であり(Casanova & Heck、1987年; Casanova ら、1989年、1994年; Heck & Casanova、1995年)、腫瘍の部位特異性と一致している(Casanova ら、1994年)。架橋はラットの嗅粘膜または骨髄では検出されなかった(Casanova-Schmitz ら、1984 年; Casanova & Heck、1987年)。DPX はホルムアルデヒド吸入ばく露後のアカゲザルで認められ ており、その濃度が最も高い部位は中鼻甲介で、次いで前側壁中隔及び鼻咽頭であった(Casanova ら、1991年)。

ラット、マウス、シリアンハムスター及びアカゲザルにホルムアルデヒドを 13 週間(マウス) または 26 週間ばく露した研究において、ラット及びアカゲザルでは鼻尖部の扁平上皮化生が 3.7 mg/m<sup>3</sup>で発現したが、シリアハムスター及びマウスでは 4.9 mg/m<sup>3</sup>でも発現しなかった(Rusch ら、 1983 年; Maronpot ら、1986 年)。細胞の複製は、ラット鼻腔上皮のうち腫瘍感受性がより高い領 域の特徴でもある(Casanova ら、1994 年)。

# グルタルアルデヒド

400 μg/m<sup>3</sup>の濃度でグルタルアルデヒドを 78 週間吸入ばく露させた結果、雌マウスの鼻前庭に 非腫瘍性の病変が生じた。その病変は、背側壁を覆う扁平上皮の過形成及びアトリオタービネート(atrioturbinate)の側方面の過形成である(Zissu ら、1998 年)。

グルタルアルデヒドの米国国家毒性プログラム (NTP)の研究では、雌雄ラットにおいて以下の ような鼻の変化が認められた。

- 鼻腔の最も吻側にある扁平上皮、外鼻腔の後ろにある扁平上皮では、過形成と炎症の発生率が 増加していた。過形成は、細胞層数の増加による上皮の不均一な肥厚と、より重篤な症例では、 様々な程度のケラチンの蓄積を特徴とする変化であった。
- 2. 呼吸上皮では、過形成、軽微な杯細胞過形成(主に鼻中隔及び腹側中隔に沿って)、炎症及び扁 平上皮化生が認められ、重度の症例では上皮表面にケラチンが蓄積していた。
- 3. 背側中膜の嗅覚上皮では、硝子変性がわずかに認められた。

扁平上皮及び呼吸上皮で観察されたグルタルアルデヒド関連の炎症は、好中球、リンパ球及び 形質細胞の多巣性から広範な浸潤にわたる様々な程度の変化があった。時折、固有層内に数個の マクロファージが存在し、重度の場合には上皮にも存在していた。この研究では雌雄のマウスに おいて、病変の質はラットと類似していたが、雌の方が雄よりも重症度は高かった。

グルタルアルデヒドは、TK6ヒトリンパ芽細胞株において DPX を誘発した(St. Clair ら、1991年)。in vivo では、グルタルアルデヒドは、吸入(Gross ら、1994年)及び鼻腔内投与(St. Clair ら、1990年)によってばく露されたラット及びマウスの鼻腔内細胞において細胞増殖(S期)を誘導した。同じ著者による並行して実施された鼻腔内投与試験において、ホルムアルデヒドでは、20 倍高いモル濃度で同様の細胞増殖を誘発した。

## C. 用量反応関係

### ホルムアルデヒド

ホルムアルデヒドをばく露したラットから得られたデータによると、key events の用量反応パタ ーンは非線形であり、2.4 mg/m<sup>3</sup>では反応を認めず、7.2 mg/m<sup>3</sup>では最小の反応を認め、12 及び 18 mg/m<sup>3</sup>では急激に反応が増加している。 ホルムアルデヒドにばく露されたラットでは、2.4 mg/m<sup>3</sup>以下の濃度に亜慢性または慢性的にば く露した後の鼻粘膜で、細胞の再生及び DNA 合成の増加は認められなかった (Rusch ら、1983 年; Zwart ら、1988 年; Monticello ら、1991 年; Casanova ら、1994 年)。Wistar ラットでは細胞の再生 速度の部位特異的な軽度の増加が 3.7 mg/m<sup>3</sup> (6 時間/日、5 日/週、13 週間) で認められた (Zwart ら、1988 年)。同様の期間ばく露した Fischer 344 ラットでは DNA 合成速度の増加が 7.2 mg/m<sup>3</sup>で 認められた (Casanova ら、1994 年)。しかしながら、これらの濃度では、6 週間後 (Monticello ら、 1991 年)または 13 週間後 (Zwart ら、1988 年) の細胞再生率がばく露 1~4 日後よりも低いため、 ラットの鼻腔上皮では適応反応が起こると思われる。単位長ラベリング指数 (ULLI) 法を用いて、 0、0.84、2.4、7.2、12、または 18 mg/m<sup>3</sup>のホルムアルデヒド濃度に 6 時間/日、5 日/週、3、6、12、 18、または 24 ヶ月間ばく露した雄の Fischer 344 ラットにおける増殖を測定した。ULLI の有意な 上昇は 12 及び 18 mg/m<sup>3</sup>群でのみ認められ、前側側鼻道及び内側上顎鼻甲介での上昇が大きかっ た。前背側中隔における ULLI の上昇は、ばく露後しばらくしてから発現した。この遅発性の ULLI 上昇は、鼻腔腫瘍の影響を受けやすい部分における、病変の成長と空隙の歪みに関連する局所的 なホルムアルデヒド濃度の変化でおこる気流変化による二次的なものと考えられた (Monticello ら、 1996 年)。

ホルムアルデヒド誘発性 DPX 形成、上皮細胞増殖及びその後の鼻腔腫瘍の非線形関係を表2に示す。Casanova ら(1994 年)が提示した高腫瘍領域と低腫瘍領域の指定は最適なものではなく、 真に腫瘍発生率の高い領域の DPX 反応は、中間的な腫瘍発生率(後方側鼻道)領域の DPX 反応 によって希釈されている可能性がある。

他の研究では、1.2 mg/m<sup>3</sup>(1日22時間、7日/週、26週間)にばく露した Fischer 344 ラットで は、検出可能な鼻腔病変は認められなかったが、3.6 mg/m<sup>3</sup>において組織学的変化は鼻甲介の扁平 上皮化生のみであった(Rusch ら、1983年)。同様に 2.4 mg/m<sup>3</sup>(6時間/日、5日/週、24ヶ月間) にばく露した Fischer 344 ラットの鼻甲介においても、軽度な扁平上皮化生の発生が示された (Kerns ら、1983年b)。これらのラットでは上皮異形成及び鼻炎も観察された。扁平上皮化生の 発生は、報告されている生体内反応の中で最も低濃度のホルムアルデヒドで発生する組織学的所 見だと思われる。

ラットにおいて正確に計算された解剖学的流体力学モデルを用いて、ホルムアルデヒドによっ て誘発される扁平上皮化生の分布が、扁平上皮の後方にある高流量領域に関係しているかどうか を検証した。50%以上が扁平上皮に置き換わっている場合、扁平上皮化生が生じているとみなさ れた。2.4 mg/m<sup>3</sup>以下でばく露されたラットの鼻には扁平上皮化生は認められなかった。7.2 mg/m<sup>3</sup> 以上のばく露では側鼻道に、12 または 18 mg/m<sup>3</sup>のばく露では気道の側壁及び内側壁に扁平上皮化 生がみられた(Kimbell ら、1997 年)。

4.8 mg/m<sup>3</sup>以上の吸入ばく露では、ラットで鼻組織内のホルムアルデヒドのグルタチオン介在性の解毒が飽和状態に達するというエビデンスがある。ホルムアルデヒド代謝の飽和は、この濃度以上のばく露における DPX、細胞増殖及び腫瘍発生率の用量反応関係の非線形性に寄与する可能性がある(Casanova & Heck、1987年)。

ホルムア ルデヒド -	<i>細胞増殖</i> ([ <sup>3</sup> H]チミジン標識細胞/mm 基底膜)ª			DNA-タンパク質 架橋形成 (pmol [ <sup>14</sup> C]-ホルムアル デヒド結合/mg DNA) <sup>b</sup>		鼻腔がんの発生率。			
ルノビト 濃度 (mg/m <sup>3</sup> )	前側 鼻道	後側 鼻道	前中隔	高腫瘍 領域	<i>低腫瘍</i> 領域	全体	前側 鼻道	後側 鼻道	前中隔
0	10.11	7.69	6.58	0	0	0/90	0/90	0/90	0/90
0.84	10.53	7.82	8.04	5	5	0/90	0/90	0/90	0/90
2.4	9.83	11.24	12.74	8	8	0/96	0/96	0/96	0/96
7.2	15.68	9.96	4.15	30	10	1/90	1/90	0/90	0/90
12	76.79	15.29	30.01	_	_	20/90	12/90	2/90	0/90
18	93.22	59.52	75.71	150	60	69/147	17/147	9/147	8/147

表 2. ホルムアルデヒドばく露の影響の比較:細胞増殖、DNA-タンパク質架橋及び 腫瘍発生率

a 指定された濃度のホルムアルデヒドに 6 時間/日、週 5 日/日、3 ヶ月間ばく露した雄の F344 ラットの鼻腔上皮の 3 箇所で測定した細胞増殖 (Monticello ら、1996 年)。

b 指定された濃度のホルムアルデヒドに6時間/日、5日/週、約12週間ばく露した雄のF344 ラットの鼻腔(呼吸器粘膜)の2つの領域で測定した DNA-タンパク質架橋形成の程度。腫瘍低発生領域は、鼻腔及び側鼻道の内側壁、後側壁、嗅覚領域を除く後背側鼻道及び鼻中隔からなる(Casanova ら、1994年)。データは引用文献中のグラフに基づいている。

c 指定された濃度のホルムアルデヒドに1日6時間、1日5日/週、24ヶ月間ばく露した雄性F344 ラットにおける鼻腔全体または鼻腔内の鼻腔腫瘍の発生率、または前側鼻道、後側鼻道、または前中隔の発生率(Monticello ら、1996年)。

# グルタルアルデヒド

グルタルアルデヒドにばく露したラット及びマウスを用いた一連の反復投与試験が NICNAS (1994 年)にまとめられている。その中で、ラットの鼻腔に病変を生じる最も低い濃度は 1000 µg/m<sup>3</sup>(6時間/日、5日/週、13週間)であった(NTP、1993 年)。最も重篤な病変は鼻腔前部に発 生し、呼吸上皮と嗅上皮の両方を障害した。過形成及び扁平上皮化生は、鼻腔の側壁及び鼻尖部 に最もよく認められた。病変は 4000 µg/m<sup>3</sup>にばく露されたラットで最も広範囲に認められたが、 1000 及び 2000 µg/m<sup>3</sup>群でも認められ、500 µg/m<sup>3</sup>群の雄 1 匹でも認められた。ラットを用いた別 の研究では、最大 776 µg/m<sup>3</sup>の濃度で 14 週間投与しても鼻の病変は観察されなかった (Bushy Run、 1983 年)。

マウスはグルタルアルデヒド吸入による感受性が高いようであり、13週間の試験で雌マウスで は最低濃度の250µg/m<sup>3</sup>で、雄マウスでは1000µg/m<sup>3</sup>でも鼻腔の炎症が観察された。このような感 受性の違いは、マウスのほうが気道が狭いためゴミなどで閉塞しやすいためと考えられる(NTP、 1993年)。呼吸器の病理組織学的病変は4000µg/m<sup>3</sup>群のマウスで最も重篤であり、喉頭上皮の扁 平上皮の軽度から中程度の扁平上皮化生、鼻腔前部の膿瘍性炎症がみられた。 鼻尖部の扁平上皮化生は軽度であった。壊死と炎症は低濃度で、主に鼻腔前部で認められた。

グルタルアルデヒドを用いた NTP(1993 年)の13週間の試験では、鼻前庭の扁平上皮と、それよりも軽度ではあるが、背側鼻道のアトリオタービネートの呼吸器上皮において、ばく露に関連した ULLI の有意な増加が認められた。ばく露に関連した細胞増殖活性は、一般的にマウスよりもラットの方が高かった。個々のマウスを調べたところ、鼻前庭の扁平上皮では、粘膜に好中球浸潤が認められたマウスでのみ細胞増殖率の増加が認められた。ただし、細胞増殖の程度と浸潤の重症度は相関していなかった。これらの観察は、特に雌マウスでは13週目に最も明らかになった。ラットでは、前庭の扁平上皮の増殖増加に加えて、背側のアトリオタービネートの呼吸器上皮でも同様に顕著な増殖増加がみられたが、マウスではこの領域での反応は弱かった。

#### D. 時間的関連性

#### ホルムアルデヒド

ラットの呼吸器上皮内の細胞増殖に対するホルムアルデヒドばく露の影響について、短期、中 期、長期の研究では、ばく露期間にかかわらず、2.4 mg/m<sup>3</sup>以上の濃度にばく露した後、鼻腔上皮 細胞の増殖が持続的に増加することが示されている。ホルムアルデヒドにばく露したラットでは、 3日間という短い期間から細胞増殖が観察された。すでに述べた ULLI 研究では、一般的に細胞増 殖の増加率は時間の経過とともに減少したが、この影響は、特定の鼻腔部位では対照群の約 2~ 10倍の増加を示し、最長で 18 ヶ月間の観察期間を含めて試験終了まで持続していた(Monticello ら、1996年)。

ほとんどのホルムアルデヒド吸入による DPX 形成の研究は短期間(すなわち、ばく露期間が1 日まで)であるため、DPX の時間的関連性に関するデータは乏しい。ホルムアルデヒド誘発性 DPX は、ラットとアカゲザルの鼻腔上皮における研究で一貫して示された(Casanova ら、1991 年)。 しかし、ホルムアルデヒドに約 12 週間ばく露したラットの急性及び累積 DPX 収量を調べる研究

(Casanova ら、1994 年)において、7.2 mg/m<sup>3</sup>以上の濃度では、事前にばく露されたラットの側鼻 道(腫瘍発生率の高い部位)の急性 DPX 収量は、対照群の約半分であったが、内側及び後側の鼻 道(腫瘍発生率の低い部位)では差が見られなかった。事前にばく露されたラットでは、DPX の 有意な蓄積は認められなかった。

ホルムアルデヒド誘発細胞毒性後の再生性細胞増殖は、DNA 複製数を増加させる。その結果、 DPX が誘発する DNA 複製エラーを増加させ、突然変異を引き起こす。この仮説は、高濃度にお けるラットの鼻での DNA 複製の阻害(Heck & Casanova、1995 年)及び前腫瘍病変における p53 発現の増加(Wolf ら、1995 年)によって支持される。

18 mg/m<sup>3</sup>に最大 2 年間ばく露して誘発されたラットの扁平上皮がんの 11 例中 5 例では、p53 相補的 DNA (cDNA) 配列の GC 塩基対に点突然変異が認められた (Recio ら、1992 年)。

# グルタルアルデヒド

ラット及びマウスの13週間のグルタルアルデヒド吸入試験(NTP、1993年)における細胞増殖 の研究では、マウスで得られた結果とは対照的に、ラットの鼻前庭における細胞増殖の増加(ULLI) は、早い時期(数日以内)に発生し、試験期間中は上昇したままであるか、またはわずかに減少 した。マウスの鼻前庭における ULLIの増加は時間の経過とともに増加する傾向があった。マウ スを用いた吸入試験(Zissuら、1994年)では、1.2 mg/m<sup>3</sup>に4日間ばく露した後、鼻中隔の呼吸 上皮及び鼻及び顎甲介に最初の病変が観察された。4.0 mg/m<sup>3</sup>ばく露終了2週間後にも重篤な病理 組織学的変化が認められた。気管及び肺ではばく露に関連した組織学的異常は認められなかった。

# E. 腫瘍発生と key events との関連性の強さ、一貫性及び特異性

## ホルムアルデヒド

ホルムアルデヒド誘発性腫瘍に関する広範な研究がある。入手可能なデータを見る限り、ラットやサル、ラットやヒトの様々な動物種や、ラットやヒトの様々な細胞の in vitro で明らかになっ ている。。同程度のばく露量では、DPX の濃度はラットよりもアカゲザルの方が約一桁低いことが 明らかになった。ホルムアルデヒドへの組織ばく露後のヒト上皮細胞増殖の亢進が、ヒトの気管 支上皮細胞を移植したラット気管を胸腺欠損マウスに異種移植したモデル系で報告された。

key events と局所的な腫瘍発生率及び腫瘍発生部位との間には相関関係がある。腫瘍が観察され ている鼻腔領域では、細胞増殖、化生及び DPX の増加が認められた。DPX、細胞毒性、細胞増殖、 化生、腫瘍についての非線形用量反応関係は一貫しており、ある試験では 2.4 mg/m<sup>3</sup> で化生の有意 な増加が認められ、4.8 mg/m<sup>3</sup> 以上の濃度では全てのエンドポイントが観察された。これはまた、 粘膜クリアランスが抑制され、グルタチオンを介した代謝が飽和する濃度、すなわち 4.8 mg/m<sup>3</sup> と 密接な相関関係を示している。Morgan ら(1986年)による雄ラットの鼻粘膜に対する吸入ホルム アルデヒドの影響を検討した研究では、2.4 mg/m<sup>3</sup>、7.2 mg/m<sup>3</sup>、18 mg/m<sup>3</sup>の濃度で1日、9日及び 14 日間ばく露後、18 時間の回復期間を設けた群が含まれていた。粘膜クリアランスの阻害は、ば く露時間が長くなるにつれて徐々に範囲が広がったが、ばく露停止 18 時間後の回復はほとんど、 あるいは全く認められなかった。 ある濃度のホルムアルデヒドにばく露後発生する鼻腔腫瘍において、マウスはラットよりも感 受性が低いようである。しかしながら、マウスでは有害化学物質の吸入に反応して換気量/分が減 少することがよく知られている(Brown ら、1986年; CIIT、1999年)。

### グルタルアルデヒド

ホルムアルデヒドと比較して、グルタルアルデヒドによって誘発された病変は、扁平上皮を含む鼻の前方に位置していた。また、それらの病変は異なる特徴を有しており、ホルムアルデヒドを 13 週間投与した動物にみられる細胞異型及び異形成を伴う局所的な角化亢進及び過形成は認められなかった(Monticello、1990年; Morgan & Monticello、1990年)。

## F. 生物学的妥当性及び整合性

#### ホルムアルデヒド

長期間にわたる再生性細胞増殖が化学物質による発がんの原因であるという仮説を支持するエ ビデンスが蓄積され続けている (PCS、2002 年)。吸入ばく露された動物におけるホルムアルデヒ ド誘発性鼻腔腫瘍に対する MOA は、生物学的に妥当性があり、利用可能なデータと一致してい る。ラットを対象とした短期・中期毒性試験や他種の研究では、鼻腔内の持続的な細胞増殖活性 が観察されている。鼻腔内の病理組織学的影響(上皮細胞の異形成と化生)は、亜慢性及び慢性 の動物実験で一貫していた。しかし、ラットの鼻腔腫瘍誘導における DPX、突然変異及び細胞増 殖のそれぞれの役割は完全には解明されていないことに留意すべきである。

### グルタルアルデヒド

吸入したグルタルアルデヒドの影響は、ホルムアルデヒドほど研究されていない。吸入試験で は、グルタルアルデヒドはラットとマウスの鼻腔腫瘍を誘発しなかった。しかし、ホルムアルデ ヒドの鼻発がん性において key events とみなされる細胞毒性と細胞増殖が、グルタルアルデヒド にばく露されたラットとマウスでも実証されている。このことは、これらの過程がホルムアルデ ヒドにとって重要であることの妥当性を低下させるように見えるかもしれない。

### G. 考えられる代替 MOA

#### ホルムアルデヒド

変異原性がホルムアルデヒド誘発性腫瘍の発生に関与している可能性がある。利用可能なデー タを評価すると、ホルムアルデヒドは in vitro では遺伝毒性はあるが、標準的な in vivo アッセイ では概して遺伝毒性はないことが示されている。しかし、DPX を産生することを示す研究は数多 くある。

ホルムアルデヒドは in vitro での遺伝毒性について広く研究されており、細菌や哺乳類細胞を用 いた試験(Ames 試験、遺伝子突然変異)で陽性結果が得られ、DNAの一本鎖切断や DPX の産生 を認めた(IARC、2005 年レビュー)。生体内では、ホルムアルデヒドはショウジョウバエで再現 性のある突然変異を誘発したが、げっ歯類の骨髄細胞試験では、その遺伝毒性について有力なエ ビデンスはない。しかし、ホルムアルデヒドのばく露が、ヒトの鼻や頬の細胞や末梢血リンパ球 における染色体異常や小核発現頻度の増加と関連しているというわずかなエビデンスはある (IARC、2005 年; 付録参照)。 DPX がホルムアルデヒドの突然変異誘発性及び発がん性にどの程度寄与しているかは不明であ る(Recio、1997年; Merk & Speit、1998年; Speit ら、2000年; Liteplo & Meek、2003年)。DPX の存在は主にばく露の指標として考えられてきたが、これらの病変の特徴は前変異原性であり、 それゆえに直接的な遺伝毒性メカニズムのエビデンスであるという見方もある。しかし、DPX は 形成された細胞にダメージを与える可能性があり、高頻度で発生すると細胞死が起こる可能性が 高い。また、本研究では細胞への影響はそれほど深刻ではないものの、タンパク質-タンパク質架 橋(PPX)が起こる可能性があることも示唆されている。もし主要なタンパク質が PPX の形成に 関与していれば、分化の制御を含む細胞の制御機構に影響を及ぼす可能性がある。化生領域が出 現しているため、このような変化は、ホルムアルデヒドにばく露されたラットの鼻腔上皮で生じ ている。遺伝毒性の MOA を示す有力なエビデンスがない限り、腫瘍は単に化生の一種と見なす ことができる。

Gaylor ら(2004 年)は、J 型濃度曲線を特定するように設計された統計手法を用いて、ラット におけるホルムアルデヒド誘発性細胞増殖の濃度-反応関係を解析し、異なるデータの解釈を提示 した。解析の際、がん発生率に関する定量的なデータが不十分であったため、細胞増殖のデータ を使用した。彼らの解析は、鼻腔腫瘍の発生に対する閾値を有する用量反応性が、低用量におけ る軽度な遺伝毒性の結果であり、これにより細胞毒性を有する高用量において J 字型の細胞増殖 曲線を呈するという仮説を支持するものである(Lutz、1998 年)。低用量では、漸増する DNA 損 傷は細胞増殖の減少によって打ち消される可能性があるため、明らかな閾値があるにもかかわら ず、データは遺伝毒性メカニズムと一致している。

ホルムアルデヒドにばく露されたラットにおいて、*p53* 遺伝子の進化的に保存された領域 II-V の cDNA 配列の GC 塩基対における点突然変異が、原発性鼻腔扁平上皮がん 11 例のうち 5 例で認 められた (Recio ら、1992 年)。この結果は、発がん過程におけるホルムアルデヒドにより誘発さ れた遺伝毒性過程を示すものと解釈される。しかし、出現した腫瘍における特定の突然変異の存 在は、それらが腫瘍の初期段階に存在していたエビデンスでも、それらが化学物質によって直接 誘導されたことを示すものではない。直接的な突然変異原性を示す可能性はあるが、これらの突 然変異は、化学物質によって誘発されたクロマチンタンパク質の機能的変化により間接的に生じ た可能性もある。これらの観察された突然変異が腫瘍の発生段階のどの段階で生じたかは推測の 域を出ない。これらの突然変異は一般的な事象であることは明らかであるが、すべて同じタイプ の腫瘍が発生するわけではないので、本質的な事象ではないこともまた明らかである。これらの 突然変異の発生は、遺伝毒性のメカニズムが排除されていないことを示しているが、必ずしも支 持するものでもない。

また、遺伝子発現の特異的な変化も生体内で観察されている。その結果、ホルムアルデヒドへのばく露は、異物代謝、細胞周期制御、DNA 合成と修復、がん遺伝子、アポトーシスを含むいくつかの機能に関与する遺伝子の発現レベルに変化をもたらすことが示された(Hester ら、2003 年)これらの変化がホルムアルデヒドにどの程度特異的であるか、あるいは発がん性においてどのような役割を果たしているのかは、現時点では明らかになっていない。

# グルタルアルデヒド

グルタルアルデヒドは、ホルムアルデヒドに比べて in vitro 及び in vivo での遺伝毒性に関する 試験があまり行われていない。グルタルアルデヒドは、in vitro 試験では軽微かつ不規則な陽性所 見を示し、in vivo 試験の大部分では活性がない。最近、グルタルアルデヒドの遺伝毒性に関する レビューが発表された(Zeiger ら、2005 年)。

グルタルアルデヒドは細菌では DNA 修復系を誘導し、サルモネラ菌や大腸菌では弱い変異原 性を示した。不定期 DNA 合成(UDS)、DPX、二本鎖切断はヒト細胞株で認められたが、ラット 初代細胞では認められなかった。哺乳類細胞を用いた染色体異常及び姉妹染色体交換(SCE)試験 では、軽微かつ一貫性のない反応がみられ、培養シリアンハムスター胚(SHE)細胞においてグル タルアルデヒドは形質転換を誘導しなかった。

生体内では、ラット及びマウスの鼻腔内投与後、グルタルアルデヒドは鼻腔内細胞にS期のDNA 合成を誘導した。グルタルアルデヒドはラット肝臓ではDNA損傷を生じず、ラット精巣DNAや 精子細胞では架橋を生じなかった。ラット及びマウスの骨髄細胞を用いた染色体異常試験では、 概ね陰性であった。グルタルアルデヒドは、マウスの骨髄細胞に小核や優性致死性突然変異を誘 発しなかった。このように、グルタルアルデヒドは遺伝毒性を有する可能性があり、データベー スはホルムアルデヒドほど豊富ではないが、接触部位における遺伝毒性の発生が予想される。し たがって、遺伝毒性がホルムアルデヒドの主要な発がんMOAであるとすれば、なぜグルタルア ルデヒドが効果を発揮しないのかを説明する必要がある。

## H. 不確実性、矛盾、データギャップ

#### ホルムアルデヒド

ホルムアルデヒドを用いたがんのバイオアッセイのほとんどは、細胞毒性の指標としての増殖 反応や DPX などの中間エンドポイントに関するデータが限られている。そのため、中間病変と腫 瘍の発生率を直接比較することには制限がある。加えて DPX と突然変異誘発との直接的な関係に 関する情報及び DPX が突然変異につながる確率に関する情報が望まれる。一方で、腫瘍の発生に 必要な突然変異の生成にはどの再生性細胞増殖が関与しているかを決定する必要がある。

ホルムアルデヒドばく露ヒト細胞における hprt 遺伝子突然変異スペクトルの研究により、突然 変異の 50%は欠失であり、50%は A:T 塩基対での点突然変異によるものであることが明らかに なった (Crosby ら、1988 年; Liber ら、1989 年)。ホルムアルデヒド誘発性扁平上皮がんにおいて、 ホルムアルデヒド突然変異スペクトルの一部として欠失が認められたことで、p53 遺伝子で観察 されるホモ塩基対変異の性質を説明できるかもしれない。しかし、変異している塩基対に関して は矛盾がある。ヒト及び哺乳類細胞株では hprt 遺伝子の A:T であり、ホルムアルデヒド誘発性 扁平上皮がんでは p53 の G:C であることが明らかになっている (Recio、1997 年)。in vitro では ホルムアルデヒドによってこのような突然変異が誘導されるが、このタイプの突然変異はホルム アルデヒドの発がん性の根本ではない可能性がある。

### グルタルアルデヒド

グルタルアルデヒドは二官能性アルキル化剤であるためか、ホルムアルデヒドよりも明らかに 細胞毒性が強い。経鼻投与試験では、水溶液を鼻粘膜に単回投与した場合、モル比でグルタルア ルデヒドはホルムアルデヒドの10~20倍の毒性があることが実証されている(St. Clair ら、1990 年)。グルタルアルデヒドの13週間吸入試験(NTP、1993年)の結果とホルムアルデヒドの同様 の吸入試験(Heck ら、1990年; Monticello ら、1990年; Monticello ら、1991年)との比較は、こ の経路でもグルタルアルデヒドはホルムアルデヒドより約20倍の毒性があることを示している。 肺障害及び壊死は、かなり高い濃度のグルタルアルデヒドで発生する。細胞毒性は、グルタルア ルデヒドを吸入した場合には外鼻に近いところで発現するため、主に影響を受ける組織はホルム アルデヒドを吸入した場合と同じではない。唯一の違いが毒性の強さであるならば、グルタルア ルデヒドは、低用量でもホルムアルデヒドと同様の影響をもたらすと予想されるため、毒性作用 部位における違いは特に重要かもしれない。

### I. 推定される MOA の評価

### ホルムアルデヒド

重要なエビデンスの観点より、ホルムアルデヒド誘発性鼻腔腫瘍の仮説 MOA は一貫して、key events すべてにおける用量反応関係の一致、データベースの生物学的妥当性と整合性など、いく つかの基準を満たしている。鼻腔内腫瘍誘発におけるホルムアルデヒドの推定される MOA は多 くの実験データと一致しているため、この MOA は信頼性が高いと考えられる。

### グルタルアルデヒド

細胞毒性、細胞増殖及び DPX 形成の key events (in vitro) は、グルタルアルデヒドへのばく露 で実証されている。しかし、グルタルアルデヒドはラットやマウスでは鼻腔内腫瘍を発生させな い。したがって、推定されているホルムアルデヒドの MOA を維持するためには、この矛盾を説 明する必要がある。この矛盾の理由は特定されていないが、仮説を提示することは可能である。 グルタルアルデヒドの二つのアルデヒド官能基は、細胞環境内で高分子のさらなる反応を阻害す る重要な因子である。これらの高分子は、生存に関与するタンパク質であり、それらの不動化は、 おそらく分化状態の変化よりも細胞死につながる。このグルタルアルデヒドの高分子を不動化さ せる特性が、ホルムアルデヒドよりも高分解能顕微鏡(例えば電子顕微鏡)用の優れた固定剤に なる理由である。そのことはほぼ確実にジアルデヒドの非常に高い毒性につながっている。また、 ホルムアルデヒドのアルデヒド官能基は細胞障害を引き起こすが、細胞の分化に関与するタンパ ク質は、腫瘍の初期変化となりうる分化方向の変化を伴うものの、その役割を維持することがで きるかもしれない。一方、これらのアルデヒドが核酸と反応する場合(グルタルアルデヒドがこ のように反応するというエビデンスは実質的なものではない)、ホルムアルデヒドが DNA と相互 作用した後にアルキル化されたヌクレオチドの修復が起こるのに対し、グルタルアルデヒドの場 合は、困難、あるいは不可能かもしれない。このように、発がん性におけるホルムアルデヒドの 作用様式が推定されている通りなのか、それとも遺伝毒性が主な原因なのかにかかわらず、グル タルアルデヒドばく露に対する反応の違いを説明することができる。

# 2. 実験動物とヒトとの間の key evants の根本的、質的な違いに基づいて、MOA のヒトとの 関連性を合理的に排除することができるか

### A. ホルムアルデヒド

7.2 mg/m<sup>3</sup>のホルムアルデヒドに 1~6 週間ばく露したアカゲザルにおいて、ホルムアルデヒド に誘発された病変は、対照群の最大 18 倍を示す細胞増殖率の増加を伴っており、6 週間のばく露 後も有意に高い値が続いた。組織学的な病変と細胞増殖の増加は鼻腔で最も広範囲に見られ、下 気道では最小限であったが、上顎洞ではホルムアルデヒドばく露に対する反応のエビデンスは認 められなかった。病変の程度及び細胞増殖のデータに基づいて、7.2 mg/m<sup>3</sup>の用量でのホルムアル デヒドの急性及び亜急性影響に対する感受性はラットよりもアカゲザルの方が高いと考えられた (Monticello ら、1989 年)。アカゲザルの上顎洞における反応がないことは、疫学研究において(あ るいは、腫瘍部位の報告において)特別な注意を払うに値する所見である。副鼻腔がんのほとん どの疫学研究では、鼻に発生した腫瘍と副鼻腔に発生した腫瘍を区別していない。そのため、副 鼻腔に発生するがんに対応するリスクがなければ、鼻腔がんのリスクは希釈される傾向にあり、 統計的に検出されない可能性がある。

多くの疫学研究では、ホルムアルデヒドばく露と気道がんとの関連が調査されている。最も有 力なエビデンスは、鼻咽頭がんで観察されている。工場労働者を対象とした最大規模のコホート 研究(Hauptmann 6, 2004 年)では、鼻咽頭がんによる死亡者数の増加が観察されており、最大 ばく露量と累積ばく露量についてばく露-反応関係を示している。死体防腐処理業者を対象とした 米国最大のコホート研究では、上咽頭がんによる死亡者の増加が死亡率分析で観察されている (Hayes 6、1990 年)。ホルムアルデヒドを製造または使用している企業の労働者を対象としたデ ンマークの研究では、鼻咽頭がんの症例数の増加が観察された(Hansen & Olsen、1995 年)。他の コホート研究では、予想よりも鼻咽頭がんの症例数は少なかったと報告されている(Walrath & Fraumeni、1983 年; Coggon 6、2003 年; Pinkerton 6、2004 年)。鼻咽頭がんに関する7件の症例 対照研究のうち、5件ではホルムアルデヒドばく露によるリスク増加が認められた。

いくつかの症例対照研究では、ホルムアルデヒドへのばく露と副鼻腔がんとの関連が調査されている。12 件のプール解析では、木粉または革粉にばく露されたことがない男女における腺がんのリスク増加が示され、累積ばく露の指標に対し、ばく露用量反応性を示す傾向が示された(Luceら、2002 年)。他の1 件の症例対照研究(Olsen & Asnaes、1986 年)と発生率研究(Hansen & Olsen、1995 年)では、副鼻腔がん、特に扁平上皮がんのリスクの増加が示された。しかしながら、工場労働者を対象とした3 件の最も有力なコホート研究では、副鼻腔がんの増加は認められなかった(Coggon ら、2003 年; Hauptmann ら、2004 年; Pinkerton ら、2004 年)。

この一連のエビデンスを評価するにあたり、国際がん研究機関(IARC)は、ホルムアルデヒドがヒトで鼻咽頭がんを引き起こすという十分な疫学的エビデンスがあるが、ホルムアルデヒドがヒトで副鼻腔がんを引き起こすという疫学的エビデンスは限られている。

また、白血病とホルムアルデヒドの職業ばく露との間に因果関係があるという十分なエビデンスはない、と結論づけた(Cogliano ら、2005)。

ホルムアルデヒドにばく露されたヒトの鼻の細胞における DPX について記述している論文は ない。ホルムアルデヒドにばく露された労働者の末梢リンパ球における DPX の評価では、全体的 なばく露との関連性が示された(Shaham ら、2003 年)。単一の DPX 試験では、14 の病院の各病 理部門で働く 399 人が参加し、ホルムアルデヒドばく露のカテゴリーは低レベル(平均 0.5 mg/m<sup>3</sup>、 範囲 0.05~0.8 mg/m<sup>3</sup>)及び高レベル(平均 2.7 mg/m<sup>3</sup>、範囲 0.86~6.7 mg/m<sup>3</sup>)であった。調整後の 平均 DPX は被曝群で有意に高かった。この研究で用いられた物理的分離法の感度及び再現性には 疑問があるようである(Heck & Casanova、2004 年)。

いくつかの研究では、ホルムアルデヒドに職業ばく露された労働者の鼻腔上皮内の組織学的変 化を調査しているが、結果はまちまちであり、いくつかの研究では木粉への共ばく露があったた め、鼻腔上皮細胞の再生性増殖がどの程度起こるのかは未解明である(Berke、1987年; Edling ら、 1988年; Holmström ら、1989年; Boysen ら、1990年; Ballarin ら、1992年)。

鼻腔前部の粘膜クリアランスは、ホルムアルデヒドの 0.30 mg/m<sup>3</sup> でのばく露後に減少した (Andersen & Mølhave、1983 年)。

ホルムアルデヒドに関する動物とヒトの key events の一致を表3にまとめた。

key events	動物におけるエビデンス	ヒトにおけるエビデンス
細胞毒性	in vivo で陽性(標的細胞)	妥当性あり
細胞増殖	in vivo で陽性(標的細胞)	妥当性あり (エビデンスはあるが、共 ばく露によって交絡されている)
遺伝毒性	DPX(in vivo 標的細胞)	DPX (非標的細胞、リンパ球など)
突然変異	in vitro では陽性、in vivo では説得 力がない	陽性(?細胞)
鼻腔腫瘍	陽性(主に前外側鼻腔)	陽性(鼻咽頭) ?(副鼻腔)

### 表3. ホルムアルデヒドの用語索引表

### B. グルタルアルデヒド

グルタルアルデヒドへのばく露とヒトのがんに関する疫学的研究はほとんどない。グルタルア ルデヒド製造に携わる男性労働者 186 名のがん死亡者の増加は認められなかった。グルタルアル デヒドに初めてばく露されてからの平均期間は 20.6 年、ばく露期間は 3~7 年であった。モニタ リングばく露期間中、大気中のグルタルアルデヒド濃度は 0.04~1.4 mg/m<sup>3</sup> の範囲であった

(NICNAS、1994年)。グルタルアルデヒドの潜在的な影響について、遺体整復師、病理医及び米 国解剖学会のメンバーを対象とした研究では、すべての研究ががんリスクの増加を示している。 しかしこのグループはホルムアルデヒドにもばく露されていた(Walrath & Fraumeni、1983年、遺 伝毒性変異;ホルムアルデヒドに関するコンセンサスワークショップ、1984年; Stroup ら、1986 年)。 ヒトの鼻組織におけるグルタルアルデヒドばく露と DPX 形成、細胞毒性及び細胞増殖を検討した研究はない。

グルタルアルデヒドに関する動物とヒトの key events の一致を表4にまとめた。

key events	動物におけるエビデンス	ヒトにおけるエビデンス
細胞毒性	陽性	妥当性あり
細胞増殖	in vivo で陽性	妥当性あり
遺伝毒性	DPX (in vitro)	不明
突然変異	in vitro で陽性	不明
鼻腔腫瘍	陰性(どの部位にもエビデンスが ない)	不明

表4. グルタルアルデヒドの用語索引表

# 3. 実験動物ととトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、MOAのとトへの関連性を合理的に排除することができるか

### A. ホルムアルデヒド

推定される MOA に関する実験動物とヒトとの間の定量的差異は、標的組織におけるホルムア ルデヒドの濃度がもたらす作用にある。細胞毒性を引き起こすのはホルムアルデヒドそのもので あり、その代謝物ではない。吸入された外因性のホルムアルデヒドは、広く分布する多数の細胞 酵素、特にホルムアルデヒド脱水素酵素によって急速に代謝されギ酸塩になる。この効率的な代 謝解毒メカニズムに加えて、粘膜絨毛機構が、その下の上皮をガスや蒸気から保護する。したが って、標的組織において細胞毒性が発生するのに十分な遊離ホルムアルデヒドの濃度を達成する ためには、これらの保護機構を克服することが必要であり、比較的高濃度のホルムアルデヒド蒸 気を標的部位に到達させる必要がある。明らかに有意な発がん性をもたらす薬力学的事象は、ラ ットのホルムアルデヒド解毒機構が飽和した用量で発生する (Casanova & Heck、1987年)。

呼吸器系におけるホルムアルデヒド誘発性毒性の定量的な種差を考慮する際には、投与量を考 慮することが重要である。吸入されたホルムアルデヒドは、生体高分子との高い反応性のため、 最初に接触する上気道の領域に多く沈着し、容易に吸収される(Heck ら、1983 年; Swenberg ら、 1983 年)。鼻の生体機構、換気量、呼吸パターン(鼻または鼻腔)の間の複雑な関係が、種によっ て上気道のどこでホルムアルデヒドの吸収が起こるかを決定する。鼻呼吸であるげっ歯類では、 沈着と吸収は主に鼻腔内で起こる。対照的に霊長類は口鼻呼吸であり、吸収と沈着は主に口腔粘 膜と鼻腔で起こる可能性が高いが、気管と気管支でも起こる可能性がある(Monticello ら、1991 年)。 この仮説は、サルの上気道内のより遠位まで病理組織学的変化、上皮細胞の増殖及び DPX 形成 が観察されることによって支持されている。

ヒトにおけるホルムアルデヒドの鼻腔発がんリスクを予測するために開発された2段階のクロ ーン増殖モデルでは、用量評価における種差が考慮されている(Conolly ら、2004年)このモデル には、ラットとヒトの正常成長曲線、細胞周期の時間及び気道の異なる領域におけるリスクのあ る細胞に関するデータも組み込まれている。

マウスは反復ばく露により呼吸速度と一回換気量の両方を低下させることができるため、マウスはラットよりもホルムアルデヒドの沈着が少なく、その結果、鼻腔上皮の組織損傷と細胞の再 生率が低下する(Chang ら、1981年、1983年)。これらの特徴は、マウスの鼻で腫瘍発生がないこ とを説明するのに役立つかもしれない。

動物とヒトとの間には、気道の用量評価の違いによる定量的な違いがあると思われるが、推定 される MOA がヒトでは発生しないことを示すような根本的な違いはないようである。

### B. グルタルアルデヒド

実験動物におけるグルタルアルデヒドの動態は、ホルムアルデヒドに比べてあまり知られていない。吸入試験は実施されていないようである。半減期は、静脈内投与(ラット10時間、ウサギ 15~30時間)と経皮投与(ラット40~110時間、ウサギ20~100時間)の両方で長くなっているが、これはグルタルアルデヒドがタンパク質に結合し、代謝物の排泄が遅いためと考えられる。 代謝物は同定されていないが、おそらくグルタルアルデヒドの代謝は、アルデヒド脱水素酵素による対応するカルボン酸への初期酸化を伴うと推定されている。酸化によって産生されたグルタ ル酸は、コエンザイムA(CoA)と反応してグルタリル CoAを産生する。そして、グルタリル CoA デヒドロゲナーゼによってグルタコニル CoA に酸化され、最終的にはアセテートを介して二酸化 炭素に分解される(Beauchamp ら、1992年; NTP、1993年; NICNAS、1994年; Ballantyne、1995 年)。

グルタルアルデヒドは、主にアミノ基間の架橋剤としてタンパク質と容易に反応する。この反応は迅速で pH に依存しており (pH>9 で反応速度が増加)、Schiff 塩基を生成する。さらに反応すると、多くの複雑な反応生成物が得られるが、架橋反応のメカニズムはまだ完全には解明されていない。

グルタルアルデヒドと DNA との相互作用についてはほとんど情報がないが、グルタルアルデ ヒドが DNA と反応するのは 60℃以上の場合のみであることが報告されており(Hopwood、1975 年)、生理的条件下では反応しないことを示唆するデータもある(Sewell ら、1984年; Douglas & Rogers、1998年; Vock ら、1999年)。

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### 4. 信頼性、解析及び帰結の記述

### A. ホルムアルデヒド

持続的な細胞毒性と細胞増殖は、いくつかの動物種における腫瘍誘発に対して提唱されている MOA にとっての key events である。ラットにおけるホルムアルデヒド誘発性鼻腔腫瘍の MOA を 支持するデータは多数存在する。細胞毒性、DPX 形成、鼻腔上皮細胞の再生性増殖、扁平上皮化 生、炎症はラットの研究で観察されており、部位特異的で非線形の濃度反応過程であり、鼻腔腫 瘍の発生率と一致している。

エビデンスの重み付けに基づいて、動物 MOA は少なくとも定性的にはヒトに関連している可 能性が高い。ホルムアルデヒド蒸気にばく露されたサルでは、上気道上皮内の細胞増殖及び DPX 形成の増加が観察されている。また、ホルムアルデヒドへの組織ばく露後のヒト上皮細胞の増殖 は、ヒト気管支上皮細胞を移植したラット気管を胸腺欠損マウスに異種移植したモデル系でも観 察されている。主にホルムアルデヒドにばく露されたヒトの鼻の病理学的病変に関する数少ない エビデンスは、定性的に類似している実験動物の上気道の病変と一致している。さらに、いくつ かの疫学研究では、ホルムアルデヒドばく露に伴う鼻腔がんのリスク増加が示唆されている。

したがって、MOA はヒトに関連していると考えられ、ヒトのリスク評価のために、動物の鼻腔 腫瘍やその他の裏付けとなるデータを活用すべきである。このプロセスには、ホルムアルデヒド が非線形で用量依存的に腫瘍を誘発することを示唆するデータを考慮することが含まれる。また、 定量法の違いにより推定される MOA については種間の反応に量的な差異があるかもしれない。

### B. グルタルアルデヒド

グルタルアルデヒドの疫学研究は非常に限られており、鼻腔腫瘍との関連性は示されていない。 動物実験では、ラットとマウスの吸入試験において、グルタルアルデヒドは細胞毒性、細胞増殖、 DPX 産生を引き起こすことが示されているが、鼻腔腫瘍は引き起こさないことが示されている。 グルタルアルデヒドの方がホルムアルデヒドよりも明らかに毒性が強いという事実は、発がん性 の違いの説明にはならない。グルタルアルデヒドはホルムアルデヒドよりも低用量での細胞死が 多いため、発がん性があるとすれば、ホルムアルデヒドよりも低い用量でそのことが証明されな ければならない。

ホルムアルデヒドで推定される MOA、すなわち持続的な細胞毒性と細胞増殖は、グルタルアル デヒドに関連しているように思われるが、腫瘍形成では実証されていない。これらのアルデヒド に対する病理学的反応の違いは、ホルムアルデヒドがモノアルデヒドであるのに対し、グルタル アルデヒドはジアルデヒドであるためであることが暫定的に示唆されている。この違いが、グル タルアルデヒドの架橋生成物が生物学的機能を保持する可能性も修復する可能性もないように、 架橋の形態が異なるという結果になっているのかもしれない。この事例研究は、動物の腫瘍デー タが不十分な場合に HRF を適用することの難しさを浮き彫りにしている。

# 参考文献

Andersen I, Mølhave L (1983) Controlled human studies with formaldehyde. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 155–165.

Ballantyne B (1995) *Toxicology of glutaraldehyde: Review of studies and human health effects*. Bound Brook, NJ, Union Carbide Corporation.

Ballarin C, Sarto F, Giacomelli L, Bartolucci GB, Clonfero E (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. *Mutation Research*, **280**:1–7.

Beauchamp ROJ, St Clair MB, Fennell TR, Clarke DO, Morgan KT, Kari FW (1992) A critical review of the toxicology of glutaraldehyde. *Critical Reviews in Toxicology*, **22**:143–174.

Berke JH (1987) Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. *Journal of Occupational Medicine*, **29**:681–684.

Boysen M, Zadig E, Digernes V, Abeler V, Reith A (1990) Nasal mucosa in workers exposed to formaldehyde: A pilot study. *British Journal of Industrial Medicine*, **47**:116–121.

Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma*, **48**:144–147.

Burgaz S, Erdem O, Cakmak G, Erdem N, Karakaya A, Karakaya AE (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, **7**:151–161.

Bushy Run (1983) *Glutaraldehyde vapour subchronic inhalation study on rats*. Export, PA, Bushy Run Research Center (Project Report 46-101).

Casanova M, Heck Hd'A (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [<sup>3</sup>H]- and [<sup>14</sup>C]formaldehyde in Fischer-344 rats: Effects of glutathione depletion. *Toxicology and Applied Pharmacology*, **89**:105–121.

Casanova M, Heck Hd'A, Everitt JI, Harrington WW Jr, Popp JA (1988) Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food and Chemical Toxicology*, **26**:715–716.

Casanova M, Deyo DF, Heck Hd'A (1989) Covalent binding of inhaled formaldehyde toDNAin the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde andDNAby high- performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundamental and Applied Toxicology*, **12**:397–417.

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck Hd'A (1991) Covalent binding of inhaled formaldehyde toDNAin the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Fundamental and Applied Toxicology*, **17**:409–428.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck Hd'A (1994) DNA–protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Fundamental and Applied Toxicology*, **23**:525–536.

Casanova-Schmitz M, Starr TB, Heck H (1984) Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [<sup>14</sup>C]- and [<sup>3</sup>H]formaldehyde. *Toxicology and Applied Pharmacology*, **76**:26–44.

Chang JCF, Steinhagen WH, Barrow CS (1981) Effects of single or repeated formaldehyde exposures on minute volume of B6C3F1 mice and F344 rats. *Toxicology and Applied Pharmacology*, **61**:451–459.

Chang JCF, Gross EA, Swenberg JA, Barrow CS (1983) Nasal cavity deposition, histopathology and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicology and Applied Pharmacology*, **68**:161–176.

CIIT (1999) Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation, rev. ed. Research Triangle Park, NC, Chemical Industry Institute of Toxicology.

Coggon D, Harris EC, Poole J, Palmer KT (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *Journal of the National Cancer Institute*, **21**:1608–1614.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F (2005) Meeting report: Summary of IARC Monographs on formaldehyde, 2-butoxyethanol and 1-*tert*-butoxy- 2-propanol. *Environmental Health Perspectives*, **113**(9):1205–1208.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2004) Human respiratory tract cancer risks of inhaled formaldehyde: Dose–response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicological Sciences*, **82**:279–296.

Consensus Workshop on Formaldehyde (1984) Report on the consensus workshop on formaldehyde. *Environmental Health Perspectives*, **58**:323–381.

Crosby RM, Richardson KK, Craft TR, Benforado KB, Liber HL, Skopek TR (1988) Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and *E. coli. Environmental and Molecular Mutagenesis*, **12**:55–166.

### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Dalbey WE (1982) Formaldehyde and tumors in hamster respiratory tract. Toxicology, 24:9-14.

Douglas MP, Rogers SO (1998)D N A damage caused by common cytological fixatives. *Mutation Research*, **401**:77–88.

Edling C, Hellquist H, Ödkvist L (1988) Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. *British Journal of Industrial Medicine*, **45**:761–765.

Feron VJ, Bruyntes JP, Woutersen RA, Immel HR, Appelman LM (1988) Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Letters*, **39**:101–111.

Feron VJ, Til HP, Woutersen RA (1990) Letter to the editor. *Toxicology and Industrial Health*, **6**:637–639.

Gaylor DW, Lutz WK, Connolly RB (2004) Statistical analysis of nonmonotonic dose– response relationships: Research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. *Toxicological Sciences*, **77**:158–164.

Gross EA, Mellick PW, Kari FW, Miller FJ, Morgan KT (1994) Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. *Fundamental and Applied Toxicology*, **23**:348–362.

Hansen J, Olsen JH (1995) Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes and Control*, **6**:354–360.

Hardman JG, Limbird LE, Gilman AG, eds (2001) *Goodman & Gilman's The pharmacological basis of therapeutics*, 10th ed. The McGraw-Hill Companies, Inc., 2025 pp.

Hauptmann A, Lubin JH, Stewart PA, Hayes RB, Blair A (2004). Mortality from solid cancers among workers in formaldehyde industries. *American Journal of Epidemiology*, **159**:1117–1130.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H (1990) Mortality of U.S. embalmers and funeral directors. *American Journal of Industrial Medicine*, **18**:641–652.

He J-L, Jin L-F, Jin H-Y (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomedical and Environmental Sciences*, **11**:87–92.

Heck H, Casanova M (1995). Nasal dosimetry of formaldehyde: Modelling site specificity and the effects of pre-exposure. In: Miller JF, ed. *Nasal toxicity and dosimetry of inhaled xenobiotics: Implications for human health.* Washington, DC, Taylor & Francis, pp. 159–175.

Heck H, Casanova M (2004) The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. *Regulatory Toxicology and Pharmacology*, **40**:92–106.

Heck Hd'A, White EL, Casanova-Schmitz M (1982) Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomedical Mass Spectrometry*, **9**:347–353.

Heck Hd'A, Chin TY, Schmitz MC (1983) Distribution of [<sup>14</sup>C]formaldehyde in rats after inhalation exposure. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 26–37.

Heck Hd'A, Casanova-Schmitz M, Dodd PB, Schachter EN, Witek TJ, Tosun T (1985) Formaldehyde (CH<sub>2</sub>O) concentrations in the blood of humans and Fischer-344 rats exposed to CH<sub>2</sub>O under controlled conditions. *American Industrial Hygiene Association Journal*, **46**:1–3.

Heck Hd'A, Casanova M, Starr TB (1990) Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**:397–426.

Hedberg JJ, Strömberg P, Höög JO (1998) An attempt to transform class characteristics within the alcohol dehydrogenase family. *FEBS Letters*, **436**:67–70.

Hester SD, Benavides GB, Yoon L, Morgan KT, Zou F, Barry W, Wolf DC (2003) Formaldehydeinduced gene expression in F344 rat nasal respiratory epithelium. *Toxicology*, **187**:13–24.

Holmquist B, Vallee BL (1991) Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. *Biochemical and Biophysical Research Communications*, **178**:1371–1377.

Holmström M, Wilhelmsson B, Hellquist H, Rosén G (1989) Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Oto-laryngologica*, **107**:120–129.

Hopwood D (1975) The reactions of glutaraldehyde with nucleic acids. *Journal of Histochemistry*, **7**:267–276.

Horton AW, Tye R, Stemmer KL (1963) Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *Journal of the National Cancer Institute*, **30**:31–43.

IARC (2005) *Formaldehyde, 2-butoxyethanol and 1*-tert-*butoxypropan-2-ol.* Lyon, International Agency for Research on Cancer, 478 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88).

### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

IPCS (2002) *Formaldehyde*. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document No. 40).

Kamata E, Nakadate E, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. *Journal of Toxicological Sciences*, **22**:239–254.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA (1983a) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Research*, **43**:4382–4392.

Kerns WD, Donofrio DJ, Pavkov KL (1983b) The chronic effects of formaldehyde inhalation in rats and mice: A preliminary report. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 111–131.

Kimbell JS, Gross EA, Richardson RB, Conolly RB, Morgan KT (1997) Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutation Research*, **380**:143–154.

Koivusalo M, Baumann M, Uotila L (1989) Evidence for the identity of glutathione- dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Letters*, **257**:105–109.

Liber HL, Benforado K, Crosby RM, Simpson D, Skopek TR (1989) Formaldehyde-induced and spontaneous alterations in human *hprtDNA*sequence and mRNA expression. *Mutation Research*, **226** 31–37.

Liteplo RG, Meek ME (2003) Inhaled formaldehyde: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health*, **B6**:85–114.

Luce D, Leclerc A, Begin D, Demers PA, Gerin M, Orlowski E, Kogevinas M, Belli S, Bugel I, Bolm-Audorff U, Brinton LA, Comba P, Hardell L, Hayes RB, Magnani C, Merler E, Preston-Martin S, Vaughan TL, Zheng W, Boffetta P (2002) Sinonasal cancer and occupational exposures: A pooled analysis of 12 case–control studies. *Cancer Causes and Control*, **13**:147–157.

Lutz WK (1998) Dose-response relationships in chemical carcinogenesis: Superposition of different mechanisms of action, resulting in linear-nonlinear curves, practical thresholds, J- shapes. *Mutation Research*, **405**:117–124.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR, Moss OR (1986) Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology*, **41**:253–266.

Merk O, Speit G (1998) Significance of formaldehyde-induced DNA–protein crosslinks for mutagenesis. *Environmental and Molecular Mutagenesis*, **32**:260–268.

Monticello TM (1990) *Formaldehyde induced pathology and cell proliferation: A thesis*.Durham, NC, Duke University.

Monticello TM, Morgan KT (1994) Cell proliferation and formaldehyde-induced respiratory carcinogenesis. *Risk Analysis*, **14**:313–319.

Monticello TM, Morgan KT, Everitt JI, Popp JA (1989) Effects of formaldehyde gas on the respiratory tract of rhesus monkeys. Pathology and cell proliferation. *American Journal of Pathology*, **134**:515–527.

Monticello TM, Miller FJ, Morgan KT (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subacute inhalation of formaldehyde. *Toxicology and Applied Pharmacology*, **111**:409–421.

Monticello TM, Swenberg JA, Gross EA, Leiniger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Research*, **56**:1012–1022.

Morgan KT, Monticello TM (1990) Formaldehyde toxicity: Respiratory epithelial injury and repair. In: Thomassen DG, Nettesheim P, eds. *Biology, toxicology, and carcinogenesis of the respiratory epithelium*. Washington, DC, Hemisphere Publishing, pp. 155–171.

Morgan KT, Jiang X-Z, Starr TB, Kerns WD (1986) More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicology and Applied Pharmacology*, **82**:264–271.

NICNAS (1994) *Glutaraldehyde. Full public report.* Canberra, Australian Government Publishing Service, National Industrial Chemicals Notification and Assessment Scheme, July (Priority Existing Chemical No. 3).

NTP (1993) *NTP technical report on toxicity studies on glutaraldehyde (CAS No. 111-30-8) administered by inhalation to F344/N rats and B6C3F1 mice*. Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Toxicity Report No. 25; NIH Publication No. 93-3348).

NTP (1999) *Toxicology and carcinogenesis studies of glutaraldehyde (CAS No. 111-30-8) in F344/N rats and B6C3F1 mice (inhalation studies).* Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 490; NIH Publication No. 99-3980).

Olsen JH, Asnaes S (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *British Journal of Industrial Medicine*, **43**:769–774.

Pinkerton L, Hein M, Stayner L (2004). Mortality among a cohort of garment workers exposed to formaldehyde: An update. *Occupational and Environmental Medicine*, **61**:193–200.

### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Recio L (1997) Oncogene and tumor suppressor gene alterations in nasal tumors. *Mutation Research*, **380**:27–31.

Recio L, Sisk S, Pluta L, Bermudez E, Gross EA, Chen Z, Morgan K, Walker C (1992) *p53* mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. *Cancer Research*, **52**:6113–6116.

Rietbrock N (1965) [Formaldehyde oxidation in the rat.] *Naunyn-Schmiedebergs Archiv für* experimentelle Pathologie und Pharmakologie, **251**:189–190 (in German).

Rusch GM, Clary JJ, Rinehart WE, Bolte HF (1983) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicology and Applied Pharmacology*, **68**:329–343.

Schlosser PM, Lilly PD, Conolly RB, Janszen DB, Kimbell JS (2003) Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA– protein cross-link dosimetry model to estimate human equivalent doses. *Risk Analysis*, **23**:473–487.

Sewell BT, Bouloukos C, von Holt C (1984) Formaldehyde and glutaraldehyde in the fixation of chromatin for electron microscopy. *Journal of Microscopy*, **136**:103–112.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003) DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occupational and Environmental Medicine*, **60**:403–409.

Soffritti M, Maltoni C, Maffei F, Biagi R (1989) Formaldehyde: An experimental multipotential carcinogen. *Toxicology and Industrial Health*, **5**:699–730.

Speit G, Schutz P, Merk O (2000) Induction and repair of formaldehyde-induced DNA– protein crosslinks in repair-deficient human cell lines. *Mutagenesis*, **15**:85–90.

St Clair MB, Gross EA, Morgan KT (1990) Pathology and cell proliferation induced by intra- nasal instillation of aldehydes in the rat: Comparison of glutaraldehyde and formaldehyde. *Toxicologic Pathology*, **18**:353–361.

St Clair MB, Bermudez E, Gross EA, Butterworth BE, Recio L (1991) Evaluation of the genotoxic potential of glutaraldehyde. *Environmental and Molecular Mutagenesis*, **18**:113–119.

Stroup NE, Blair A, Erikson GE (1986) Brain cancer and other causes of deaths in anatomists. *Journal of the National Cancer Institute*, **77**:1217–1224.

Swenberg JA, Gross EA, Martin J, Popp JA (1983) Mechanisms of formaldehyde toxicity. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 132–147.

Swenberg JA, Gross EA, Martin J, Randall HA (1986) Localization and quantitation of cell proliferation following exposure to nasal irritants. In: Barrow CS, ed. *Toxicology of the nasal passages*. Washington, DC, Hemisphere Publishing, pp. 291–300.

Takahashi M, Hasegawa R, Furukawa F, Toyoda K, Sato H, Hayashi Y (1986) Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with *N*-methyl-*N*<sub>ℓ</sub>-nitro-*N*-nitrosoguanidine. *Japanese Journal of Cancer Research*, **77**:118–124.

Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE (1989) Two-year drinking- water study of formaldehyde in rats. *Food and Chemical Toxicology*, **27**:77–87.

Titenko-Holland N, Levine AJ, Smith MT, Quintana PJ, Boeniger M, Hayes R, Suruda A, Schulte P (1996) Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutation Research*, **371**:237–248.

Tobe M, Naito K, Kurokawa Y (1989) Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology*, **56**:79–86.

Uotila L, Koivusalo M (1974) Formaldehyde dehydrogenase from human liver. Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *Journal of Biological Chemistry*, **249**:7653–7663.

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and *S*- formylglutathione hydrolase activities in different rat tissues. *Advances in Experimental Medicine and Biology*, **414**:365–371.

Van Miller JP, Hermansky SJ, Neptun DA, Loscoa PE, Ballantyne B (1995) Combined chronic toxicity/oncogenicity study with glutaraldehyde (GA) in the drinking water of rats. *Toxicologist*, **15**:203 (abstract).

Vargová M, Janota S, Karelová J, Barancokova M, Šulcová M (1992) Analysis of the health risk of occupational exposure to formaldehyde using biological markers. *Analysis*, **20**:451–454.

Vock EH, Lutz WK, Ilinskaya O, Vamvakas S (1999) Discrimination between genotoxicity and cytotoxicity for the induction of DNA double-strand breaks in cells treated with aldehydes and diepoxides. *Mutation Research*, **441**:85–93.

Walrath J, Fraumeni JF Jr (1983) Mortality patterns among embalmers. *International Journal of Cancer*, **31**:407–411.

Wolf DC, Gross EA, Lycht O, Bermudez E, Recio L, Morgan KT (1995) Immunohistochemical localization of p53, PCNA, and TGF-Į proteins in formaldehyde- induced rat nasal squamous cell carcinomas. *Toxicology and Applied Pharmacology*, **132**:27–35.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron VJ (1989) Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *Journal of Applied Toxicology*, **9**:39–46.

Ying C-J, Yan W-S, Zhao M-Y, Ye X-L, Xie H, Yin S-Y, Zhu X-S (1997) Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomedical and Environmental Science*, **10**:451–455.

Zeiger E, Gollapudi B, Spencer P (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde—A review. *Mutation Research*, **589**:136–151.

Zissu D, Gagnaire F, Bonnet P (1994) Nasal and pulmonary toxicity of glutaraldehyde in mice. *Toxicology Letters*, **71**:53–62.

Zissu D, Bonnet P, Binet S (1998) Histopathological study in B6C3F1 mice chronically exposed by inhalation to glutaraldehyde. *Toxicology Letters*, **95**:131–139.

Zwart A, Woutersen RA, Wilmer JWGM, Spit BJ, Feron VJ (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. *Toxicology*, **51**:87–99.

# 付表:ホルムアルデヒドにばく露されたヒトにおける小核及び染色体異常に関する研究の 概要(IARC, 2005 年)

		エンド ポイン			
参考文献	標的組織	$\mathbb{F}$	反応(対照群vsばく露群)	コメント及びばく露	
Vargováら (1992)	PBL	MN	3.6% vs 3.08%	n = 20;対照群では高頻度;木材の破片製造 ホルムアルデヒド 8-h TWA 0.55-10.36 mg/m <sup>3</sup> 5- >16年	
Ballarin ら (1992)	鼻粘膜	MN	$0.25 \pm 0.22\%$ vs $0.90 \pm 0.47\%$ (P < 0.01)	木粉の同時ばく露; 用量反応性なし	
Burgazら (2001)	鼻粘膜	MN	$0.61 \pm 0.27\%$ vs $1.01 \pm 0.62\%$ (P < 0.01)	ばく露、n=23; 非ばく露、n=27; 用量反応性なし	
Burgazら (2002)	口腔粘膜	MN	$0.33 \pm 0.30\%$ vs $0.71 \pm 0.56\%$ pathology laboratory (P < 0.05) $0.33 \pm 0.30\%$ vs $0.62 \pm 0.45\%$ shoe factory (P < 0.05)	ばく露、n=22の可変ばく露; ホルムアルデヒドにばく露、n=28; 非ばく露、n=28; ばく露期間との相関性あり	
Titenko-Holland ら (1996)	口腔粘膜 鼻粘膜	MN MN	0.6 ± 0.5% vs 2.0 ± 2.0% (P = 0.007) 2.0 ± 1.3% vs 2.5 ± 1.3% (NS)	ばく露、n=28; ばく露前とばく露後の比較; 喫煙習慣の詳細は不明; ホルムアルデヒド濃度 口腔:1.2 mg/m <sup>3</sup> -h vs 18 mg/m <sup>3</sup> -h、90日間 鼻腔:2.4 mg/m <sup>3</sup> -h vs 20 mg/m <sup>3</sup> -h、90日間	
Yingら් (1997)	鼻粘膜 口腔粘膜	MN MN	$\begin{array}{l} 1.20 \pm 0.67 \ \text{vs} \ 3.84 \pm 1.48 \\ (P < 0.001) \\ \\ 0.57 \pm 0.32 \ \text{vs} \ 0.86 \pm 0.56 \\ (P < 0.001) \end{array}$	ばく露、n=25; ばく露前とばく露後の比較; 年齢、性及び喫煙習慣のコントロールに関する質問; ホルムアルデヒド濃度 0.508±0.299 mg/m <sup>3</sup> vs. 0.012±0.0025 mg/m <sup>3</sup>	
	PBL	MN	$0.91 \pm 0.39 \text{ vs } 1.11 \pm 0.54 \text{ (NS)}$		
НеБ (1988)	PBL	СА	$3.40 \pm 1.57\%$ vs $5.96 \pm 2.40\%$ (P < 0.01)	染色体異常には破壊や断片化が含まれており、解 釈が難しくなっている	
		MN	$3.15 \pm 1.46\%$ vs $6.38 \pm 2.50\%$ (P < 0.01)		

CA、染色体異常; MN、小核; NS、有意差なし; PBL、末梢血リンパ球; TWA、時間加重平均

# 第2部

# 非発がん MOA のヒトへの関連性を解析するための IPCS フレームワーク

ヒトに対するがん MOA の妥当性を解析するための IPCS フレームワーク(第1部参照)の完成 後、2006 年にジュネーブで専門家会議が開催され、化学物質リスク評価に IPCS フレームワーク を一般的に適用できるか否か(すなわち、がん以外のフレームワークを開発すること)という問 題が議論された。この専門家会議の参加者は、フレームワークはすべてのエンドポイントに適用 可能であるべきであると結論付け、会期外に出版物の草案を作成した。この草案は、ハーモナイ ゼーションプロジェクト運営委員会のメンバーによって査読され、その後、受領した査読コメン トを考慮して著者によって修正された。

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### 頭字語と略語のリスト

- ACE アンジオテンシン変換酵素
- CSAF 化学物質特異的調整係数
- EMS 好酸球增多筋痛症候群
- HBOC ヘモグロビン系酸素運搬体
- HRF ヒト関連性フレームワーク
- ILO 国際労働機関
- ILSI 国際生命科学研究機構
- IPCS 国際化学物質安全性計画
- MOA Mode of Action (作用モード)
- MPTP 1-メチル-4-フェニル-1,2,3,6-テトラヒドロピリジン
- RSI リスクサイエンス研究所 (ILSI)
- SLE 全身性エリテマトーデス
- UNEP 国際連合環境計画
- WHO 世界保健機関

# 非発がん MOA のヒトへの関連性を解析するための

# IPCS フレームワーク<sup>1</sup>

# Alan R. Boobis, John E. Doe, Barbara Heinrich-Hirsch, M.E. (Bette) Meek, Sharon Munn, Mathuros Ruchirawat, Josef Schlatter, Jennifer Seed, & Carolyn Vickers

体系化されたフレームワークは、化学物質のリスク評価の透明性と調和のとれたアプローチを促進する上で非常に有用である。これが成功している分野の一つに、発がん化学物質の MOA の実験動物とヒトの間の関連性の解析がある。最近、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)はヒトとの関連性に対処するために、動物における MOA フレームワークの更新版を発表した(がんヒト関連フレームワーク、または HRF)。この作業は現在、発がん以外の影響にまで拡大されており、最終的には、発がんと非発がんの両方のエンドポイントに対するフレームワークの適用を調和させることを目的としている。発がん HRF と同様に、最初のステップは、実験的観察に基づくエビデンスの重み付けが、仮説 MOA を確立するのに十分であるか否かを判断することである。これは、毒性影響に因果関係のある一連の key events からなり、Bradford Hill 基準に基づくアプローチを用いて同定される。実験動物とヒトの間でこれらの事象を、先ずは定性的に、それから定量的に比較する。解析により、結果の信頼性、解析及び帰結とともに、明確な結論が記述される。このフレームワークは、データの透明性のある評価、主要なデータギャップの特定、用量反応関係などの化合物のリスク評価に価値のある情報の特定、ライフステージの考慮などに基づく潜在的に感受性の高いサブグループを認識するための手段を提供している。

本論文に記載されているフレームワークは、非発がんヒト関連性フレームワーク(HRF)であ り、国際化学物質安全性計画(IPCS)(WHO/ILO/UNEP)の化学物質へのばく露によるリスク評価 のアプローチの調和プロジェクトによって作成されたものである。この世界的な「調和プロジェ クト」は、リスク評価手法の一貫性の向上と国際的なガイダンス文書の構築を通じて、化学物質 のリスク評価に対する世界的適用を目的としている。このプロジェクトは、環境と開発に関する 国際連合会議(1992年、国際連合)、化学物質の安全性に関する政府間フォーラム(1994年)、持 続可能な開発に関する世界首脳会議(2002年、UNEP)、国際的化学物質管理に関する戦略的アプ ローチ(2006年、WHO)などで合意された化学物質のリスク評価手法の調和に関する公約を達成 できるようにするものである。このプロジェクトには、化学物質の評価が行われる様々な分野の 専門家が参加しているため、作成された文書は、工業用化学物質、殺生物剤、殺虫剤、動物用化 学物質、医薬品、化粧品、天然毒物、食品添加物ならびに、食品中、水中、大気中及び消費者製品 中の環境汚染物質の評価に適用することができる。

調和プロジェクトの主な成果として、化学発がんに対する MOA 評価のための IPCS フレームワ

<sup>&</sup>lt;sup>1</sup>本論文は、WHO が著作権を有するもので、2008年に Critical Reviews in Toxicology, Volume 38, pages 87-96に掲載された。本 論文はWHO の出版物のために編集された。

ーク(Sonich-Mullin ら、2001 年)と、それに続くがん MOA のヒトへの関連性を解析するための IPCS フレームワーク(IPCS cancer HRF)(Boobis ら,2006 年;本文書の第1部も参照)がある。 MOA 解析は、因果関係の Bradford Hill 基準 (Hill、1965 年)に基づくエビデンスの重み付け (weight of evidence) アプローチを利用している。これは、腫瘍の原因に沿った一連の key events の特定を 必要とするエビデンスの重み付けアプローチを適用することで、動物実験で観察された発がん作 用に対する MOA を確立できるか判断することを目的としている。実験動物で MOA が確立され た場合、がん HRF は、MOA のヒトへの関連性を検討するために透明性のあるデータ評価を可能 にする解析ツールを提供できる。

これを受けて、IPCS は、必要に応じて修正を加えながら、他のエンドポイントとそれに関連す る MOA にもがんのフレームワークを適用できるか否かを検討することにした。国際生命科学研 究機構(ILSI)のリスクサイエンス研究所(RSI)は、がん以外のリスク評価に適用するために、 同様のフレームワークを開発した。また、IPCSは2006年3月にジュネーブで国際会議を開催し た。そこで、ILSIのフレームワークが一般的に化学物質のリスク評価に適用できるかという問題 を探るために、ILSIの出版物(Seed ら、2005年)と、ILSIのがんHRF(Boobis ら、2006年;本 文書の第1部も参照)を評価、検討した。要約すると、このIPCS会議では、フレームワークが発 がんと非発がんの両方のエンドポイントに適用されるべきであり、これを実践に移すための更な る作業を推奨している。そしてこれには、本稿にも記載しているが、フレームワークのより一般 的適用のための根拠の文書化及びフレームワークの利用と利用を促進するための手順が含まれて いる。

IPCS 会議では、化学物質リスク評価において非発がん HRF は下記のような複数の有用性を持つことが認識された。

- 実験動物における MOA の確立とヒトとの関連性について、国際的に調和のとれたアプローチ を提供する。
- MOA の基準が作成され、後続のケースを考慮することができるようになる。すなわち、ある 化合物に確立された MOA を適合できるかを示すことができるようになる。
- MOA のヒトとの関連性に関する重要な情報が明らかになり、MOA を共有する他の化学物質の 評価に情報を提供できるようになる。
- 一般的に、このフレームワークを適用することで、重要データの不足や必要な研究が明らかに なり、定性的・定量的な評価に役立てることができるようになる。

### 非発がんとト関連性フレームワークの必要性

非発がん HRF は、動物 MOA のヒトとの関連性を評価するための体系化されたアプローチを提供する手段であり、エビデンスの重み付けの文脈の中で、動物において推定される MOA のヒト への関連性を評価する。その後、推定される MOA のヒトへの外挿性を明確に検討することが含 まれており、多くの場合、種間の解剖学的、生理学的及び生化学的な差異などの一般的情報の検 討に基づいている。このようにして、フレームワークは、透明性のある解析方法で、化学物質に 特異的な情報と一般的な情報の両方を最大限に利用することを奨励している。

フレームワークを用いてヒトへの関連性を決定する際の透明性を確保するために極めて重要な のは、key events の様々な種におけるエビデンスの性質を明確にし、検討することである。すなわ ち、推定される MOA における、測定可能で毒性学的反応の誘発に重要であると想定される key events に関するエビデンスの性質を明らかにし、考慮することである。実験動物とヒトの差異を 明確に考慮した上での key events の一致性の評価は、ヒトとの関連性に関するエビデンスの重み 付けを考慮する上での透明性を保つための基礎となる。

主にハザード評価において、非発がん HRF は、その後のヒトに関連すると考えられる MOA の 用量反応解析に関わる適切な key events の明確な定義とデータの検討を通じて、リスク評価の透 明性を高めることにも貢献している。実験動物における MOA がヒトと定性的に関連があると判 断された場合には、ヒトとの関連性が否定されるか否かを判断するために、実験動物とヒトの双 方からの動態学的及び薬力学的情報を考慮に入れた、より定量的な評価が必要である。

これらのデータは、その後の関連があると考えられる MOA の用量反応解析に極めて重要であ る(IPCS、2005年)。例えば、化学物質特異的調整係数(CSAFs)の定義づけにおいて、デフォル トの不確実係数を置き換えるための基礎として、利用可能な情報の妥当性を考慮する場合である。 この情報は、標的組織における反応代謝物の形成速度の種差を考慮して、毒物動態の種間差に対 するデフォルトのサブファクターを CSAF で置き換えるための適切な根拠となり得る(IPCS、2005 年)。

また、この非発がんHRFの使用はすべてのエンドポイントのリスク評価適用の調和を促進する。 例えば、発がん影響と非発がん影響に関するこれまでの異なるアプローチの架け橋となる。ここ でいう調和とは、関連情報を最大限に活用するために生物学的関連性を探ることが重要であり、 すべてのエンドポイントのリスク評価における一貫したアプローチを意味する。例えば多くの場 合、臓器毒性は同一部位に腫瘍を誘発すると推定される MOA において key events となる。非発が ん HRF は、その後の用量反応解析における種間差及び個体間のばらつきの定量化に関連する、重 要な前がん病変の key events を特定するための段階を設定する。他にも、推定される MOA が複数 の臓器に毒性効果をもたらす可能性があれば同じ非発がん HRF 解析で検討されることになる。

さらに、透明性のあるフレームワークで検討することで、それ自体は毒性学的影響に必須では ない(すなわち key events ではない)が、key events を修飾し、種や個体間の差異に寄与する因子 を特定できる可能性がある。そのような要因には、代謝経路の遺伝的差異、競合する代謝経路、 及び同時進行する病理学的要因により誘発される細胞増殖が含まれる。

このような解析は、特定の用量範囲でのみ、作動する可能性のある推定される MOA の構成要

素に関する指標も提供するかもしれない。MOA に必須な事象を引き起こすのに、対象の化合物の 高用量ばく露が必要とされる場合、ヒトのリスクとの関連性はばく露量に依存する問題となる。 このように、リスク評価におけるばく露評価は、包括的な評価を行う上で非常に重要である。

重要なことは、非発がん HRF を適用することで、リスクが高い集団(例えば遺伝的素因を持つ 人)を特定し、様々なライフステージにおける相対リスクに関連する情報を提供できるようにな るということである。これは多くの場合、化学物質固有の情報に基づくのではなく、MOA の知識 に基づき特定の年齢層でのリスクが増加しているか減少しているかを推論することに基づいてい る。このためには、ヒト及び動物モデルにおける発達及び老化過程を比較し、事象を明確に検討 する必要がある。これらの検討事項は、後続のリスク評価過程において用量反応解析など、どこ に重点を置くべきか決定する上で重要となる。

推定される MOAs のエビデンスの重み付けと透明性のあるヒトとの関連性を示すこと(利用可 能なデータベースの長所と短所を明確に検討し、種間の質的・量的な類似性と相違点及び関連す る不確実性を強調することを必要とする)は、利用可能なデータの矛盾を特定し、重要なデータ ギャップと研究の必要性を明らかにすることにもなる。これは、各ステップにおいて、解析の基 礎となるデータの質と量、フレームワーク内での解析の一貫性、データベースの一貫性(つまり 研究が互いに矛盾していないこと)、そして一致解析の質的・量的な信頼性を明確に評価する必要 があることに由来している。

すべてのデータが利用可能になる前であっても、推定される MOA 及びヒトとの関連性の解析 に、非発がん HRF を繰り返して適用することは、追加情報の獲得とともに研究戦略を策定・改良 するための基礎として有益である。すなわち、フレームワークは動物における推定される MOA の ヒトへの関連性解析を支援するデータの性質について、リスク評価者と研究者が共に理解を深め ること、データ取得に向け次の一手を決める手助けとなるに違いない。また、研究戦略を立案す る際に MOA を繰り返し検討することで、重要な分野に資源を集中させ、より階層的で集中的な アプローチを行うことができ、効率性を高めることが期待される。

知識の進歩に伴い、MOA は化学物質に特化したものから、関連する主要な生物学的プロセスに 基づいて、ある化合物から別の化合物へとヒトとの関連性をより一般化させることになるだろう。 MOA を構成する key events が発生していることを厳密に立証することが常に必要であるものの、 MOA を確立する際に化学物質固有のデータを必要とすることは少なくなるだろう。

非発がん HRF を適用した推定 MOA の透明性は、解析と推論を裏付ける明確で一貫性のある文 書化を奨励している。そして、それを満たすことで結果の信頼性を高める重要なデータギャップ を特定し、データの矛盾や不確実性を明らかにし、到達した結論に対する信頼性を向上させる。 このような透明性は、リスク評価者と研究者との議論を促進させるだけでなく、異なる規制機関 間の意見合意にも貢献すると期待される。 また非発がん HRF は、専門家の関与やピアレビューのために改善されたプロセスや内容の基礎 を提供し、推定される MOA 及びそのヒトへの関連性についての助言や承認を得るための基礎と して、解析の明確性や透明性の最低限の基準を明らかにする。これらの基準に従うことで、他者 が key events や他の MOA の除外、対象 MOA のヒトへの関連性の結論の根拠を、明確に認識でき るようになる。

### 非発がん HRF はどのような場合に適用されるのか

非発がん HRF は MOA を評価するための貴重なツールを提供するが、かなりの労力と実験的作 業を必要とするため、すべての化学物質の評価に用いられるものではない。HRF の主な目的は、 動物でみられるすべての影響がヒトに関連するというデフォルトの仮定を適用するか否かを決定 することであろう。この疑問は、リスク評価の過程で上記の仮定を適用した場合に評価結果がヒ トに有害な影響が発生する可能性が高いことを示す場合に重要性が増す。例えば、検討中の影響 が発生する最低用量とヒトの推定ばく露量との差が小さい場合、特にヒトのばく露量の推定値が 既に精緻化されている場合である。そしてその場合、リスク管理対策が必要かを知ることが重要 になる。このことは、新たな影響を特定したデータ、化学物質の用量反応関係に関する追加デー タ、または使用パターンの変更やばく露推定値の変更など、既に使用されている化学物質のリス ク評価を変更するような新たなデータが出てきた場合に最も懸念されることである。

非発がん HRF の使用は、動物での影響が神経毒性や催奇形性など、ヒトで発生した場合に重大 な影響を及ぼす可能性がある場合にも有用である。これらの影響は非常に厳格なリスク評価プロ セスの対象となるため、比較的頻繁にリスク管理措置の必要性が示唆される。

がん以外にHRFの使用を検討すべきもう一つの状況は、影響の種類または影響が発生する用量 レベルのいずれかに種差がある場合である。このような場合には、どの種がヒトへの外挿におい て最も適切なものであるかを理解することが重要である。この指針は、同一種の性別又は系統間 の違いにも適用される。

これらの状況は、さらなる検討が必要であることを示しており、非発がん HRF はこれを行う方 法を提供している。このフレームワークは、影響を検討するプロセスのどの段階でも適用でき、 科学者の道しるべとなるよう、影響を調査する過程で反復的に適用されるべきである。最初に影 響が観察され、懸念が生じたとき、フレームワークは、調査者が対処すべき問いを示すことによ って作業プログラムを構成できるようにする。研究の進展とともに、HRF はデータが作成された ときには、データを評価する研究者を導き、また他のデータが必要かどうか、どのようなデータ が必要かを決定する際の指標となる。

大量のデータがある状況では、フレームワークにより、評価者はその重要性と量に応じてエビ デンスの重み付けが可能となる。 非発がん HRF は、既にフレームワークを用いて確立されている MOA によって、化学物質が影響を 起こすことが観察された場合や、化学物質が既に確立された MOA を持つ化学物質と構造的類似があ る場合にも有用である。MOA を確立するために非発がん HRF を早期に使用することで、MOA を新し い化学物質に適用させるために調査する必要がある重要なステップを特定することができる。これは、 新しい研究や試験を設計する際の前向きな方法としても、データを遡及的に評価する方法でも、価値 あるものになるだろう。

### 非発がん HRF の検討

非発がん HRF は、推定される MOA とヒトとの関連性を評価するための全体的なエビデンスの重み 付けのための手段を体系化する解析ツールである。このフレームワークは、状況に応じて異なるため、 情報が十分であるか否かに対する絶対的な答えを提供するようには設計されていない。これは基準の チェックリストではなく、あくまでもデータの評価と提示の手段である。フレームワーク適用による 成果は、化合物のリスク評価を継続するための基礎となる。

非発がん HRF は、臓器、組織、細胞の構造と機能の変化(生理学的影響や神経行動学的影響を含む) を含む幅広い毒性学的エンドポイントに適用されることが想定されている。このフレームワークを用 いて対処できる毒性の種類には、以下のものが含まれるが、これらに限定されるものではない。

- 臓器毒性:例)ベンゼンによる血液毒性(再生不良性貧血)、パラコートによる肺毒性、クロロ キンによる眼毒性など
- 生殖毒性:例)フタル酸による男性不妊、ダイオキシンによる女性の受胎能力障害など
- 発達毒性:例)メチル水銀による発達神経毒性、レチノイドによる催奇形性など
- 神経毒性:例)鉛による末梢神経障害、アクリルアミドによる軸索障害、1-メチル-4-フェニル -1,2,3,6-テトラヒドロピリジン(MPTP)によるパーキンソン病など
- 免疫毒性:例)オルガノチンによる免疫抑制、イソニアジドによる全身性エリテマトーデス(SLE) 様症候群、汚染された L-トリプトファンによる好酸球増多症症候群(EMS)など

### MOA の紹介

非発がん HRF 解析に着手する前に、実験動物における化学物質へのばく露による毒性学的反応のエ ビデンスの重みを慎重に評価する必要がある。非発がん HRF の特徴は一度に1つの MOA のみを解析 する点である。したがって、例えば、化学物質の投与に関連した異なる毒性学的影響は、たとえ同じ動 物で観察されたとしても、各影響の MOA を識別するために別のフレームワーク解析を必要とする。代 謝的活性化と解毒における種や組織の違いと同様に、一部の毒性物質では毒性発現部位の一致性が低 い場合がある。動物とヒトのデータを比較する際には、この点に留意する必要がある。

### 推定されるMOA (事例における仮説)

ここで、被験物質の毒性学的影響の原因となっていると推定される MOA における一連の事象

の概要を簡単に説明している。この記述は次のセクションにつながり、MOAの中で「重要」と考えられる事象(すなわち、必要かつ測定可能な事象)を特定するものである。

#### Key events

MOAにおける「key events」を簡潔に説明する。key events とは、推定される MOA で仮定され た毒性学的反応の誘発に重要な事象であり、測定可能な事象である。ある事象を key events とし て支持するためには、その事象を特徴づける、一貫した測定実験データが必要である。関連する 可能性のある情報の種類には、例えば、類似の細胞における毒性学的反応と関連する key events、 理論上関連する作用部位、特定の生化学的事象、酵素の発現または活性の変化、受容体とリガン ドの相互作用、補因子レベルへの影響、組織学における特異的変化、細胞増殖の変化(増加また は減少)、ホルモンのホメオスタシスまたは他のシグナル伝達経路(細胞内または細胞外)、セカ ンドメッセンジャー、またはイオン流束、高分子の分解性の増加、さらには膜透過性または膜統 合性の変化などがある。

### 用量反応関係の一致

それぞれの key events と毒性学的反応の量的反応関係を特徴づけ、Bradford Hill 基準(Hill、1965 年)を参考にして相互関係を検討すべきである。理想としては、key events の大きさ(または頻度) の増加の用量依存性と、その後に発生する他の key events の重症度(例えば、病変の進行)の増 加、最終的な毒性学的反応に関連づけられるべきである。key events と毒性学的反応の変化の大き さを比較することは、用量反応の一致を検討するのに役立つことが多い。

用量反応曲線の異なる部分において生物学的反応に根本的な違いがあるか否かを考慮することが重要である(Slikker ら、2004 年)。もしあれば、用量反応曲線の異なる部分に関連する key events を定義し、フレームワーク解析に用いる必要がある。

### 時間的関連性

各 key events と毒性反応の時間的関係を特徴付ける必要がある。key events は毒性が明らかにな る前に観察可能であり、時間的に互いに一貫している必要がある。これは、データが推定される MOA を支持しているか否かを判断する上で不可欠なステップである。毒性反応と同時に(試験終 了時など) key events を観察しても、時間的関連性についての結論は出ないが、次のセクションで 説明する解析に貢献することができる。

### kev events と毒性学的反応との関連の強さ、一貫性及び特異性

key events、前駆病変及び毒性学的反応を結びつけるエビデンスの重みに注目すべきである(エ ビデンスの重みの意味についての考察は Weed [2005 年]を参照)。key events を遮断または減少さ せた場合に毒性が発現しない、または減少したことを示す中止/回復試験は関連性の試験として特 に有用である。

様々な実験計画は未知のバイアスや交絡を減らすことができるため、実際デザインの異なる多

くの研究で一貫した結果が得られることは、MOA の支持を高める。一貫性(Consistency)は、異なる研究において仮定された MOA の key events の再現性であるが、整合性(coherence)とは異なるものであり、仮定された MOA と観察との関係をより広く扱うものである(次のポイントを参照)。ここで価値があると思われる観察には、類似の細胞における毒性学的反応や関連する key events、論理的に関連する作用部位、中止/回復試験の結果などが含まれる。

### 生物学的妥当性及び整合性

MOA が、ターゲットとなる順序/場所の生物学一般についての知見と一致しているかどうか (生物学的妥当性)、また物質の全体的な生物学的影響について具体的に知られていることとの関 連性(整合性)を考慮すべきである。推定される MOA とそれに関連する key events が生物学的に 妥当であるためには、現在の生物学的知見と一致している必要がある。しかし、生物学的妥当性 をエビデンスの重みを評価する基準として用いる場合には、我々の知識との間にギャップが生じ る可能性を考慮することが重要である。推定される MOA と化学物質固有の観察結果との関係を より広く、例えば、毒性学的反応の MOA と他のエンドポイントの MOA との関連性などは、整合 性(前項で述べた)とは区別する必要がある。一貫性を評価する際には、構造類似体に関する情 報に価値があるかもしれない(すなわち、構造活性解析)。また、推定される MOA を共有する他 の化合物から得た性差、種差、系統の違いや key events との関係などに関する情報も有用であろ う。さらに、このセクションでは、推定される MOA を支持するために、その物質に関するデータ ベースが内部的に一貫しているかどうかを検討すべきである。

### その他のMOA

論理的に存在する代替 MOA を検討すべきである。代替 MOA が支持される場合、それらは 別の非発がん HRF 解析を必要とする。これらは、検討中の MOA の一部として扱われるため、 単一の MOA の追加的な構成要素とは区別されるべきである。

### 不確実性、矛盾及びデータギャップ

不確実性は完全かつ明示的に記載すべきである。不確実性には、毒性反応の生物学的性質に関 連するものと、評価対象の化合物のデータベースに関するものが含まれるべきである。矛盾する ものはすべて記載し、データギャップを特定すべきである。また特定されたデータギャップが、 推定される MOA を支持する上で重要であるか否かも明記すべきである。

### 推定されるMOA の評価

推定される MOA に対する高、中、低など信頼度を示す解析結果の明確な記述があるべきであ る。新規 MOA が推定されている場合は、これを明確に示すべきである。しかし、MOA が以前に 説明されたものと同じである場合は、key events がこの MOA に適合する程度を明示的に記載する 必要がある。主要な相違点はすべて記載し、MOA の適用が与える影響を議論すべきである。

### ライフステージの考察

化学物質ばく露に対する生物の反応は、その一生を通じて変化する可能性があるため、すべて の毒性エンドポイントの MOA 解析において、ライフステージを考慮することが重要である。器 官の感受性は臨界期、主要な代謝酵素の発生時期、あるいは母体と胎児の相互作用に依存するか もしれないため、特に発生期のばく露で生じる影響において重要である(Zoetis & Walls、2003 年 を参照)。さらに、発生過程の障害はその下流に影響をもたらす可能性がある。

老化を考慮することも、いくつかの理由から重要である。第一に、発育期のばく露では、かな り後にならないと毒性が検出されないことがある。さらに、器官系ごとに種特異的な老化パター ンが存在する可能性がある。例えば、生殖器はげっ歯類とヒトでは病因が異なり、げっ歯類の系 統によっても異なることがある。

### ヒトとの関連性

動物において毒性学的影響の MOA を確立することができれば、次の段階でヒトへの関連性を 評価する必要がある。IPCS の非発がん HRF は、一連の3つの(または4つの)問いに答えるア プローチとして提示されており、毒性学的影響の根底にある MOA のヒトへの外挿性に関する文 書化された論理的な結論を導き出すことができる。このガイダンスを適用すると、4つ(または5 つ)のセクションで構成された説明文が作成され、リスクアセスメントのハザード評価に組み込 むことができる。

1. 動物における MOA を確立するのにエビデンスの重み付けは十分か。この問いは、因果関係の Bradford Hill 基準(Hill、1965年)に基づいた、上記のような MOA 解析を行うことで解決される。可能性のある代替 MOA におけるエビデンスの重みを考慮し、検討中の MOA を支持するエビデンス全体の強さに基づいて結論を出す必要がある。このようなアプローチは、重要なデータギャップを特定する。このデータギャップが埋められることにより、提示された MOA はより信頼性を高めるであろう。推定される MOA が他の化学物質について既に記述されている場合、そのヒトへの外挿性は評価されているだろう。推定される MOA が新規である場合、ヒトへの外挿性は新たに評価する必要がある。

2. 実験動物とヒトとの間のkey events の根本的、質的な違いに基づいて、MOA のヒトとの関連 性を合理的に排除することができるか。この問いでは、ヒトに対する MOA の関連性の質的評価 を行う。動物の MOA で発生する重要な key events をリストアップし、各 key events がヒトで発生 する可能性があるかどうかを直接評価することで、評価が容易になり、プロセスの透明性が高ま る。一致表と呼ばれる表形式での提示は特に有用である。この表に従って証明するため、表の情 報は比較的簡潔でなければならない。1 つの列には、key events のそれぞれについてのヒトへの影 響が評価されている。毒性をもたらさない異なる系統、種、性、または異なる投与経路の結果に 関する列は、比較するのに有用である。それ自体は重要ではないが、key events を修飾し、種差ま たは個体差に寄与する因子が同定される場合がある。例としては、代謝経路における遺伝的差異、 競合する代謝経路及び同時並行的な病態によって誘発される影響などが挙げられる。特定されたそのような要因はすべて、一致表の脚注に記載する必要がある。

代謝経路における遺伝的差異、競合する代謝経路及び同時並行的な病態によって誘発される影響などが挙げられる。特定されたそのような要因はすべて、一致表の脚注に記載する必要がある。 ある化学物質のヒトにおける MOA の key events の一致性の評価は、特定の化学物質の評価という よりは、ヒトにおける MOA の評価である。一般的に、初期の key events の詳細は、より化学物質 に特化したものになる可能性が高い。その後の事象は、MOA により一般的に当てはまる事象とな る。ヒトにおける key events を評価するための情報は、物質そのものに関する in vitro 及び in vivo 研究から得られるが、解剖学、生理学、内分泌学、遺伝的疾患、疫学、その他ヒトにおける key events に関して知られているあらゆる基本的な情報が重要となる。

この問いに答える際には、エビデンスの重みとヒトの情報における信頼度の評価を記述した説 明文を作成する必要がある。有用な情報の具体例としては、以下のようなものがある。

- 必要に応じて、年齢、性別、人種差、化学物質やその他環境因子などの危険因子を含む、対象 となる解剖学的部位や細胞種における発生率の背景値
- 生理的、細胞的、生化学的レベルの制御機構や発生、構造(肉眼、組織)を含む標的部位の性質と機能に関する知識
- 標的器官の制御と応答に関する考察をもたらすヒト及び動物の疾患状態
- 対象となる臓器や影響を含む、短期、中期、または長期ばく露後の、評価対象の化学物質また は構造類似体に対するヒト及び動物の反応

MOA がヒトには関連性がないと明確に結論づけるためには、相当量の情報が必要である。その ような結論がデータによって強く支持されている場合、その MOA のみで毒性を生じる化学物質 はヒトにリスクをもたらさず、このエンドポイントに関する追加のリスク評価は必要ない。

関連性の問題は、すべての集団とライフステージを考慮している。MOA が作動する条件が、主 に感受性の高い集団やライフステージにいる人に発生する可能性がある。例えばすでにウイルス に感染しているか、ホルモンバランスの不均衡を有する人または疾患を持っている人である。感 受性の質的または量的差異を示唆する情報は、リスクの特徴付けに使用するため強調される。

非発がんHRF解析では、ライフステージに関連して考慮すべきいくつかの側面がある。第一に、 解析ではヒトと動物モデルで起こる発生過程と事象を比較検討すべきである (Zoetis & Walls、2003 年参照)。この比較は、ヒトと動物モデルにおいて発生過程がどの程度類似しているかを示すもの である。一般的に、発生過程はそんなに変化しておらず、このような場合には、動物 MOA はヒト MOA にも当てはまるという結論が導き出される。しかし、いくつかの種には特有の発生過程があ り、その場合種特異的な MOA となり、ヒトにもあてはまるとは限らない。 第二に、解析では、ヒトと動物モデルにおける発生過程や事象の相対的な時期や段階の特異性 を考慮する必要がある。重要な発生過程における事象は、発生期の異なる時期に生じる可能性が ある。いくつかの発生過程における事象は、動物では出生前の早い時期に発生し、ヒトでは出生 前の遅い時期に発生する可能性がある。その他発生過程における事象は、ヒトでは出生前に発生 し、動物では出生後に発生することもあれば、その逆もある。胎盤移行と乳汁移行に大きな差が ある場合には、発生時期の違いが線量基準に影響を与える可能性がある。同様に、発生過程に関 連する主要な代謝酵素の発生時期を比較すると、ヒトと動物モデルの間にかなりの差があること が明らかになる可能性がある。このような考察から、ヒトでは動物モデルの MOA は妥当ではな いという結論が導き出されるかもしれない。

実験動物とヒトとの間の薬力学的または動態的要因のいずれかの量的差異に基づいて、MOA 3. のヒトヘの関連性を合理的に排除することができるか。実験動物における MOA がヒトと定性的 に無関係であると判断できない場合(問い2に「No」)、実験動物とヒトから得られるあらゆる動 態学的及び薬力学的情報を考慮して、より定量的な評価が行われる。このようなデータは、必然的 に化学物質と MOA に固有のものであり、可能であれば、毒性を引き起こす薬力学的影響が発現 する用量を含むべきである。動態学的考察には、吸収の速度及び程度、組織分布、代謝及び排泄が 含まれる。胎生の違いは、胎盤移行及び乳汁移行において実質的な種差をもたらす可能性があり、 これは定量的な影響を及ぼす。したがって、これによりヒトと実験動物との間で MOA の量的差 異がもたらされる可能性がある。同様に、主要な代謝酵素の発現時期の違いにより、ヒトと実験 動物の間ではかなりの差が生じる可能性がある。薬力学的な考慮事項には、化学物質と細胞、組 織及び器官との相互作用が含まれる。量的差異に基づいてヒトへの外挿性を否定することができ るのは、ごくまれである。定量的ばく露評価は HRF よりもむしろその後のリスクの判定の一部で あるため、その差はヒトへのばく露がそのようなレベルに達するとは考えられないほどの大きさ でなければならない。ほとんどの場合、後続のリスクの判定で正式なばく露評価を行わなければ、 そのような結論に達することはできない。したがって、問いの答えは「No」であるが、その後の リスク評価では、リスクは無視できる程度であると結論づけられるかもしれない。メラミン誘発 性膀胱発がんは、この点を説明する有用な事例研究を提供している(Meek ら、2003 年)ここで も、実験動物とヒトからのデータを表形式で比較することが評価に役立つ。同様または類似の MOA によって作用する他の化合物の研究から得られた情報も価値がある。実験動物とヒトの間の 反応の違いの基礎的な理解が深まれば、定性的であると考えられていた key events の違いが、定 量的な違いによるものであることが示されるかもしれない。

量的差異に基づいて毒性 MOA がヒトと関連性がないと結論づけることはできないかもしれな いが、評価の中で、これらの差異の程度はリスク評価に顕著な影響を与えるのに十分である。し たがって、この問いへの回答の説明文は包括的であり、可能な限り定量的な情報を把握しておく ことが重要である。

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問い2と同様に、この質問への回答が「Yes」であれば、この MOA のみで毒性を示す化学物質 へのばく露はヒトにリスクをもたらさず、追加のリスク特性評価は必要ない。

前述の3つの質問は、決定ツリーを構成している(図1参照)。

動物における MOA を立証するのに エビデンスの重み付けは十分か? NO リスク評価を続ける

### ↓ YES

MOA は関係ない YES

実験動物とヒトとの間の key events の
 基本的な質的差異に基づいて
 MOA のヒトへの関連性を合理的に排除できるか?

#### ↓ NO

	VES	実験動物とヒトとの間の動態学的または薬力学的 要因のいずれかの量的な違いに基づいて MOA のヒ	NO	
MOA は関係ない	$\leftarrow$	要因のいずれかの量的な違いに基づいて MOA のヒ	$\xrightarrow{\text{INO}}$	リスク評価を続ける
	•	トへの関連性を合理的に排除することができるか?		

# 図 1. 実験動物で観察された毒性に対する MOA のヒトへの関連性を判断するための 決定ツリー

### 用量反応評価への影響

リスク評価を進める前に、毒性に対する MOA のヒトへの関連性を除外することはできないが、 更なる問いに対処すべきである。それは以下の点である。これは、4. 種や個体差の不確実性因子 のデフォルト値を変更できるような、key events に量的な違いがあるか。このような情報は、動態 学又は薬力学のいずれかに関するものである。これは CSAF を計算するために使用することがで き、その場合動態学又は薬力学における種又は個体差のデフォルト値の1つ以上が、化学物質固 有の情報に基づく値に置き換えられる (IPCS、2005 年)。調整係数の他の要素は変更しない。この ような情報は、通常のデフォルト値と比較して調整係数の増加または減少をもたらす可能性があ る。

### 発表された事例研究

がん以外のエンドポイントに対する MOAs のヒトへの関連性を評価するためのフレームワーク を決定するにあたり、ILSI/RSI はまた、一連の例示的な事例研究を実施した。これらの事例研究 は以下の通り。

モリネートによる精子形成阻害(Kavlock & Cummings、2005年a)、
エチレングリコールの腎及び発育への影響(Corley ら、2005年)、
ニコチンの発達神経毒性(Slikker ら、2005年)
フタル酸エステルの男性の生殖発達に対する影響(Foster、2005年)、
ビンクロゾリン誘発性奇形(Kavlock & Cummings、2005年b)、
バルプロ酸の発育への影響(Wiltse、2005年)、

ヘモグロビン系酸素運搬(HBOC)関連先天性奇形(Holson ら、2005 年)、
 アンジオテンシン変換酵素(ACE)阻害剤の発生影響(Tabacova、2005 年)、
 ポリハロゲン化芳香族炭化水素の発達聴器毒性(Crofton & Zoeller、2005 年)、
 プロピルチオウラシルが神経発達に及ぼす影響(Zoeller & Crofton、2005 年)などがある。

これらの事例は、様々なエンドポイントを対象としているが、そのほとんどは発生段階の影響 を対象としている。したがって、上記のような他のエンドポイントに関する追加の事例研究が必 要である。このフレームワークの使用経験の積み重ねにより、公開された事例のいくつかは、フ レームワーク適用ための例示的事例を提供できるように、さらに洗練されていく可能性がある。

一般的に、これらの事例は、この非発がん HRF の基礎となっているいくつかの重要な問題を強 調する上で非常に有用である。例としては、一致解析の重要性、ヒトへの関連性を排除できない 場合のフレームワーク適用中に特定された量的情報の価値、フレームワーク解析の結論を報告す る際の透明性のある包括的な構成の必要性、重要なデータギャップの特定(例:モリネートと HBOC の事例研究)、研究ニーズの特定(例:ビンクロゾリンの事例研究)、特定の代謝物の形成を理解 することの重要性、Bradford Hill 基準(Hill、1965 年)を key events に適用して堅牢な MOA を確 立することの重要性などが挙げられる。

### 信頼性、解析及び帰結の記述

非発がん HRF を適用し、3つ(または4つ)の質問に回答した後、解析の基礎となるデータの 質と量、フレームワーク内での解析の一貫性、データベースの一貫性及び一致性解析の性質と程 度に対応した信頼性を示す文書を提供すべきである。必要に応じて代替的な MOAs は、同様に厳 密に評価されるべきである。重要な成果は、解析の信頼性を高めるために実験的に対処できるデ ータギャップを特定することである。

非発がん HRF を行うことにより、実験動物における毒性 MOA がヒトに関連するかどうかを判 断するだけでなく、それ以上に有用な情報が得られる。また、関連する影響のリスクの判定の次 の段階で重要な情報を得ることができる。例えば、得られた情報に基づいて CSAFs を開発するこ とができるかもしれない。フレームワークを適用することで、リスクに影響を与える可能性のあ る調節因子の情報も提供できる。さらに、推定される MOA の要素のうち、特定の用量範囲での み作用するものを特定することができる。MOA で必須の段階が、ある化合物の高用量投与後にの み発生する場合、ヒトのリスクに対する MOA の関連性はばく露量によって決定される。したが って、効果的なばく露評価は、このような毒性によるヒトリスクの評価にとって特に重要である。

解析もまた、リスクが増加する可能性のある集団(例えば、遺伝的素因を持つ人々)の特定に 寄与し、多くの場合様々なライフステージにおける相対的なリスクを考慮するのに有用な情報を 提供している。これは必ずしも化学物質固有の情報に基づくものではなく、むしろ MOA の知識 に基づいて、年齢によってリスクが異なるかどうかについての推論に基づくものである。 非発がん HRF を用いたデータとその解析は、他の人が到達した結論の根拠を判別できるように 明確かつ包括的な方法で報告されるべきである。

提示の具体的な形式は利用可能なデータの種類によって異なるが、可能であれば、フレームワ ークの主要な見出しを含む形式的な報告書を提供すべきである。発表は、到達した結論の透明性 を確保するために、文脈と思考プロセスの詳細を含めるべきである。一致表を含めることが強く 推奨される。これにより、透明性を高め専門家同士の協力が容易になる。

### フレームワークとその成果の利用

IPCS の非発がん HRF は、主に推定される MOA の key events の堅牢な一致解析に基づいている。以下のようないくつかの分野で調和に貢献するための基礎として、リスク評価と研究コミュニティの双方に価値があると考えられている。

- 動物における推定 MOA の妥当性とエビデンスの重み付けの性質及びヒトへの関連性
- エンドポイント間の MOA の統合
- 専門家の参加とレビューを十分に確保するための透明性のある基準

非発がん HRF の強みは、その柔軟性、透明性及びエンドポイント間で適用できることである。 これには、用量反応曲線の性質と形状の決定、個々の key events に対する生物学的閾値と位置の 特定及びそれらの影響が含まれる。さらに、各 key events に関与する動態学的及び薬力学的要因 を考慮することは、特定の集団、例えば、若年期、特定の疾患を有する人々、または特定の多型 を有する人々への関連性の有無に関して有益である。あるいは、フレームワークを適用すること で、そのようなグループ間の差異に関する定量的な情報を提供することもできる。また、ヒトへ の関連性の有無についての解析において、ある種の生物が、用量制限のために潜在的に関連する エンドポイントの評価には不適切である場合もある。

### 次のステップ

非発がん HRF を効果的に適用するためには、その適用及びその利用の解釈において個人を訓練 する必要がある。発がん性 HRF の利用においては経験が得られており、その得られた専門知識は、 非発がん HRF 使用における訓練にも適用できる。訓練は、適切な事例研究がより多く利用可能と なることによって促進されるであろう。これまでに発表された事例は、この目的のための適切な 基盤となる (Seed ら、2005 年)。さらに、より広範囲のエンドポイントに関する事例を調査する必 要がある。非発がん HRF 解析の経験を持つ組織が指針を作り、訓練を支援するために適切な専門 家がその教材を利用できるようにすることは有用であろう。

一般的に受け入れられている MOA のデータベースを、有益な事例研究とともに編集し、持続 させるべきである。このようなデータベースは、MOA の開発において事例は更新され続けられ、 ある化合物の MOA が新規か、あるいは他の化合物について以前に記載されていたものかを判断 する上でも重要なものとなるだろう。

IPCS の発がん性 HRF から生まれた現在の非発がん HRF は、がん以外のエンドポイントに焦点を 当てている。しかし、発がんと非発がんの効果を評価するための哲学や戦略には顕著な類似点が ある。化学物質のリスク評価の調和における次のステップの一つとして、がんを含むすべての毒 性学的エンドポイントに適用可能な統一的な HRF を作成することが強く推奨される。リスク評価 過程へのフレームワーク適用は、さらに精緻化されるべきであり、その際には、例示が価値ある ものとなるであろう。このようなフレームワーク文書には、リスク評価における HRF 解析の使用 に関するガイダンスと同様に、HRF 解析に着手する前の問題の定式化に関するガイダンスが含ま れるべきである。また、フレームワークを適用することで、用量反応関係の理解が深まることが 多いが、これはハザード評価に反映されるべきである。上述のように、用量の変化に関する知識 は、ばく露データを解釈する上で非常に重要である。MOA における key events を特定することで、 種差及び個体差の原因や程度に関する知見を得ることができる。

### 参考文献

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Critical Reviews in Toxicology*, **36**:781–792.

Corley RA, Meek ME, Carney EW (2005) Mode of action: Oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—Renal and developmental effects of ethylene glycol. *Critical Reviews in Toxicology*, **35**:691–702.

Crofton KM, Zoeller RT (2005) Mode of action: Neurotoxicity induced by thyroid hormone disruption during development—Hearing loss resulting from exposure to PHAHs. *Critical Reviews in Toxicology*, **35**:757–769.

Foster PM (2005) Mode of action: Impaired fetal Leydig cell function—Effects on male reproductive development produced by certain phthalate esters. *Critical Reviews in Toxicology*, **35**:713–719.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Holson JF, Stump DG, Pearce LB, Watson RE, DeSesso JM (2005) Mode of action: Yolk sac poisoning and impeded histiotrophic nutrition—HBOC-related congenital malformations. *Critical Reviews in Toxicology*, **35**:739–745.

Intergovernmental Forum on Chemical Safety (1994) *The International Conference on Chemical Safety—Final report.* Geneva, World Health Organization (http://www.who.int/ ifcs/documents/forums/forum1/en/FI-report en.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability:

Guidance document for use of data in dose/concentration-response assessment.

Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document No. 2; http://whqlibdoc.who.int/publications/2005/9241546786\_eng.pdf).

Kavlock R, Cummings A (2005a) Mode of action: Reduction of testosterone availability— Molinateinduced inhibition of spermatogenesis. *Critical Reviews in Toxicology*, **35**:685–690.

Kavlock R, Cummings A (2005b) Mode of action: Inhibition of androgen receptor function— Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, **35**:721–726.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

Seed J, Carney E, Corley R, Crofton K, DeSesso J, Foster P, Kavlock R, Kimmel G, Klaunig J, Meek E, Preston J, Slikker W, Tabacova S, Williams G (2005) Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Critical Reviews in Toxicology*, **35**:663–672.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity. *Toxicology and Applied Pharmacology*, **201**:203–225.

Slikker W Jr, Xu ZA, Levin ED, Slotkin TA (2005) Mode of action: Disruption of brain cell replication, second messenger, and neurotransmitter systems during development leading to cognitive dysfunction—Developmental neurotoxicity of nicotine. *Critical Reviews in Toxicology*, **35**:703–711.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Tabacova S (2005) Mode of action: Angiotensin-converting enzyme inhibition— Developmental effects associated with exposure to ACE inhibitors. *Critical Reviews in Toxicology*, **35**:747–755.

UNEP (2002) *Plan of implementation of the World Summit on Sustainable Development*. New York, NY, United Nations Environment Programme (http://www.un.org/esa/sustdev/documents/WSSD POI PD/English/WSSD PlanImpl.pdf).

United Nations (1992) Agenda 21: United Nations Conference on Environment and Development. New York, NY, United Nations Division for Sustainable Development (http://www.un.org/esa/sustdev/documents/agenda21/english/Agenda21.pdf).

Weed DL (2005) Weight of evidence: A review of concept and methods. Risk Analysis, 25:1545–1557.

WHO (2006) *Strategic Approach to International Chemicals Management (SAICM)*. Geneva, World Health Organization (http://www.who.int/ipcs/features/iccm\_crp.pdf).

Wiltse J (2005) Mode of action: Inhibition of histone deacetylase, altering WNT-dependent gene expression, and regulation of beta-catenin—Developmental effects of valproic acid. *Critical Reviews in Toxicology*, **35**:727–738.

Zoeller RT, Crofton KM (2005) Mode of action: Developmental thyroid hormone insufficiency— Neurological abnormalities resulting from exposure to propylthiouracil. *Critical Reviews in Toxicology*, **35**:771–781.

Zoetis T, Walls I, eds (2003) *Principles and practices for direct dosing of preweaning mammals in toxicity testing and research. A report of the ILSI Risk Science Institute Expert Working Group on Direct Dosing of Preweaning Mammals in Toxicity Testing.* Washington, DC, ILSI Press.

ハーモナイゼーションプロジェクト文書シリーズ

IPCS risk assessment terminology (No. 1, 2004)

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Principles of characterizing and applying human exposure models (No. 3, 2005)

Part 1. IPCS framework for analysing the relevance of a cancer mode of action for humans and case-studies; Part 2. IPCS framework for analysing the relevance of a non-cancer mode of action for humans (No. 4, 2007)

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Harmonization Project Document No. 4

### PART 1: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS AND CASE-STUDIES

### PART 2: IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS

This project was conducted within the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.

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### ハーモナイゼーションプロジェクト文書第4号

第1部:

発がん MOA のヒトへの関連性を解析するための IPCS フレ ームワーク及び事例研究

### 第2部:

非発がん MOA のヒトへの関連性を解析するための IPCS フ

レームワーク

本プロジェクトは、化学物質へのばく露によるリスク評価のアプローチの調和に関する IPCS プロジェクトの一環として実施された。

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The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)は、1980年に設立され た国連環境計画(UNEP)、国際労働機関(ILO)、世界保健機関(WHO)の共同事業である。IPCSの全 体的な目的は、化学物質の安全性の向上のための必要条件として、国際的なピアレビューを通じて、化 学物質へのばく露によるヒトの健康と環境へのリスクを評価するための科学的基盤を確立し、化学物 質の健全な管理のために各国の管理能力を強化する技術支援を提供することである。

化学物質の適正な管理に関する国際機関間プログラム (IOMC) は、1992 年の国連環境開発会議 (UNCED) において表明された化学物質の安全性の分野での協力及び連携の強化を目的とした勧告に 従い、UNEP、ILO、国連食糧農業機関 (FAO)、WHO、国際連合工業開発機関 (UNIDO)、国連訓練調 査研究所 (UNITAR)、経済協力開発機構 (OECD) (以上参加機関) によって 1995 年に設立された。 IOMC の目的は、ヒトの健康と環境に関連した化学物質の健全な管理を達成するために、参加機関が共 同で、あるいは個別に遂行する政策と活動の連携を促進することである。

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IPCS MOA フレームワーク (IPCS ハーモナイゼーションプロジェクト文書 ; no.4)

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 発がん性試験 5. 発がん物質 6. 腫瘍-化学的に誘発される腫瘍
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#### FOREWORD

Harmonization Project Documents are a family of publications by the World Health Organization (WHO) under the umbrella of the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP). Harmonization Project Documents complement the Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as authoritative documents on methods for the risk assessment of chemicals.

The main impetus for the current coordinated international, regional, and national efforts on the assessment and management of hazardous chemicals arose from the 1992 United Nations Conference on Environment and Development (UNCED). UNCED Agenda 21, Chapter 19, provides the "blueprint" for the environmentally sound management of toxic chemicals. This commitment by governments was reconfirmed at the 2002 World Summit on Sustainable Development and in 2006 in the Strategic Approach to International Chemicals Management (SAICM). The IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter 19, and contributes to the implementation of SAICM. In particular, the project addresses the SAICM objective on Risk Reduction and the SAICM Global Plan of Action activity to "Develop and use new and harmonized methods for risk assessment".

The IPCS Harmonization Project goal is to improve chemical risk assessment globally, through the pursuit of common principles and approaches, and, hence, strengthen national and international management practices that deliver better protection of human health and the environment within the framework of sustainability. The Harmonization Project aims to harmonize global approaches to chemical risk assessment, including by developing international guidance documents on specific issues. The guidance is intended for adoption and use in countries and by international bodies in the performance of chemical risk assessments. The guidance is developed by engaging experts worldwide. The project has been implemented using a stepwise approach, first sharing information and increasing understanding of methods and practices used by various countries, identifying areas where convergence of different approaches would be beneficial, and then developing guidance that enables implementation of harmonized approaches. The project uses a building block approach, focusing at any one time on the aspects of risk assessment that are particularly important for harmonization.

The project enables risk assessments (or components thereof) to be performed using internationally accepted methods, and these assessments can then be shared to avoid duplication and optimize use of valuable resources for risk management. It also promotes sound science as a basis for risk management decisions, promotes transparency in risk assessment, and reduces unnecessary testing of chemicals. Advances in scientific knowledge can be translated into new harmonized methods.

This ongoing project is overseen by a geographically representative Harmonization Project Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment. ハーモナイゼーションプロジェクト文書とは、国際化学物質安全性計画(IPCS)(WHO/ILO/UNEP) のもと世界保健機関(WHO)によって出版された出版物群である。ハーモナイゼーションプロジ ェクト文書は、化学物質のリスク評価方法に関する正式な文書として、環境保健クライテリア (EHC)の方法論シリーズ(黄表紙)を補完するものである。

有害化学物質の評価と管理に関する現在の国際的、地域的及び各国内の協調的な取り組みの主 なきっかけは、1992年の国連環境開発会議(UNCED)にあった。UNCEDアジェンダ21の第19 章において、有害化学物質の環境的に健全な管理のための「青写真」が示されている。各国政府 によるこのコミットメントは、2002年の持続可能な開発に関する世界首脳会議で、さらに2006年 の国際的な化学物質管理のための戦略的アプローチ(SAICM)でも再確認された。化学物質への ばく露によるリスク評価のアプローチの調和に関する IPCS プロジェクト(ハーモナイゼーショ ンプロジェクト)は、このアジェンダ21第19章の下で実施されており、SAICMの実施に貢献し ている。特に、本プロジェクトは、SAICMの目標である「リスク低減」と SAICM 世界行動計画 の活動である「リスク評価のための新たな調和された手法の開発と利用」に対応している。

IPCS ハーモナイゼーションプロジェクトの目的は、共通の原則とアプローチを追求することに よって化学物質のリスク評価を世界的に改善し、持続可能性というフレームワークの中でヒトの 健康と環境をより良く保護するための国内及び国際的な管理手法を強化することである。ハーモ ナイゼーションプロジェクトは、特定の問題に関する国際的なガイダンス文書を作成することを 含め、化学物質リスク評価に対する世界各国のアプローチを調和させることを目的としている。 このガイダンスは、化学物質のリスク評価を実施する際に各国や国際機関で採用され、使用する ことを目的としている。このガイダンスは、世界中の専門家の協力を得て作成されている。本プ ロジェクトは、まず各国の手法や慣行について情報を共有し、理解を深め、異なるアプローチの 統一化が有益となる分野を特定した上で、調和のとれたアプローチの実施を可能にするガイダン スを作成するという段階的アプローチを用いて採用している。本プロジェクトでは、アプローチ の調和を図る上で、特に重要なリスク評価の側面に焦点を当てたビルディングブロック法を採用 している。

このプロジェクトにより、国際的に認められた手法を用いてリスク評価(またはその構成要素) を実施することを可能にし、これらの評価を共有することで重複を避け、リスク管理のための貴 重な資源を最適に利用することが可能となる。また、リスク管理の意思決定の基礎となる健全な 科学を促進し、リスク評価の透明性を高め、不必要な化学物質の試験を減らすことができる。ま た、科学的知識の発展が新たな調和のとれた手法の開発へとつながりうる。

この進行中のプロジェクトは、各地域の代表的なハーモナイゼーションプロジェクト運営委員 会と、詳細な作業を管理するいくつかの特別作業部会によって監督されている。最終的には、国 際的なピアレビュー及びパブリックコメントといった厳格なプロセスが含まれている。

### PART 1

第1部

発がん MOA のヒトへの関連性を解析するための IPCS フレームワーク及び事例研究

### IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS AND CASE-STUDIES

#### PREFACE

Following publication of the International Programme on Chemical Safety (IPCS) Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (in animals),<sup>1</sup> an IPCS Cancer Working Group convened on 3–5 March 2004 in Arlington, Virginia, USA. The working group agreed that the issue of human relevance of animal tumours should be further explored with the goal of developing a unified IPCS Human Relevance Framework for use of mode of action information in risk assessment for regulatory and other purposes, and it provided initial guidance for this task. The members of this working group, including secretariat support and a representative of the Organisation for Economic Co-operation and Development, were as follows:

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Extending the Mode of Action Framework to include consideration of human relevance, taking into account guidance from the Arlington meeting, was the subject of an IPCS international workshop convened in Bradford, United Kingdom, from 21 to 23 April 2005. This workshop prepared draft text for an IPCS Human Relevance Framework, including updating the 2001 Mode of Action Framework. The workshop participants, including

#### 序文

国際化学物質安全性計画(IPCS)の(動物における)<sup>1</sup>「化学物質の発がん作用機序の評価に関 する概念的フレームワーク」の公表を受けて、2004年3月3日から5日に米国バージニア州アー リントンで IPCS がんワーキンググループが開催された。このワーキンググループは、規制やその 他の目的でリスク評価において作用機序に関する情報を利用するための統一された IPCS ヒト関 連性フレームワークを開発することを目標に、動物における腫瘍のヒトへの関連性の問題をさら に検討する必要性があることに合意し、この作業のための初期のガイダンスを提供した。このワ ーキンググループのメンバーは、事務局サポートと経済協力開発機構(OECD)の代表者を含めて 以下の通りである。

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2005 年 4 月 21 日から 23 日に英国のブラッドフォードで開催された IPCS 国際ワークショップ においてアーリントン会議のガイダンスを考慮し、MOA フレームワークに人間への関連性を考慮 した内容に拡張することが検討された。このワークショップでは、2001 年の MOA フレームワー クの更新を含む IPCS ヒト関連性フレームワークの草案が作成された。

<sup>&</sup>lt;sup>1</sup> Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

<sup>&</sup>lt;sup>1</sup> Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, 34:146–152.

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#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

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#### Harmonization Project Document No. 4

The draft was published on the Internet for public comment and sent to a number of WHO Collaborating Centres and IPCS Participating Institutions for peer review. An expert meeting that convened in London in December 2005 considered the comments received and finalized the framework. The expert meeting participants were as follows:

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#### Harmonization Project Document No. 4

草案はパブリックコメントのためにインターネット上で公開され、ピアレビューのために多く の WHO 協力機関と IPCS 参加機関に送付された。2005 年 12 月にロンドンで開催された専門家会 議では、寄せられたコメントを検討し、最終的にフレームワークが決定された。専門家会議の参 加者は以下の通りである。

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### LIST OF ACRONYMS AND ABBREVIATIONS

ADH	alcohol dehydrogenase
ANOVA	analysis of variance
bw	body weight
CAR	constitutively active receptor
cDNA	complementary deoxyribonucleic acid
CoA	coenzyme A
CpG	cytosine and guanine separated by a phosphate
CYP	cytochrome P-450
dA	deoxyadenosine
dG	deoxyguanosine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPX	DNA-protein cross-links
FAO	Food and Agriculture Organization of the United Nations
HRF	Human Relevance Framework
IARC	International Agency for Research on Cancer
ILO	International Labour Organization
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
IU	International Units
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K <sub>M</sub>	Michaelis-Menten constant
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NAT	<i>N</i> -acetyltransferase
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program (USA)
OAT	<i>O</i> -acetyltransferase
PCNA	proliferating cell nuclear antigen
PPX	protein–protein cross-linkage
RNA	ribonucleic acid
RSI	
rT3	Risk Science Institute (ILSI)
	reverse triiodothyronine
S9	$9000 \times g$ supernatant from rat liver
SCE	sister chromatid exchange
SHE T2	Syrian hamster embryo
T3	triiodothyronine
T4 TCDD	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TGF	tumour growth factor
TSH	thyroid stimulating hormone
UDP	uridine diphosphate
UDS	unscheduled DNA synthesis
UGT	uridine diphosphate glucuronosyltransferase

#### 頭字語と略語のリスト

ADH	アルコール脱水素酵素
ANOVA	分散分析
bw	体重
CAR	恒常的活性化受容体
cDNA	相補的デオキシリボ核酸
CoA	補酵素 A
CpG	シトシンとグアニンの2塩基配列
CYP	シトクロム P-450
dA	デオキシアデノシン
dG	デオキシグアノシン
DMSO	ジメチルスルホキシド
DNA	デオキシリボ核酸
DPX	DNA-タンパク質架橋
FAO	国連食糧農業機関
HRF	ヒト関連性フレームワーク
IARC	国際がん研究機関
ILO	国際労働機関
ILSI	国際生命科学研究機構
IPCS	国際化学物質安全性計画
IU	国際単位
JMPR	残留農薬に関する FAO/WHO 合同会議
KM	ミカエリス・メンテン定数
LOAEL	最小毒性量
MOA	Mode of Action(作用モード)
NAT	N-アセチルトランスフェラーゼ
NOAEL	無毒性量
NTP	米国国家毒性プログラム
OAT	0-アセチルトランスフェラーゼ
PCNA	増殖性細胞核抗原
PPX	蛋白質間架橋
RNA	リボ核酸
RSI	リスク科学研究所(ILSI)
rT3	リバーストリヨードサイロニン
S9	9000g で遠心分離したラット肝臓上清(肝ミクロソーム)
SCE	姉妹染色分体交換
SHE	シリアンハムスター胚
Т3	トリヨードサイロニン
T4	チロキシン
TCDD	2.3.7.8-ジベンゾ-p-ダイオキシン
TGF	<u>「</u> 」 腫瘍成長因子
TSH	甲状腺刺激ホルモン
UDP	ウリジンニリン酸
UDS	不定期 DNA 合成
000	

UGT ウリジン二リン酸グルクロン酸転移酵素

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IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

UNEPUnited Nations Environment ProgrammeUSAUnited States of AmericaUSEPAUnited States Environmental Protection AgencyWHOWorld Health Organization	UNEP USA USEPA WHO	国際連合環境計画 アメリカ合衆国 米国環境保護庁 世界保健機関
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### IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Alan R. Boobis, Samuel M. Cohen, Vicki Dellarco, Douglas McGregor, M.E. (Bette) Meek, Carolyn Vickers, Deborah Willcocks, & William Farland

The use of structured frameworks can be invaluable in promoting harmonization in the assessment of chemical risk. The International Programme on Chemical Safety (IPCS) has therefore updated and extended its Mode of Action (MOA) Framework for cancer to address the issue of human relevance of a carcinogenic response observed in an experimental study. The first stage is to determine whether it is possible to establish an MOA. This comprises a series of key events along the causal pathway to cancer, identified using a weight-of-evidence approach based on the Bradford Hill criteria. The key events are then compared first qualitatively and then quantitatively between the experimental animals and humans. Finally, a clear statement of confidence, analysis, and implications is produced. The IPCS Human Relevance Framework for cancer provides an analytical tool to enable the transparent evaluation of the data, identification of key data gaps, and structured presentation of information that would be of value in the further risk assessment of the compound, even if relevancy cannot be excluded. This might include data on the shape of the dose-response curve, identification of any thresholds, and recognition of potentially susceptible subgroups, for example, the basis of genetic or life stage differences.

Fundamental to the evolution of cancer risk assessment over the last three decades has been our increasing understanding of the biology of cancer and the identification of key events in carcinogenesis. Through the mid-1980s, national and international assessments of human cancer hazard and risk depended primarily on lifetime assays in rodents of potentially carcinogenic agents. For few agents was there sufficient human evidence on which to base retrospective cancer assessments, and fewer still would be expected to be detected prospectively, given modern controls on general exposures in the workplace and in the environment generally. Inherent in rodent-based assessments was the assumption that the observation of tumours in laboratory animals could be meaningfully extrapolated to identify potential human carcinogens and, by the use of mathematical models, to provide upper-bound estimates of risk at human doses of regulatory significance. During the same period, the potential significance of mutagenesis in carcinogenesis was becoming accepted by the scientific community. Subsequently, it has become increasingly apparent that an appreciable number of chemicals cause cancer in laboratory animals by processes that do not involve direct interaction with DNA. These developments in our understanding of the biological basis of carcinogenesis in both laboratory animals and humans have benefited risk assessment processes by providing more data on the pharmacokinetics and pharmacodynamics of suspect carcinogenic agents. Consideration of the biological processes involved in the carcinogenesis of specific compounds has led to the concept of mode of action (MOA).

#### 発がん MOA のヒトへの関連性を解析するための

IPCS フレームワーク

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体系化されたフレームワークの利用は、化学物質のリスク評価における調和を促進する上で非常 に重要である。したがって、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)は、実験的研究で観察された発がん性反応のヒトへの関連性の問題に対処するために、発が ん Mode of Actioin (MOA)フレームワークを更新し、拡張した。第一段階は、MOA を確立する ことが可能かどうかを判断することである。MOA は、Bradford Hill 基準に基づいたエビデンスの 重み付けアプローチを用いて特定された発がんの原因経路に沿った一連の key events で構成され ている。次に、実験動物とヒトの間で key events を定性的に、次に定量的に比較する。最後に、信 頼性、解析的検討及び意義についての明確な報告書が作成される。IPCS 発がん性ヒト関連性フレ ームワークは、データの透明性のある評価、重要なデータギャップの特定及び関連性が排除できな い場合であっても、化合物のさらなるリスク評価において価値のある情報の体系的な提示を可能 にする解析ツールを提供するものである。この価値ある情報には、用量反応曲線の形状に関するデ ータ、閾値の特定及び遺伝的またはライフステージの違いなどに基づいた潜在的に感受性の高い 集団を認知することが含まれる。

過去30年間のがんリスク評価の発展の基礎となったのは、がんの生物学的理解の深まりと発が んにおける key events の特定である。1980 年代半ばまでは、ヒトにおけるがんのハザードとリス クに関する国内及び国際的な評価は、主に発がん性のある物質のげっ歯類を用いた生涯試験に依 存していた。後ろ向きがん研究の基礎となるに十分なヒトにおけるエビデンスが存在する薬剤は ほとんどなく、職場や環境における一般的なばく露に対する現代の規制を考えれば、将来的に発 がん性が検出される薬剤はさらに少ないと予想される。げっ歯類を用いた評価において、実験動 物で観察された腫瘍は、潜在的にヒトに対して発がん性を有する物質を特定するために意味のあ る外挿が可能であり、数学的モデルを用いて規制上重要となるヒトにおけるリスクの上限推定値 を提供することができるという前提があった。同時期に、発がんにおいて変異原性が重要な意義 を有する可能性があるということが科学界に受け入れられつつあった。その後、かなりの数の化 学物質が、DNA との直接的な相互作用を伴わないプロセスによって実験動物にがんを引き起こす ことが次第に明らかになってきた。実験動物とヒトの両方における発がんの生物学的基盤に関す る我々の理解を深めることは、発がん性が疑われる薬剤の薬物動態及び薬理作用に関するより多 くのデータを提供することにより、リスク評価の過程を有益なものとした。特定の化合物の発が んに関与する生物学的プロセスを考察することは、Mode of action(以下、MOA)の概念につなが っている。

<sup>&</sup>lt;sup>1</sup> This article, to which WHO owns copyright, was originally published in 2006 in *Critical Reviews in Toxicology*, Volume 36, pages 781–792. It has been edited for this WHO publication and includes corrigenda.

<sup>&</sup>lt;sup>1</sup> この論文は、WHO が著作権を有するものであり、元々は 2006 年に Critical Reviews in Toxicology, Volume 36, pages 781-792 に掲載されたものです。この論文は WHO の出版物のために編集されたもので、正誤表が含まれています。

A postulated MOA for carcinogenesis is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed carcinogenicity—in a logical framework. MOA contrasts with mechanism of action, which generally involves a sufficient understanding of the molecular basis for an effect and its detailed description so that causation can be established in molecular terms.

In 2001, as part of its efforts to harmonize risk assessment practices, the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP) published a framework for assessment of MOA for carcinogenesis in laboratory animals (animal MOA), based on Bradford Hill criteria for causality. The IPCS Human Relevance Framework (HRF) presented in this document updates this MOA Framework and extends it to consider human relevance. It is an analytical tool to provide a means of evaluating systematically the data available on a specific carcinogenic response to a chemical in a transparent manner. While it is envisaged that the framework will be of value to risk assessors both within and outside of regulatory agencies, it will also be a valuable tool to the research community. Among reasons for using the framework are:

- to provide a generic approach to the analysis of data to contribute to harmonization;
- to encourage transparency of the consideration and use of available data and reasons for the conclusions drawn;
- to provide guidance in the presentation of data;
- to identify critical data deficiencies and needs;
- to inform the quantitative assessment of carcinogenic risk to humans.

These and other topics will be discussed in more detail below.

#### THE ROLE OF IPCS IN DEVELOPING THE FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MOA FOR HUMANS

IPCS has been leading an effort to harmonize approaches to cancer risk assessment as part of its larger project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. The first phase of this work resulted in the publication of the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis in experimental animals (Sonich-Mullin et al., 2001). As described in that publication, a major impediment to harmonization identified in the consideration of weight of evidence was the evaluation of MOA in animals. Sonich-Mullin et al. (2001) provided a framework for evaluating MOA of chemical carcinogenesis in animals and recognized the importance of moving on to the next step in the overall characterization of cancer hazard and risk in humans: the assessment of relevance of the MOA of animal carcinogenesis to humans. Adoption of the MOA Framework concept is proceeding through its incorporation in the revised United States Environmental Protection Agency (USEPA) Guidelines for Carcinogen Risk Assessment (USEPA, 1999, 2005), and the framework is now commonly used by other regulatory agencies and international organizations. In the United Kingdom, the framework is being used for the assessment of pesticides and industrial chemicals. The United Kingdom

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

推定される発がん MOA とは、試験で観察された影響につながる生物学的に妥当な key events の 一連の流れであり、堅牢な実験観察とメカニズムデータに裏付けられ、重要な細胞学的及び生化 学的事象、すなわち、観察された発がん性に必要かつ測定可能な事象を論理的なフレームワーク で記述している。MOA は作用機序 (mechanism of action)とは対照的であり、作用騎乗では一般的 に、効果の分子的基盤を十分に理解し、その詳細を記述することで、分子的観点から因果関係を 確立することができる。

2001 年に、リスク評価手法の調和の一環として、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)(WHO/ILO/UNEP)は、因果関係に関する Bradford Hill 基準に基づいた実験動物の発がん MOA(動物 MOA)評価のためのフレームワークを発表した。本文書書で紹介している IPCS ヒト関連性フレームワーク(HRF)は、この MOA フレームワークを更新し、ヒトとの関連性を考慮するために拡張したものである。これは、化学物質の特定の発がん性について利用可能なデータを透明性のある方法で体系的に評価する手段を提供する解析ツールである。フレームワークは、規制当局の内外のリスク評価者にとって価値のあるものであると同時に、研究者にとっても貴重なツールとなることを想定している。フレームワークを利用する理由には、以下のようなものがある。

- データ解析の一般的なアプローチを提供し、調和の推進に貢献する。
- 利用可能なデータの検討と使用及び導き出された結論の論拠の透明性を促進する。
- データの提示におけるガイダンスを提供する。
- 重要なデータの不足と必要な追加データを特定する。
- ヒトに対する発がん性リスクの定量的評価において情報を提供する。

これら及びその他のトピックについて、以下でより詳細に議論する。

# 発がん MOA のとトへの関連性を解析するためのフレームワークを開発する上での IPCS の役割

IPCS は、化学物質へのばく露によるリスク評価のアプローチの調和に関するより大きなプロジ ェクトの一環として、発がんリスク評価への手法を調和させる取り組みを主導してきた。この作 業の第一段階では、実験動物における化学物質の発がん MOA を評価するための「IPCS の概念的 なフレームワーク」の公表につながった(Sonich-Mullin ら, 2001 年)。この出版物に記載されて いるように、エビデンスの重み付けを検討する際にみられる調和への障害は、主に動物 MOA の 評価であった。Sonich-Mullin ら(2001 年)は、動物における化学物質の発がん MOA を評価する ためのフレームワークを提供し、ヒトにおけるがんのハザードとリスクの総合的な判定の次のス テップ、すなわち、動物における発がん MOA のヒトへの関連性の評価に進むことの重要性を認 識した。MOA フレームワークの概念は、米国環境保護庁(USEPA)発がん物質リスク評価ガイド ライン改訂版(USEPA、1999 年、2005 年)に盛り込まれており、MOA フレームワークは現在、 他の規制当局や国際機関でも一般的に用いられている。英国では、このフレームワークが農薬や 工業用化学物質の評価に利用されている。

Committee on Carcinogenicity (2004) has noted the framework's value with regard to both harmonization between agencies and internal consistency in its latest guidelines. The framework has also been adopted and is being used by agencies in Australia and in Canada, in the evaluation of Existing Chemicals under the Canadian Environmental Protection Act. The European Union has incorporated the framework into its technical guidance documents on evaluating new and existing industrial chemicals and biocides, including carcinogenicity. With regard to international organizations, of particular note is the use of the framework by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), for example, in its evaluation of pyrethrin extract and its incorporation into the resulting monograph.

The step to extend the MOA Framework to include consideration of human relevance has been undertaken by IPCS in cooperation with international partners. It was the subject of an IPCS international workshop convened in Bradford, United Kingdom, from 21 to 23 April 2005. This workshop prepared draft text for an IPCS HRF, including updating the 2001 MOA Framework. The draft was published on the Internet for public comment and sent to a number of WHO Collaborating Centres and IPCS Participating Institutions for peer review. An expert meeting convened in London in December 2005 considered the comments received and finalized the framework. The framework text and the steps leading to its development are discussed in detail in the following sections.

# THE 2001 IPCS CONCEPTUAL MOA FRAMEWORK FOR EVALUATING ANIMAL CARCINOGENESIS

#### **Purpose of the framework**

The IPCS MOA Framework for evaluating carcinogenesis in animals (Sonich-Mullin et al., 2001) remains a fundamental basis for the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans. The animal MOA Framework provides a generic approach to the principles commonly used when evaluating a postulated MOA for tumour induction in animals by a chemical carcinogen. Thus, the framework is a tool that provides a structured approach to the assessment of the overall weight of the evidence for the postulated MOA. In this context, a supported MOA would have evidence provided by robust experimental observations and mechanistic data to establish a biologically plausible explanation.

The framework is designed to bring transparency to the analysis of a postulated MOA and thereby promote confidence in the conclusions reached through the use of a defined procedure that encourages clear and consistent documentation supporting the analysis and reasoning and that highlights inconsistencies and uncertainties in the available data. The purpose of the framework is to provide a systematic means of considering the weight of the evidence for an MOA in a given situation; it is not designed to give an absolute answer on sufficiency of the information, as this will vary depending on the circumstance. It is not a checklist of criteria, but rather an analytical approach. However, the process can be greatly aided by the presentation of tabular summaries of comparative data on incidence of key events and tumours.

The animal MOA Framework analysis is an important step in the hazard characterization. It is envisaged that the animal MOA Framework will contribute to risk assessments of chemical

#### Harmonization Project Document No. 4

英国発がん性委員会(2004年)は、機関間の調和と最新のガイドラインにおける内部の整合性の 両方の点でフレームワークの価値を指摘している。このフレームワークは、オーストラリアの機 関でも採用され、カナダではカナダ環境保護法の下での既存化学物質の評価に用いられている。 欧州連合(EU)は、新規及び既存の工業用化学物質及び殺生物剤のの評価について、発がん性を 含む技術ガイダンス文書に本フレームワークを組み込んでいる。国際機関では、FAO/WHO合同 残留農薬専門家会議(JMPR)が、例えばピレスリン抽出物の評価やその結果として得られた研究 論文に、このフレームワークを組み入んでいることが。特筆される。

MOA フレームワークを拡張し、ヒトへの関連性の考慮を含めるためのステップは、IPCS が国際的なパートナーと協力して実施されてきた。これは 2005 年 4 月 21 日から 23 日まで英国ブラッドフォードで開催された IPCS の国際ワークショップの議題であった。このワークショップでは、2001 年の MOA フレームワークの更新を含む IPCS HRF の草案が作成された。この草案はパブリックコメントのためにインターネット上で公開され、また、ピアレビューのために多くの WHO 協力機関と PCS 参加機関に送付された。2005 年 12 月にロンドンで開催された専門家会議では、受け取ったコメントが検討され、フレームワークが最終決定された。フレームワークの内容とその発展へとつながるステップについては、以下のセクションで詳述する。

#### 2001年の動物における発がん性評価のための IPCS 概念的 MOA フレームワーク

#### フレームワークの目的

動物における発がん性評価のための IPCS MOA フレームワーク (Sonich-Mullin ら, 2001 年) は 今もなお発がん MOA のヒトとの関連性を解析するための IPCS フレームワークの基本原理とな っている。動物 MOA フレームワークは、発がん性化学物質による動物の腫瘍誘発に対して、想 定される MOA を評価する際に一般的に適用される原則への汎用的アプローチを提供するもので ある。したがって、このフレームワークは想定される MOA に対する総合的なエビデンスの重み 付けの評価に際して体系的なアプローチを提供するツールである。すなわち、この文脈では支持 された MOA は、生物学的に妥当な説明を確立するためにしっかりとした実験的観察とメカニズ ムデータのエビデンスを持つことになる。

このフレームワークは、推定される MOA の解析に透明性をもたらし、その結果、明確にされ た手法(解析と推論を裏付ける明確で一貫性のある文書化を促し、利用可能なデータの矛盾と不 確実性を明らかにする手法)を用いることにより到達した結論の信頼性が増すように設計されて いる。フレームワークの目的は、特定の状況における MOA のエビデンスの重み付けを検討する 体系的な手段を提供することであり、情報が十分か否かに関して絶対的な回答を与えるようには 設計されていない。なぜなら情報は状況によって異なるためである。このフレームワークは基準 のチェックリストではなく、むしろ解析的なアプローチである。しかし、key events 及び腫瘍の発 生に関する比較データの表形式の要約はこのプロセスの大きな助けとなる。

動物 MOA フレームワーク解析は、ハザードの特性評価の重要なステップである。動物 MOA フレームワークは、すべての分野(医薬品、工業用化学物質、農薬、食品添加物など)にわたる発がん化学物質のリスク評価に貢献することが想定されている。

carcinogens across all sectors (drugs, industrial chemicals, pesticides, food additives, etc.). In the resulting risk assessment documentation, the framework analysis would be appropriately positioned within the hazard characterization section. In the absence of adequate epidemiological data, it may be regarded as an essential component in any discussion of human relevance, dose–response relationships, and risk characterization. It is also envisaged that the framework will be useful to both regulators and researchers in identifying research needs based on clear delineation of data gaps and inconsistencies.

MOA analysis can be used to establish either that a compound has an MOA that has been described previously or that it has a novel MOA. Thus, the output of an MOA analysis may serve to support the evaluation of a specific compound or contribute to the generation of a novel MOA. In the former, chemical-specific data play a vital role in the concordance analysis for human relevance. In the latter, it will be important to identify which events are key to the biological processes that represent the MOA.

Thus, an MOA comprising the same set of key events may apply to many different compounds. The evidence necessary to establish that a specific MOA is responsible for a given carcinogenic response will be substantial the first time such an MOA is proposed. As subsequent compounds are found to share this MOA, the "barrier" to acceptance will be lower, although it will always be necessary to establish rigorously that the key events comprising the MOA occur and that they fulfil the criteria described below. It will also be important to exclude other possible MOAs.

Scientific peer participation is a prerequisite for the development and acceptance of a novel postulated MOA. Peer participation includes both peer involvement in the development of an MOA and peer review by scientists who are independent of the process of development of the MOA. Publication in the scientific literature and presentation and discussion at scientific meetings and workshops constitute peer involvement that contributes to acceptance of an MOA by the scientific community.

While acceptance does not necessarily mean unanimity, most of the scientists reviewing an MOA analysis should agree that the relevant scientific information has been identified and appropriately analysed, that "key events" have been identified and are supported by the information presented, that their relationship to carcinogenesis has been clearly established in the hypothesized MOA, and that alternative MOAs have been considered and rejected.

As knowledge advances, the characterization of an MOA will change. Additional key events may be identified, and others may be refined or even dropped. Nevertheless, significant changes to the key events also need some general acceptance, through peer review, such as described above.

#### **Update of framework guidelines**

In development of the IPCS HRF, the 2001 animal MOA Framework text has been updated, and this revised version is presented here.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

結果として得られるリスク評価文書では、フレームワーク解析はハザードの特性評価のセクショ ン内に適切に配置されるであろう。適切な疫学データがない場合には、ヒトとの関連性、用量反 応関係、リスクの特性を議論する上で、フレームワーク解析は不可欠な要素とみなされるかもし れない。また、このフレームワークは、データギャップや矛盾の明確な定義に基づいて研究の必 要性を特定する上で規制当局と研究者の双方にとって有用であると考えられる。

MOA 解析は、化合物が以前に記述されている MOA を有すること、または新規の MOA を有す ることを確認するために用いることができる。したがって、MOA 解析の結果は、特定の化合物の 評価に役立つこともあれば、新規の MOA の確立に貢献することもある。前者において、化学物 質に特有のデータが、ヒトへの関連性を考慮した一致解析において重要な役割を果たす。後者に おいては、どのような事象が MOA を表す生物学的プロセスの鍵となるのかを特定することが重 要になる。

このように、同様の一連の key events で構成された MOA は、多くの異なる化合物にも適用でき る可能性がある。ある MOA が特定の発がん作用の原因であることを立証するために必要なエビ デンスは、そのような MOA が初めて提示される場合には重要である。後発の化合物がこの MOA を共有することが発見される場合に、受け入れに対する「障壁」は低くなるが、MOA を構成する key events が発生すること、また、以下に説明する基準を満たしていることを厳密に立証する必要 がある。また、他の可能性のある MOA を除外することも重要である。

科学的な専門家の関与は、新規に想定される MOA の確立と承認のための必要条件である。「専 門家の関与」には、MOA の確立への専門家の関与と、MOA の研究プロセスから独立した科学者 によるピアレビューの両方が含まれる。科学雑誌への発表、科学会議やワークショップでの発表 や議論も、科学界が MOA を受け入れるための「専門家の関与」を意味する。

容認は必ずしも科学者全員が合意したことを意味するわけではないが、容認されたということは、MOA をレビューした科学者の大部分が、提示された MOA に関して関連する科学的情報が特定され適切に解析されていること、「key events」が特定され提示された情報によって支持されていること、発がんとの関係が仮説の MOA において明確に説明されていること及びその代替 MOA が検討され、それらが除外されていることに合意したということである。

知識の向上につれて MOA は変化するだろう。追加の key events が特定され、他の事象は見直さ れるか、あるいは取り除かれるかもしれない。しかしながら、key events に関する大きな変更は、 上記のようなピアレビューを通してある程度一般に認められることも必要である。

#### フレームワークガイドラインの更新

IPCS HRF の発展において、2001 年の動物 MOA フレームワークの内容が更新されたので、その 改訂版を以下に提示する。

#### Introduction to framework analysis

This section describes the cancer end-point or end-points that have been observed and identifies which of these are addressed in the analysis. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals. The nature of the framework is such that only one MOA is analysed at a time; hence, for example, different tumour types associated with chemical treatment, even if recorded in the same animals, will require separate framework analyses to discern each tumour's MOA. However, in considering the pathogenesis of a single type of tumour, it should be recognized that it is possible that a chemical could induce that tumour type by more than one MOA. Hence, it might be necessary to undertake an analysis of more than one MOA for the same tumour type for a single chemical. Consistent with species- and tissue-specific variation in metabolic activation and detoxication, there is often only poor site concordance for genotoxic carcinogens. This will need to be kept in mind when comparing animal and human data. In contrast, consistent with the observation that most carcinogens acting by a non-genotoxic MOA perturb physiological processes that tend to be site specific, site concordance is reasonably assumed, at least as an initial premise in the HRF.

#### 1. Postulated mode of action (theory of the case)

This section comprises a brief description of the sequence of events on the path to cancer for the postulated MOA of the test substance. This explanation of the sequence of events leads into the next section, which identifies the events considered "key" (i.e. necessary and measurable), given the database available for the analysis.

#### 2. Key events

This section briefly identifies and describes the "key events"—measurable events that are critical to the induction of tumours as hypothesized in the postulated MOA. To support an association, a body of experiments needs to define and measure an event consistently. Pertinent observations include, for example, tumour response and key events in the same cell type, sites of action logically related to event(s), increased cell growth, specific biochemical events, changes in organ weight and/or histology, proliferation, perturbations in hormones or other signalling systems, receptor–ligand interactions, effects on DNA or chromosomes, and impact on cell cycle. For example, key events for tumours hypothesized to be associated with prolonged regenerative proliferation might be cytotoxicity as measured histopathologically and an increase in labelling index. As another example, key events for induction of urinary bladder tumours hypothesized to be due to formation of urinary solids composed primarily of calcium phosphate might include elevated urinary free calcium, phosphate, and pH and formation of urinary solids, followed by irritation and regenerative hyperplasia of the uro-thelium.

#### 3. Concordance of dose-response relationships

This section should characterize the dose–effect/response relationships for each of the key events and for the tumour response and discuss their interrelationships, in the context of the Bradford Hill criteria. Ideally, one should be able to correlate the dose dependency of the increases in incidence of a key event with increases in incidence or severity (e.g. lesion progression) of other key events occurring later in the process, and with the ultimate tumour incidence. Comparative tabular presentation of incidence of key events and tumours is often helpful in examining dose–response. In the case of complex data sets, this is almost essential.

#### 序文

このセクションでは、観察された発がんエンドポイントまたはその他エンドポイントについて 記述し、これらのうちのどれが解析で扱われているかを特定する。フレームワーク解析に着手す る前に、実験動物における発がん性反応のエビデンスの重み付けを慎重に評価する必要がある。 フレームワークの性質上、一度に1つの MOA のみが解析される。したがって、例えば、化学物 質の投与に関連した異なる種類の腫瘍は、かりに同じ動物で記録されていても、各腫瘍の MOA を 識別するために別々のフレームワーク解析が必要となる。しかしながら、あるひとつの腫瘍の病 態を検討する際には、化学物質が複数の MOA によってそのタイプの腫瘍を誘発する可能性があ ることを認識しておくべきである。したがって、ある化学物質におけるひとつの腫瘍について複 数の MOA の解析を行う必要があるかもしれない。遺伝毒性発がん物質の場合、代謝活性化と解 毒における種や組織特異的な違いと同様に、発がん部位が異なることも多い。動物とヒトのデー タを比較する際にはこのことについて留意する必要がある。対照的に、非遺伝毒性 MOA に則っ て作用するほとんどの発がん物質は、特異的な傾向のある生理学的プロセスに影響を及ぼすとい う観察結果から、少なくとも HRF の初期の前提として、部位の一致が合理的に想定されている。

#### 1. 推定される MOA (事例における仮説)

このセクションでは、被験物質において推定される MOA について、腫瘍になるまでの一連の 事象を簡単に説明している。この一連の事象の説明は、次のセクションにつながり、解析に利用 可能なデータベースから、「鍵」と考えられる事象(すなわち、必要かつ測定可能な事象)を特定 する。

#### 2. Key events

このセクションでは、「key events」一推定される MOA において仮定された腫瘍の誘発に極めて 重要な測定可能な事象について簡潔に特定し、説明する。関連せを裏付けるためには、一連の実 験で事象を定義し、それを一貫して測定する必要がある。関連する観察項目には、例えば、同じ 種類の細胞における発がん性と key events、事象に論理的に関連する作用部位、細胞増殖の増加、 特定の生化学的事象、臓器重量や組織学的変化、増殖、ホルモンや他のシグナル伝達系の変動、 受容体-リガンド相互作用、DNA や染色体への影響、細胞周期への影響などが含まれる。例えば、 再生性増殖の延長と関連していると推定される腫瘍の発生における key events は、病理組織学的 検索における細胞毒性の検出と標識指標の上昇であるかもしれない。別の例として、リン酸カル シウムを主成分とする尿固形物の形成が原因であると推定される膀胱腫瘍の誘発の key events に は、尿中の遊離カルシウム、リン酸塩、pH の上昇と尿固形物の形成、それに続く尿路上皮への刺 激及び再生性過形成が含まれる。

#### 3. 用量反応関係の一致性

このセクションでは Bradford Hill 基準の観点から、それぞれの key events と発がんの用量-効果/ 反応関係を特徴づけ、それらの相互関係について議論するべきだろう。理想的には、key events の 発生率の増加の用量依存性と、その後に発生する他の key events の発生率または重症度(病変の 進行など)の増加及び最終的な腫瘍の発生率とを相関させることができるようにすべきである。 Key events と腫瘍の発生率を表形式で比較表示することは、しばしば用量反応性を検討する上で しばしば有用である。複雑なデータセットの場合、これはほぼ必須である。 It is important to consider whether there are fundamental differences in the biological response (i.e. dose transitions) at different parts of the dose–response curve for tumour formation (Slikker et al., 2004). If so, key events relevant to the different parts of the dose–response curve will need to be defined and used in the framework analysis.

#### 4. Temporal association

This section should characterize the temporal relationships for each of the key events and for the tumour response. The temporal sequence of key events leading to the tumour response should be determined. Key events should be apparent before tumour appearance and should be consistent temporally with each other; this is essential in deciding whether the data support the postulated MOA. Observations of key events at the same time as the tumours (e.g. at the end of a bioassay) do not contribute to considerations of temporal association, but can contribute to analysis in the next section. Most often, complete data sets to address the criterion of temporality are not available.

### 5. Strength, consistency, and specificity of association of tumour response with key events

This section should discuss the weight of evidence linking the key events, precursor lesions, and the tumour response. Stop/recovery studies showing absence or reduction of subsequent events or tumour when a key event is blocked or diminished are particularly important tests of the association. Consistent observations in a number of such studies with differing experimental designs increase that support, since different designs may reduce unknown biases or confounding. Consistency, which addresses repeatability of key events in the postulated MOA for cancer in different studies, is distinguished from coherence, however, which addresses the relationship of the postulated MOA with observations in the broader database (see point 6). Pertinent observations include tumour response and key events in the same cell type, sites of action logically related to event(s), and results from multistage studies and from stop/recovery studies.

#### 6. Biological plausibility and coherence

One should consider whether the MOA is consistent with what is known about carcinogenesis in general (biological plausibility) and also in relation to what is known for the substance specifically (coherence). For the postulated MOA and the events that are part of it to be biologically plausible, they need to be consistent with current understanding of the biology of cancer. However, the extent to which biological plausibility can be used as a criterion against which weight of evidence is assessed may be limited due to gaps in our knowledge. Coherence, which addresses the relationship of the postulated MOA with observations in the broader database-for example, association of MOA for tumours with that for other end-points—needs to be distinguished from consistency (addressed in point 5), which addresses repeatability of key events in the postulated MOA for cancer in different studies. For coherence, likeness of the case to that for structural analogues may be informative (i.e. structure-activity analysis). Information from other compounds that share the postulated MOA may be of value, such as sex, species, and strain differences in sensitivity and their relationship to key events. Additionally, this section should consider whether the database on the agent is internally consistent in supporting the purported MOA, including that for relevant non-cancer toxicities. Some MOAs can be anticipated to evoke effects other than cancer, such as reproductive effects of certain hormonal disturbances that are carcinogenic.

腫瘍形成に対する用量反応曲線の異なる部分において、生物学的反応(すなわち線量推移)に基本的な相違があるか否かを検討することが重要である(Slikker ら, 2004 年)。もしそうであれば、 用量反応曲線の異なる部分に関連する key events を明らかにし、フレームワーク解析に用いる必要がある。

#### 4. 時間的関連性

このセクションでは、それぞれの key events と腫瘍反応の時間的関係の特徴について述べなけ ればならない。腫瘍反応に至る key events を時系列的に明らかにすべきである。key events は腫瘍 の発現前に明らかにしなければならず、互いに時間的な整合性が取れていなければならない。す なわち、時間的関連性についてはデータが想定される MOA を支持しているかどうかを判断する 上で不可欠である。腫瘍と同時に key events が観察された場合(例えば、バイオアッセイの終了時 など) は、時間的関連性の検討には役立たないが、次のセクションに述べる解析に役立てること ができる。多くの場合、時間的関連性についての基準を満たす完全なデータセットは入手できな い。

#### 5. 腫瘍反応とkey events との関連性の強さ、一貫性、特異性

このセクションでは、key events、前がん病変及び腫瘍反応を結び付けるエビデンスの重み付け について議論しなければならない。Key events を遮断または低減した場合に、その後の事象または 腫瘍が発生しない、または減少することを示す中止/回復試験は、関連性を検証する上で特に重要 である。デザインが異なることによって未知のバイアスや交絡因子を減少させると考えられるた め、異なる実験デザインの研究において一貫した結果が観察されることは、key events と腫瘍の関 連性をより強く裏付けることになる。しかし、さまざまな研究で推定される MOA の key events の 再現性で示される一貫性 (consistency) は、より広範なデータベースでの結果と推定 MOA の相関 性で示される整合性 (coherence) とは区別される (6 参照)。関連する観察項目には、同じ種類の 細胞における腫瘍反応と key events、事象に論理的に関連する作用部位並びに多段階試験及び中止 /回復試験の結果が含まれる。

#### 6. 生物学的妥当性及び整合性

その MOA が、一般的にがんの発生について一般的に知られていることと一致している(生物 学的に妥当である)かどうか、また、その物質について知られていることと関連している(理論 的な整合性がある)かどうかを検討しなければならない。推定される MOA とその一部である事 象が生物学的に妥当であるとするためには、それらが現在のがんについての生物学的理解と一致 している必要がある。しかしながら、我々の知識には限りがあるために生物学的妥当性について エビデンスの重み付けを評価する際の基準として用いることができる範囲は限られているであろ う。推定される MOA とより広範なデータベースの観測値との関係 一例えば、発がん MOA と他 のエンドポイントの MOA との関連性一を扱う「整合性」は、異なる研究における推定される発 がん MOA における key events の再現性を扱う「一貫性」(ポイント5参照)とは区別される必要 がある。理論的な整合性については、構造的アナログにおける事例との類似性が参考になるかも しれない(すなわち、構造-活性解析)。推定される MOA を共有する他の化合物からの情報、たと えば、感度における性差、種差及び系統差や key events との関係に関するような情報は重要とな るだろう。さらに、このセクションでは、その薬剤に関するデータベースが内部で一貫して関連 している発がん以外の毒性を含めた MOA を支持しているかどうかを検討しなければならない。 MOA の中には、発がんにつながるホルモン障害による生殖への影響のような、発がん以外の影響 を引き起こすことが予想されるものがある。

#### 7. Other modes of action

This section discusses alternative MOAs that logically present themselves in the case. If alternative MOAs are supported, they need their own framework analysis. These should be distinguished from additional components of a single MOA that likely contribute to the observed effect, since these would be addressed in the analysis of the principal MOA.

#### 8. Uncertainties, inconsistencies, and data gaps

Uncertainties should include those related to both the biology of tumour development and those for the database on the compound of interest. Inconsistencies should be flagged and data gaps identified. For the identified data gaps, there should be some indication of whether they are critical as support for the postulated MOA.

#### 9. Assessment of postulated mode of action

This section should include a clear statement of the outcome with an indication of the level of confidence in the postulated MOA—for example, high, moderate, or low. If a novel MOA is being proposed, this should be clearly indicated. However, if the MOA is the same as that proposed for other compounds, the extent to which the key events fit this MOA needs to be stated explicitly. Any major differences should be noted, and their implications for the MOA should be discussed.

#### ADDRESSING THE ISSUE OF HUMAN RELEVANCE

In 2000, an IPCS Harmonization Project Cancer Planning Work Group convened in Carshalton, United Kingdom (IPCS, 2000). (This initial IPCS working group differed in membership from the subsequent IPCS working group convened to work on the human relevance project.) Among the recommendations of that meeting was the suggestion that IPCS and the International Life Sciences Institute (ILSI) move forward together and in parallel on the development of the extension of the IPCS MOA Framework towards addressing human relevance. It was recognized that ILSI could provide much help in technical workshops. In June 2001, the ILSI Risk Science Institute (RSI) with support from the USEPA and Health Canada formed a working group to examine key issues in the use of MOA information to determine the relevance of animal tumours. These efforts have resulted in several published reports that are described below. An IPCS Cancer Working Group, convened on 3–5 March 2004 in Arlington, Virginia, USA, agreed that these reports should form the starting point for further exploration of the issue of human relevance of animal tumours by IPCS with the goal of developing a unified IPCS HRF for use of MOA information in risk assessment for regulatory and other purposes (IPCS, 2004).

To address the issue of the human relevance of the MOAs determined in animals, ILSI/RSI charged its working group with expanding the IPCS MOA Framework to include evaluation of the human relevance of a cancer MOA determined in animals. The details of the process, the case-studies, and the framework were published as a series of papers in the November 2003 issue of *Critical Reviews in Toxicology* (Cohen et al., 2003; Meek et al., 2003). These articles describe the ILSI/RSI HRF and provide guidance for its application. In addition, references to specific examples on which the framework is based are included. Several iterations of case-studies of chemicals with generally well known MOAs were used to

#### 7. その他のMOA

このセクションでは、事例の中で論理的に提示された代替 MOA について議論する。代替 MOA が支持されている場合は、それ自体のフレームワーク解析が必要である。これら代替 MOA は観察された影響に寄与していると考えられる推定される MOA の追加的な構成要素とは区別し、異なる独立した MOA として解析しなければならない。さもなくば、代替 MOA ではなく、元々の解析の対象であった MOA に関する解析になってしまう。

#### 8. 不確実性、矛盾、データギャップ

不確実性には、腫瘍発生の生物学的知見に関連するものと、対象の化合物のデータベースに関 連するものの両方を含まなければならない。矛盾点にはフラグを立て、データギャップについて 特定しなければならない。特定されたデータギャップについては、推定される MOA の裏付けと して重要か否かを示すべきである。

#### 9. 推定される MOA の評価

このセクションには、推定される MOA の信頼度を示す指標と共に(例えば、高、中、低など) 結果に関する明確な記述を含まなければならない。新規の MOA が推定されている場合は、新規 であることを明確に示すべきである。しかし、MOA が他の化合物について提示されているものと 同じである場合には、key events がこの MOA にどの程度合致しているかを明示する必要がある。 大きな違いがあれば、それを明記し、MOA に対するその違いの意味合いについて議論すべきであ る。

#### ヒトへの関連性の問題

2000 年に、英国のカーシャルトンで IPCS ハーモナイゼーションプロジェクトがん計画ワーキ ンググループが招集された (IPCS、2000 年) (この最初の IPCS ワーキンググループは、ヒト関連 性プロジェクトの作業のために招集されたその後の IPCS ワーキンググループとはメンバーが異 なっていた)。この会議において、ヒトへの関連性への対応に向けて IPCS MOA フレームワークの 拡張を IPCS と国際生命科学研究機構 (ILSI) が協力して並行して進めていくことが提案された。 ILSI は技術ワークショップにおいて多くの支援を提供できることが認識された。2001 年 6 月、 ILSI リスクサイエンス研究所 (RSI) は、USEPA とカナダ保健省の支援を受けて、動物における 腫瘍とヒトとの関連性を判断するための MOA 情報の使用における重要な問題を検討するための ワーキンググループを結成した。これらの努力の結果、以下に記載されているいくつかの報告書 が公表された。2004 年 3 月 3 日から 5 日に米国バージニア州アーリントンで開催された IPCS が んワーキンググループは、これらの報告書を出発点として、規制目的やその他の目的で MOA 情 報をリスク評価に使用するための統一的な IPCS HRF を作成することを目標に、動物における腫 瘍のヒトへの関連性の問題を IPCS がさらに調査することに合意した (IPCS、2004 年)。

動物で決定された MOA のヒトへの関連性の問題に対処するために、ILSI/RSI は同ワーキング グループに、動物で決定された発がん MOA のヒトへの関連性の評価を含めるために IPCS MOA フレームワークを拡張することを求めた。このプロセス、事例研究及びフレームワークの詳細は、 Critical Reviews in Toxicology 誌の 2003 年 11 月号に一連の論文として発表された(Cohen ら, 2003 年)。これらの論文では ILSI/RSI HRF を説明し、その適用のための指針を提供している。さらに、 フレームワークが基づいた具体的な例への参照も含まれている。統合フレームワークを開発する ために、一般的によく知られている MOA を有する化学物質を複数回にわたって繰り返し検討し た事例研究が使用された。その意図は、動物での MOA と各 key event についてヒトへの関連性を

develop the integrated framework. The intent was to provide guidance for a disciplined, transparent process evaluating the MOA in animals and each key event with respect to human relevance.

The ILSI/RSI HRF is based on three fundamental questions:

- 1. Is the weight of evidence sufficient to establish the mode of action (MOA) in animals?
- 2. Are key events in the animal MOA plausible in humans?
- 3. Taking into account kinetic and dynamic factors, are key events in the animal MOA plausible in humans?

Questions 2 and 3 involve qualitative and quantitative considerations, respectively, in a concordance analysis of human information in relation to the animal MOA and its key events.

These are followed by an explicit description of confidence in the evaluation, identification of specific data gaps, and the implications for risk assessment. It was emphasized by ILSI/RSI that use of this framework would form part of the hazard characterization step of the overall risk assessment process.

# DEVELOPMENT OF AN IPCS HRF GUIDANCE DOCUMENT BASED ON THE IPCS MOA FRAMEWORK AND THE ILSI/RSI HRF

The 2004 IPCS Cancer Working Group discussed the type of document that would be produced as a result of its task to extend the IPCS MOA Framework to address human relevance. It was recognized that one integrated guidance document that worked as a whole would be needed to facilitate uptake and use by regulatory and other risk assessment bodies. The guidance could be supplemented by publication of the other materials generated through the process (e.g. issue papers and case-studies).

There was general agreement among working group members that the questions identified as the critical components of the ILSI/RSI HRF were important and in general appropriate for addressing the human relevance of an MOA determined in animals. However, several issues were identified that could benefit from additional clarification, development, or expansion.

These refinements of the ILSI/RSI HRF were developed through discussions of the IPCS Cancer Working Group and at a workshop convened for this purpose in Bradford, United Kingdom, on 21–23 April 2005 (IPCS, 2005). The resulting IPCS HRF is presented as an approach to answering a series of three questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumours. The application of the guidance results in a narrative with four sections that may be incorporated into the hazard characterization of a risk assessment. The sections are as follows (see Figure 1):

- 1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?
- 2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

評価する規律ある透明なプロセスの指針を提供することであった。

ILSI/RSI HRF は3つの基本的な問いかけに基づいている:

- 1. 動物における Mode of action (動物 MOA) を確立するのにエビデンスの重み付けは十分か?
- 2. 動物の MOA に含まれる key events はヒトにおいて妥当であるか?
- 3. 動態的、薬力学的要因を考慮した上でも、動物の MOA における key events はヒトにおいて妥 当か?

問2と3に関して、動物の MOA とその key events に関連したヒトでの情報の一致解析において、それぞれ定性的な検討と定量的な検討が必要となる。

これらに続いて、ILSI/RSI HRF は評価の信頼性、特異的なデータギャップの特定、それらのリ スク評価の意味合いを明確に記述している。ILSI/RSI では、このフレームワークの利用が全体的 なリスク評価プロセスのハザードの特性評価ステップの一部を形成することを強調した。

#### IPCS MOA フレームワークと ILSI/RSI HRF に基づく IPCS HRF ガイダンス文書の開発

2004 年の IPCS がんワーキンググループでは、ヒトへの関連性に対処するために IPCS MOA フレームワークを拡張する作業の報告書として作成される文書のタイプについて議論された。規制 当局や他のリスク評価機関による採用と利用を促進するためには、全体を一つに統合されたガイ ダンス文書が必要であると認識された。ガイダンスは、発行論文や事例研究といったプロセスを 経て作成された他の公表資料を追加することができる。

ワーキンググループのメンバー間では、ILSI/RSI HRF の重要な構成要素として特定された問い かけは重要であり、動物で決定された MOA のヒトへの関連性に対処するのに適切であるという 点で概ね合意されていた。しかし、フレームワークの補足説明、進展、または拡大が有効と思わ れるいくつかの課題が明らかとなった。

ILSI/RSI HRF のこれらの改良は IPCS がんワーキンググループの議論と、2005 年4月21日から 23 日に英国ブラッドフォードで開催されたワークショップでの議論を経て進められた(IPCS、 2005 年)。結果として得られた IPCS HRF は、動物の腫瘍における MOA のヒトへの関連性に関す る文書化された論理的な結論へとつながる一連の3つの問いかけに答えるためのアプローチとし て提示されている。このガイダンスを適用すると、リスク評価のハザードの特性評価に組み入れ ることができる4つのセクションからなるストーリーができあがる。これらのセクションは以下 の通りである(図1を参照)。

- 1. 動物における Mode of action (動物 MOA) を立証するためにエビデンスの重み付けは十分か?
- 2. 実験動物とヒトとの間の key events の根本的、質的な違いに基づいて、MOA のヒトへの関連性 を合理的に排除できるか?
- 3. 実験動物とヒトとの間の動態的または薬力学的要因のいずれかの量的な違いに基づいて、MOA のヒトへの関連性を合理的に排除できるか?

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4. 結論:信頼性、解析的検討及び帰結の記述。

- 3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?
- 4. Conclusion: Statement of confidence, analysis, and implications.

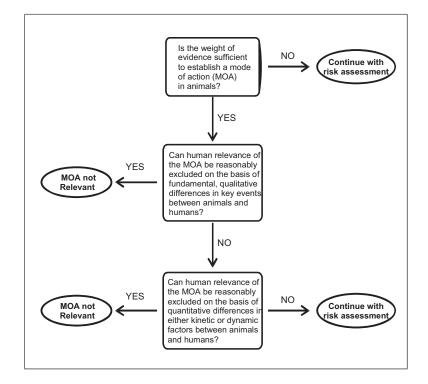
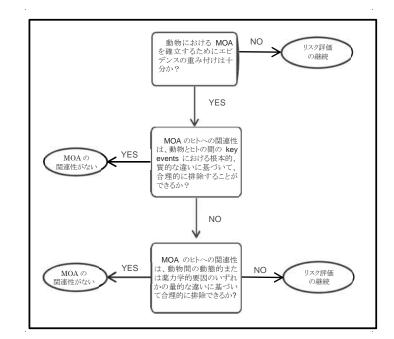


Figure 1. IPCS general scheme illustrating the main steps in evaluating the human relevance of an animal MOA for tumour formation. The questions have been designed to enable an unequivocal answer *yes* or *no*, but recognizing the need for judgement regarding sufficiency of weight of evidence. Answers leading to the left side of the diagram indicate that the weight of evidence is such that the MOA is not considered relevant to humans. Answers leading to the right side of the diagram indicate either that the weight of evidence is such that the MOA is not considered relevant to humans. Answers leading to the right side of the diagram indicate either that the weight of evidence is such that the MOA is likely to be relevant to humans or that it is not possible to reach a conclusion regarding likely relevance to humans, owing to uncertainties in the available information. In these cases, the assessment would proceed to risk characterization. It should be noted that only at this stage would human exposure be included in the evaluation.

In applying this framework for a given chemical, tumours of each animal target organ observed are evaluated independently, with the assumption that different MOAs are possible in different organs, although based on this analysis, MOAs in different tissues may be similar. Similarly, an evaluation of the likelihood of congruence between target organ(s) in different species and in humans needs to be made, based on the MOA analysis.



#### 図 1. IPCS の一般的なスキーム:腫瘍形成に対する動物 MOA のヒトへの関連性を評価す る際の主なステップを示す

質問は、Yes か No かを明確に答えられるように設計されているが、エビデンスの重み付けが十分 であるかどうかを判断する必要がある。図の左側に示された回答は、エビデンスに十分な重みがあ り MOA がヒトに関連するとは考えられないことを示している。図の右側の回答は、MOA がヒト に関連する可能性が高いと考えられるという十分に重みのあるエビデンスの存在を示しているか、 利用可能な情報に不確実性があるため、ヒトへの関連性の可能性についての結論を出すことができ ないことを示している。このような場合、評価はリスクの判定に進むことになる。この段階でのみ、 ヒトへのばく露評価が含まれることに留意すべきである。

このフレームワークをある化学物質に適用する場合、観察された各動物の標的臓器の腫瘍は、 異なる臓器では異なる MOA が存在しうるという仮定の下、独立して評価される。しかし、この 解析手法に基づいたとしても、異なる組織での MOA が類似している可能性はある。同様に、異 なる種とヒトにおいて、標的臓器における MOA が一致する可能性についての評価も MOA 解析 に基づいて行う必要がある。

# Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?

Answering this first question in the IPCS HRF requires application of the (updated) IPCS MOA Framework described previously in this document. The steps in the MOA Framework, which are based on the Bradford Hill criteria for causality, are:

- 1. postulated MOA;
- 2. key events; associated critical parameters;
- 3. dose-response relationships;
- 4. temporal association;
- 5. strength, consistency, and specificity of association of key events and tumour response;
- 6. biological plausibility and coherence;
- 7. possible alternative MOAs;
- 8. uncertainties, inconsistencies, and data gaps;
- 9. conclusion about the MOA.

This process incorporates an evaluation of the weight of evidence for possible alternative MOAs at a given site and an evaluation of the overall strength of evidence supporting the MOA under consideration. Ultimately, a decision concerning the weight of evidence supporting the MOA and the level of confidence in that decision must be made. The process also identifies critically important data gaps that, when filled, would increase confidence in the proposed MOA. It is also necessary to establish whether the postulated MOA has already been described for other chemicals, in which case human relevance will already have been evaluated, or whether the proposed MOA is novel, in which case human relevance needs to be assessed de novo.

For a given chemical, the primary sources of information for evaluating an MOA are likely to be data generated for that specific chemical in the animal model in which tumours were produced. Obviously, data from other sources can and should also be used, as appropriate, along with data on chemicals with similar chemical structures, the same or similar MOAs, or both. If the MOA for a chemical is novel, considerably more data will be required to support the conclusion that it is related to the carcinogenic process of the tumours induced by that chemical than for subsequent examples of chemicals acting by the same MOA. The ILSI/RSI working group and the IPCS Bradford workshop did not address the issue of how many data are sufficient to support a specific MOA for a given chemical per se, except by way of example within the case-studies and recognition that acceptance of a novel MOA requires scientific consensus (described above). Consideration at this stage of the MOA analysis of potential variations between animals and humans also facilitates addressing subsequent steps in the framework.

# Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS workshop on the implications of a *yes* or a *no* answer to the original question. In answering the original question, only an unequivocal *no* would be sufficient to

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#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

# 動物における Mode of action (動物 MOA)を確立するのにエビデンスの重み付けは十分 か

IPCS HRF のこの最初の問いかけに答えるためには、上述の(更新された) IPCS MOA フレーム ワークを適用する必要がある。因果関係を説明するための Bradford Hill 基準に基づいた MOA フ レームワークのステップは、次のとおりである:

- 1. 推定される MOA
- 2. Key events; 関連する重要なパラメータ
- 3. 用量反応関係
- 4. 時間的関連性
- 5. Key events と腫瘍反応との関連の強さ、一貫性、特異性
- 6. 生物学的妥当性と整合性
- 7. 可能性のある代替 MOA
- 8. 不確実なこと、矛盾すること及びデータギャップ
- 9. MOA に関する結論

このプロセスには、状況に応じた考え得る代替 MOA のエビデンスの重み付けの評価と、検討 中の MOA を支持するエビデンスの全体的な強度の評価が組み込まれている。最終的には、MOA を支持するエビデンスの重み付けと、それに対する信頼度について決定しなければならない。ま た、このプロセスは、ギャップを満たすことで推定される MOA の信頼性を高めるであろう決定 的に重要なデータギャップを特定する。また、推定される MOA が他の化学物質において既に記 述された MOA であり、ヒトへの関連性について既に評価されているかどうか、または、新規の MOA であり、ヒトへの関連性について新たに評価する必要があるかどうかを確認することも必要 である。

ある化学物質について、MOA を評価するための主な情報源は、腫瘍が発生した動物モデルにお けるデータである可能性が高い。当然、類似の化学構造を持つ化学物質に関するデータや、同じ または類似の MOA に関するデータ、もしくはその両方のデータといった他の情報源からのデー タも必要に応じて利用することができるし、利用すべきである。ある化学物質の MOA が新規で ある場合、その化学物質が誘発された腫瘍の発がんプロセスに関連しているという結論を裏付け るためには、同じ MOA で作用する後続の化学物質よりもかなり多くのデータが必要である。 ILSI/RSI ワーキンググループと IPCS ブラッドフォードワークショップでは、対象の化学物質の MOA を支持するのに十分なデータの数はどのくらいかという問題に関して、事例研究の中で例を 示し、新しい MOA を受け入れるには科学的コンセンサスが必要であるとの認識(上述)を示し てはいるが、それら以外についての方針等は何ら表明していない。この段階における動物とヒト との間の潜在的な違いについての MOA 解析の検討は、フレームワークにおける次のステップの 対応を容易にする。

# 実験動物ととトとの間の key events の根本的、質的な違いに基づいて、MOA のとトとの関連性を合理的に排除することができるか

この質問の文言は、IPCS ワークショップにおける質問に対する回答の意味合いについての議論 を受けて、ILSI/RSI HRF に記載されていた文言から変更された。元の質問への回答では、動物 MOA がヒトへの関連性を持たないという結論を出すには、はっきりとした「No」しか許容されていな かった。また、「plausible」という単語が他の言語に翻訳する際に問題になる可能性があることも

permit the conclusion that the animal MOA was not relevant to humans. Also, it was recognized that translation of the word "plausible" into other languages could be problematic. The question was therefore reworded to enable a *yes/no* answer, but qualified by the descriptor "reasonably", based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve scientific judgement based on transparent analysis of the available data.

This step represents a qualitative assessment of the relevance of the MOA to human cancer potential. Listing the critical specific key events that occur in the animal MOA and directly evaluating whether each of the key events might or might not occur in humans facilitate consideration and transparent presentation of the relevant information. Presentation in tabular form, referred to as a concordance table, can be helpful in delineating the relevant information (for an example, see Meek et al., 2003, case-study 6: kidney and liver tumours associated with chloroform exposure, Table 7: McGregor et al., current document, case-study on formaldehyde, Table 3). The key events (and possibly some of the critical associated processes) are listed with the information regarding these events for the animals in which the tumour was observed. It is intended that the information in these tables be brief, since a narrative explanation is expected to accompany the table. In the right-hand column, the effect on humans for each of the key events is evaluated. An additional column for the results in a different strain, species, sex, or route of administration that does not result in tumours can be useful if information is available for comparison with the model that leads to tumours. In addition, factors may be identified that, while not key themselves, can modulate key events and so contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology. Any such factors identified should be noted in a footnote to the concordance table.

The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical specific—for example, the enzyme induction response by phenobarbital in rodent liver, or the formation of a cytotoxic metabolite from chloroform by specific cytochrome P-450 enzymes. Later events are more generic to the MOA—for example, pleiotropic stimulation of hepatic proliferation or regenerative hyperplasia. Information that can be utilized to evaluate the key events in humans can come from in vitro and in vivo studies on the substance itself, but also can involve basic information regarding anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans. Information concerning an evaluation of the key event in humans exposed directly to the specific chemical is frequently unavailable.

As knowledge concerning the development of cancer evolves, it may become possible to combine some MOAs on the basis of the basic biology of the processes involved, thus relying less on chemical-specific information to reach a conclusion on the human relevance of a given MOA.

認識された。そのため、質問は Yes/No の回答を可能にするように書き換えられたが、エビデンス の重み付けの妥当性に関する決定は絶対的なものではなく、利用可能なデータの透明性のある解 析に基づいた科学的判断が必要であるという認識に基づき、「合理的に」という語句が付け加えら れた。

このステップでは、ヒトにおける発がんの可能性と MOA の関連性を定性的に評価することで ある。動物 MOA で発生する重要な特定の key events をリストアップし、それぞれの key events が ヒトで発生するか否かを直接評価することで、関連する情報の検討と透明性のある提示が容易に なる。一致表 (concordance table) と呼ばれる表形式での提示は、関連情報を明確にするのに役立 つ (例として、事例研究 6:クロロホルムばく露に伴う腎臓及び肝臓の腫瘍、Meek ら、2003 年、表 7;本書、ホルムアルデヒドに関する事例研究、McGregor ら、表 3 を参照)。腫瘍が観察された動 物におけるこれらの事象に関する情報とともに、key events (及び重要と思われるいくつかの関連 プロセス) がリストアップされる。これらの表の情報は簡潔にすることが意図されており、表に は説明文が添付されることが予想される。右側の列では、各 key events についてヒトへの影響を 評価している。追加の列として、腫瘍が発生しない別の系統、種、性、または投与経路での結果 についての情報は、腫瘍が発生するモデルとの比較情報が得られる場合に有用である。さらに、 それ自体は key events ではないが、key events を変化させ、種差または個体差に寄与する因子が同 定されることがある。そのような因子には、代謝経路の遺伝的差異、競合する代謝経路及び同時 進行する病態によって誘導される細胞増殖が含まれる。特定されたそのような要因はすべて、一 致表の脚注に記載されるべきである。

ヒトにおけるある化合物の MOA の key events の一致性の評価は、その特定の化学物質の評価と いうよりは、ヒトにおける MOA の評価である。一般的に、初期の key events の詳細は化学物質に 特異的である可能性が高い。例えば、げっ歯類の肝臓におけるフェノバルビタールによる酵素誘 導反応や、特定のチトクローム P-450 酵素によるクロロホルムからの細胞毒性代謝物の生成など である。その後の事象は MOA においてより一般的なものであり、例えば、多面的な肝増殖刺激 や再生性過形成などである。ヒトにおける key events の評価に利用できる情報は、化学物質の in vitro 及び in vivo 試験に由来するもののほか、解剖学、生理学、内分泌学、遺伝学、疫学、その他 ヒトの key events に関する既知の情報などの基本的な情報が含まれる。特定の化学物質に直接ば く露されたヒトにおける key events の評価に関する情報は、入手できないことが多い。

がんの発生・進行に関する知見が蓄積されるにつれて、関与するプロセスの基本的な生物学的 知見に基づいていくつかの MOA を組み合わせることが可能になるかもしれない。それにより、 MOA のヒトへの関連性について結論付ける際に、その化学物質の特異的な情報への依存度が低く なるかもしれない。

In evaluating the concordance of the information in humans to that in animals, a narrative describing the weight of evidence and an evaluation of the level of confidence for the human information need to be provided. Some specific types of information that are useful include the following:

- 1. cancer incidences at the anatomical site and cell type of interest, including age, sex, ethnic differences, and risk factors, including chemicals and other environmental agents;
- 2. knowledge of the nature and function of the target site, including development, structure (gross and microscopic), and control mechanisms at the physiological, cellular, and biochemical levels;
- 3. human and animal disease states that provide insight concerning target organ regulation and responsiveness;
- 4. human and animal responses to the chemical under review or analogues following short-, intermediate-, or long-term exposure, including target organs and effects.

Obviously, a substantial amount of information is required to conclude that the given MOA is not relevant to humans. If such a conclusion is strongly supported by the data, then chemicals producing animal tumours only by that MOA would not pose a cancer hazard to humans, and no additional risk characterization for this end-point is required. Since there is no cancer hazard, there is no cancer risk for the tumour under consideration.

The question of relevance considers all groups and life stages. It is possible that the conditions under which an MOA operates occur primarily in a susceptible subpopulation or life stage—for example, in those with a pre-existing viral infection, hormonal imbalance, or disease state. Special attention is paid to whether tumours could arise from early-life exposure, considering various kinetic and dynamic aspects of development during these life stages. Any information suggesting quantitative differences in susceptibility is identified for use in risk characterization.

#### Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

The wording of this question was changed from that in the ILSI/RSI HRF, following discussion at the IPCS workshop on the implications of a *yes* or a *no* answer to the original question. In answering the original question, only an unequivocal *no* would be sufficient to permit the conclusion that the animal MOA was not relevant to humans. The question was therefore reworded to enable a *yes/no* answer, but qualified by the descriptor "reasonably", based on recognition that decisions about the adequacy of weight of evidence are not absolute but involve judgement based on transparent analysis of the available data.

For purposes of human relevance analysis, if the experimental animal MOA is judged to be qualitatively relevant to humans, a more quantitative assessment is required that takes into account any kinetic and dynamic information that is available from both the experimental animals and humans. Such data will of necessity be both chemical and MOA specific and will include the biologically effective doses required to produce the relevant dynamic responses from which neoplasia can arise. Kinetic considerations include the nature and time course of

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ヒトの情報と動物の情報との一致性を評価する際には、エビデンスの重み付けを説明し、ヒト の情報の信頼度の評価をする必要がある。有用な情報の具体的な種類としては、以下のようなも のがある。

- 1. 対象の解剖学的部位及び細胞の種類におけるがんの発生率:年齢、性別、人種及び化学物質や その他の環境因子などのリスク因子を含む。
- 標的部位の性質と機能に関する知見:生理的、細胞的、生化学的レベルでの発生、構造(肉眼 的、顕微鏡的)、制御機構を含む。
- 3. ヒト及び動物の疾患状態:標的臓器の調節及び応答性に関する洞察を提供する。
- 4. 短期、中期、または長期ばく露後の、対象となる化学物質または類似物質に対するヒト及び動物の反応:標的臓器及び影響を含む。

当然、対象の MOA がヒトに関連していないと結論づけるには、相当量の情報が必要である。 そのような結論がデータによって強く支持されているならば、その MOA によってのみ動物の腫 瘍を誘発される化学物質は、ヒトに対するがんに関するハザードを有さず、このエンドポイント に対するリスク判定を追加で行う必要はない。がんに関するハザードがないため、検討中の腫瘍 に対する発がんリスクはない。

関連性の質問は、すべての集団とライフステージを考慮している。MOA が作用する状況は、例 えばすでにウイルスに感染しているヒト、ホルモンバランスの不均衡のあるヒト、または病を患 っているヒトなど、主に感受性の高い集団またはライフステージで起こる可能性がある。ライフ ステージの早期における発達に関する様々な動態的及び薬力学的側面を考慮して、腫瘍がライフ ステージの早期におけるばく露から発生するかどうかに特別な注意が払われている。感受性の量 的な違いを示唆するいかなる情報も、リスク判定に用いるために特定される。

#### 実験動物ととトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、 MOA のとトへの関連性を合理的に排除することができるか

この質問の文言は、IPCS ワークショップにおける質問に対する回答の意味合いについての議論 を受けて、ILSI/RSI HRF に記載されていた文言から変更された。元の質問への回答では、動物 MOA がヒトへの関連性を持たないという結論を出すには、はっきりとした「No」しか許容されていな かった。そのため、質問は Yes/No の回答を可能にするように書き換えられたが、エビデンスの重 み付けの妥当性に関する決定は絶対的なものではなく、利用可能なデータの透明性のある解析に 基づいた科学的判断が必要であるという認識に基づき、「合理的に」という語句が付け加えられた。

ヒトへの関連性解析の目的では、実験動物の MOA がヒトに定性的に関連性があると判断され る場合には、実験動物とヒトの両方から得られるあらゆる動態的及び薬力学的情報を考慮して、 より定量的な評価が必要である。このようなデータは、必然的に化学物質及び MOA に特有のも のであり、腫瘍形成を起こしうる薬力学的反応を引き起こすために必要な生物学的影響を及ぼす 用量を含むものである。動態的考察には、化学物質の取り込み、分布、代謝及び排泄の性質及び 経時的変化が含まれ、一方、薬力学的考察には、化学物質と細胞、組織及び器官との相互作用の 結果が含まれる。

chemical uptake, distribution, metabolism, and excretion, while dynamic considerations include the consequences of the interaction of the chemical with cells, tissues, and organs. On occasion, the biologically effective dose that would be required to create these conditions would not be possible in humans. It may also be that quantitative differences in a biological process involved in a key event-for example, the clearance of a hormone-are so great that the animal MOA is not relevant to humans. However, the IPCS workshop recognized that only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. As with the qualitative assessment, a tabular comparison of quantitative data from the experimental animals and humans can facilitate the evaluation (for example, see Meek et al., 2003, case-study 5, thyroid tumours associated with exposure to phenobarbital, Table 6; Dellarco et al., current document, case-study on thiazopyr, Table 4). Useful comparisons can also be made with key events identified from studies of other compounds believed to induce effects by a similar MOA. For example, in the case of thiazopyr, information on the effects of phenobarbital in humans was particularly informative in evaluating the relevance of the MOA. As molecular and kinetic approaches continue to evolve, understanding of the similarities and differences of responses in animals and humans will be improved. It may become apparent that qualitative differences in a key event between an animal model and humans will be identified as being due to a specific quantitative difference, thus changing the answer to the second question (described above) to no.

As with question 2, if the conclusion to this question is *yes*, then chemicals producing animal tumours only by that MOA would not pose a cancer hazard to humans, and no additional risk characterization for this end-point is required.

#### Statement of confidence, analysis, and implications

Following the overall assessment of each of the three questions, a statement of confidence is necessary that addresses the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database, and the nature and extent of the concordance analysis. An evaluation of alternative MOAs, using comparable analyses and rigour, is also essential. A critically important outcome of adequate consideration of the weight of the evidence for an overall MOA and the qualitative and quantitative concordance is the identification of specific data gaps that can be addressed experimentally in future investigations to increase confidence.

Infrequently, there may be conclusive epidemiological data on the cancer risk from a chemical that shares the MOA of the compound under consideration—that is, the compound does or does not cause cancer in humans. Obviously, such data would lend considerable weight to the conclusion of the human relevance evaluation. However, there may be occasions when, despite it being possible to establish an MOA in animals, there is insufficient information on the key events in humans to reach a clear conclusion on human relevance. In such circumstances, it might be possible to bridge this data gap by using epidemiological data. For example, the database on key events in humans for compounds that act like phenobarbital via activation of the constitutively active receptor (CAR) to induce hepatic tumours is incomplete. However, there are robust epidemiological data showing that exposure to phenobarbital for prolonged periods at relatively high doses does not cause cancer in humans. One possibility, therefore, is to "read across" from these findings with phenobarbital to any other 時折、これらの条件を作り出すために必要とされる生物学的に影響を及ぼす用量が、ヒトにおい て不可能であることがある。また、ホルモンのクリアランスなど key events に関与する生物学的 プロセスにおける定量的な違いが大きすぎるため、動物の MOA がヒトには関連しないというこ ともあり得る。しかし、量的な差異に基づいて MOA のヒトへの関連性を否定することが可能に なるのは、ごくまれであると IPCS ワークショップでは認識されている。定性評価と同様に、実験 動物とヒトの定量データを表形式で比較することで、評価を容易にすることができる(例えば、 事例研究 5、フェノバルビタールばく露に伴う甲状腺腫瘍、Meek ら、2003 年、表 6;チアゾビル に関する事例研究、Dellarco ら、本書、表 4 参照)。同様の MOA によって効果を誘導すると考え られている他の化合物の研究から同定された key events との比較も有用である。例えば、チアゾ ピルの場合、ヒトにおけるフェノバルビタールの効果に関する情報は、MOA の関連性を評価する 上で特に有用であった。分子学的及び動態学的アプローチが進化し続けるにつれ、動物及びヒト における反応の類似性及び相違点の理解が向上するであろう。動物モデルとヒトとの間の key events における定性的な違いが、特定の定量的な違いによるものであることが明らかになるかも しれない。その場合、第2の問いかけ(上述)への回答を「No」に変更する。

問2と同様に、この問いかけへの結論が「Yes」であれば、そのMOAによってのみ動物に腫瘍 を誘発させる化学物質はヒトにがんに関するハザードをもたらさず、このエンドポイントにおけ る追加のリスク判定は必要ない。

#### 信頼性、解析及び帰結の記述

3 つの問いかけのそれぞれの総合評価に続いて、解析の基礎となるデータの質と量、フレームワ ーク内での解析の一貫性、データベースの一貫性、一致解析の性質と程度についての信頼性につ いての記述が必要である。また、同等の解析法や厳密な評価を用いた代替的な MOAs の評価も不 可欠である。全体的な MOA と質的・量的一致のエビデンスの重み付けを十分に考慮し、その結 果として、MOA の信頼性を高めることができる将来の研究で実験的に特異的なデータギャップを 特定することが極めて重要である。

まれに、検討中の化合物と共通する MOA を有する化学物質の発がんリスクに関する決定的な 疫学的データ、すなわち、その化合物がヒトに発がん性を有するか否かのデータが存在すること がある。当然、そのようなデータは、ヒトへの関連性評価の結論にかなりの重みを与えるであろ う。しかし、動物では MOA を確立することが可能であるにもかかわらず、ヒトでの key events に 関する情報が不十分であり、ヒトへの関連性について明確な結論を出すことができない場合があ る。そのような場合には、疫学データを利用することで、このデータギャップを埋めることがで きるかもしれない。例えば、構成的活性化受容体 (CAR) の活性化を介してフェノバルビタール と同様の作用をして肝腫瘍を誘発する化合物のヒトでの key events に関するデータベースは不完 全である。しかし、比較的高用量のフェノバルビタールに長期間ばく露しても、ヒトでは発がん しないことを示す確固たる疫学的データがある。したがって、1 つの可能性としては、これらのフ ェノバルビタールに関する知見から、げっ歯類に肝腫瘍を誘導する動物 MOA を共有する他の化 合物について「類推」し、そのような化合物によって引き起こされる腫瘍は、ヒトにおける化合 物のリスク評価には関係ないと結論づけることである (Holsapple ら、2006 年)。

compound that shares its MOA in animals in inducing rodent liver tumours and to conclude that the tumours caused by such a compound are not relevant to the risk assessment of the compound in humans (Holsapple et al., 2006). Such a conclusion would be critically dependent on the reliability of the epidemiological data and the similarity between the MOA for the chemical under test to that of the compound for which there are epidemiological data available.

In applying the framework to case-studies, it is apparent that much current research does not address key questions that would facilitate an analysis of an animal MOA or its relevance to humans. Often this has been because of lack of transparent delineation of key data gaps based on consideration of the data in analytical frameworks such as that presented here. Thus, use of the HRF can be very informative to researchers from the outset in the design of their studies.

The output of formal human relevance analysis provides information that is useful for more than just determining whether or not an end-point in animals is relevant to humans. Rather, consideration of the relevant information in a transparent, analytical framework provides much additional information that is critically important in subsequent steps in the risk characterization for relevant effects. Based on a human relevance analysis for a proposed MOA for relevant effects, it may be possible to predict, for example, site concordance or not of observed tumours in animals to humans. Application of the HRF also often provides information on relevant modulating factors that are likely to affect risk, such as hepatitis B and aflatoxin  $B_1$  (see Cohen et al., current document, case-study on 4-aminobiphenyl). Analysis often also provides an indication of those components of a proposed MOA that may operate only over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in an MOA, then the relevance to human risk becomes a matter of exposure. Thus, the exposure assessment step of the subsequent risk characterization is critical to the proper evaluation of human cancer potential. In addition, information identified during the framework analysis can prove invaluable in hazard quantification based on the key events for the MOA.

Importantly, the human relevance analysis also contributes to identification of any special subpopulations (e.g. those with genetic predisposition) who are at increased risk and often provides information relevant to consideration of relative risk at various life stages. In some cases, this may be based not on chemical-specific information but rather on inference, based on knowledge of the MOA, as to whether or not specific age groups may be at increased or decreased risk.

The data and their analysis using the framework should be reported in a transparent manner, enabling others to determine the basis of the conclusions reached with respect to the key events, the exclusion of other MOAs, and the analysis of human relevance. As the specific form of presentation will vary with the type of data available, it is not helpful to be prescriptive on how the information should be reported. However, presentation should include sufficient details on the context and thought processes to ensure transparency of the conclusions reached. The use of appropriate tables can be helpful in presenting certain data, such as comparative analysis of key events in experimental animals and humans.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

このような結論は、疫学的データの信頼性及び試験対象の化学物質の MOA と疫学的データが利用可能な化合物の MOA との類似性に極めて依存している。

このフレームワークを事例研究に適用すると、現在の研究の多くは、動物 MOA の解析及びヒトとの関連性の解析を容易にするような重要な問いに対処していないことは明らかである。多くの場合、このことは、ここで紹介したような解析フレームワークでのデータの検討に基づいて、主要なデータギャップを透明性をもって記述していないことが原因である。このように、HRFを用いることは試験設計の初期段階から研究者にとって非常に有益である。

統一された形式のヒト関連性解析の結果は、動物のエンドポイントがヒトに関連するかどうか を判断するだけでなく、それ以上に有用な情報を提供してくれる。むしろ、透明性のある解析的 フレームワークの中で関連情報を検討することで、関連する影響のリスク判定における次の段階 で多くの極めて重要な追加情報が提供される。推定される関連影響に関する MOA のヒト関連性 解析に基づくことで、例えば、動物で観察された腫瘍がヒトにおいて発生部位が一致するかどう かを予測することが可能である。また、HRF を適用することで、B型肝炎やアフラトキシン B<sub>1</sub>な どのように、リスクに影響を与える可能性のある関連する調節因子に関する情報が得られること が多い(4 Aminobiphenyl に関する事例研究、Cohen ら、本書を参照)。解析はまた、推定される MOA の構成要素のうち、一定の用量以上でのみ作用する可能性のあるものについての指標を提供 することが多い。MOA に必須なステップを引き起こすために対象の化合物の実験的高用量のばく 露が必要とされる場合、ヒトのリスクとの関連性はばく露の問題となる。このように、その後の リスク判定におけるばく露評価ステップは、ヒトでの発がん性を適切に評価するために重要であ る。さらに、フレームワーク解析で特定された情報は、MOA の key events に基づくハザードの定 量化において非常に貴重なものとなりうる。

重要なことだが、ヒト関連性解析は、リスクが増大している特別な集団(遺伝的素因を持つ集 団など)を特定することにも寄与し、しばしば、様々なライフステージにおける相対リスクの検 討に関連する情報を提供してくれる。場合によっては、これは化学物質固有の情報に基づくので はなく、特定の年齢層のリスクが増加しているか減少しているかについて、MOAの知識に基づい ている場合もある。

フレームワークを用いたデータ及びその解析は、透明性のある方法で報告され、他の者が key events、他の MOA の除外及びヒトとの関連性の解析に関して到達した結論の根拠を判断できるよ うにすべきである。具体的な提示形式は利用可能なデータの種類によって異なるため、情報がど のように報告されるべきかを規定することに意味はない。しかし、到達した結論の透明性を確保 するために、文脈や考察のプロセスに関して十分な詳細情報が含まれていなければならない。実 験動物とヒトにおける key events の比較解析など、特定のデータを提示する際には、適切な表を 使用することが有用である。

#### **Dissemination of the framework**

To assist in the dissemination and application of the IPCS HRF, a database of generally accepted MOAs and informative cases should be constructed and maintained. This would comprise a series of MOAs and their associated key events, for reference by those developing framework analyses for compounds that may act by similar MOAs. The case-studies would comprise worked examples that have been analysed using the framework, to provide an indication of the relevant level of detail of the analyses and nature of the weight of evidence required to support acceptance of a proposed MOA in causing the carcinogenic response. Such cases would be particularly valuable early in the development of a new MOA.

#### Application of the IPCS HRF to DNA-reactive carcinogens

Because of similarities in the carcinogenic process between rodents and humans and the comparable initial interactions with DNA by DNA-reactive carcinogens, it would be expected that, in general, DNA-reactive carcinogens would be assessed as progressing to the step of "ves, the key events in the animal MOA could occur in humans" in the ILSI/RSI HRF, as was the case for ethylene oxide (Meek et al., 2003), and "no" to the equivalent step in the IPCS HRF that asks the question, "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?", as was the case for 4-aminobiphenyl (Cohen et al., current document). In a recent paper, Preston & Williams (2005) presented a set of key events for tumour development that provided a guide for the use of the ILSI/RSI HRF with DNA-reactive carcinogens. This guide supported the view that for most DNA-reactive chemicals, the animal MOA would be predicted to be relevant to humans. However, it was also argued that there could be exceptions and that the ILSI/RSI HRF would be a valuable tool for identifying these. Use of the ILSI/RSI HRF and the IPCS HRF can also assist in quantifying differences in key events between rodents and humans that may be of value in extrapolating risk to humans. Not all rodent DNA-reactive carcinogens have been established to be human carcinogens, as judged by the International Agency for Research on Cancer (IARC) review process. For some of these exceptions, this human-rodent difference in tumour response is attributable to lower exposure of humans to the agent or to the relative insensitivity of epidemiological studies to detect tumour responses at low exposure levels. However, there are other reasons for such differences that are based on biological considerations. For example, if a DNA-reactive carcinogen induces tumours *only* in a species-specific organ, it is possible that the animal MOA based on key events might not be relevant to humans, although available data on MOA would need to be considered to permit such a conclusion. Similarly, the generally more proficient DNA repair processes that occur in humans compared with rodents (Cortopassi & Wang, 1996; Hanawalt, 2001) or a unique pathway of bioactivation in rodents could result in there being ves answers to the steps in the IPCS HRF that address the queries "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?" and/or "Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?" Alternatively, the IPCS HRF could provide quantitative information on these processes for use later in the risk characterization step.

#### フレームワークの普及

IPCS HRF の普及と適用を支援するために、一般的に受け入れられている MOA と有益な事例の データベースを構築し、維持する必要がある。これは、類似の MOAs で作用する可能性のある化 合物のフレームワーク解析を進めている人が参照できるよう一連の MOAs とそれに関連した key events で構成されている。事例研究は、実際の事例で構成されており、それらは発がん性反応を引 き起こす推定 MOA の受入れを支持するために必要な解析の詳細の関連度とエビデンスの重み付 けの性質を示すために、フレームワークを用いて解析されている。そのような事例研究は、新し い MOA を開発する初期段階では特に貴重であろう。

#### DNA 反応性発がん物質への IPCS HRF の適用

げっ歯類とヒトの発がんプロセスに類似性があり、DNA 反応性発がん物質による DNA との初 期相互作用が類似しているため、一般的に DNA 反応性発がん物質は、エチレンオキサイドの場合 (Meek ら、2003 年)のように ILSI/RSI HRF の「動物の MOA の key events はヒトで起こりうる」 という質問に対し「Yes」のステップに進むと評価され、IPCS HRF における上記質問に相当する 「実験動物とヒトとの間の key evants の根本的、質的な違いに基づいて、MOA のヒトとの関連性 を合理的に排除することができるか」の質問に対し 4-Aminobiphenyl の場合 (Cohen ら、本書) 「No」のステップへと進むと評価されると予想される。最近の論文では、Preston & Williams (2005 年)は、DNA 反応性発がん物質に対する ILSI/RSI HRF の使用の指針となる腫瘍発生の key events のセットを提示した。この指針は、ほとんどの DNA 反応性化学物質について、動物 MOA はヒト に関連すると予測されるという見解を支持した。しかし、例外がある可能性があり、ILSI/RSI HRF はこれらを特定するための貴重なツールになるとも指摘された。ILSI/RSI HRF と IPCS HRF を使 用することは、ヒトへのリスクを外挿する際に価値があるであろうげっ歯類とヒトの間の kev events の違いを定量化するのにも役立つ。国際がん研究機関(IARC)のレビュープロセスで判断 されたように、すべてのげっ歯類における DNA 反応性発がん物質がヒトの発がん物質であると 確立されているわけではない。これらの例外のいくつかについては、発がん性におけるこのヒト-げっ歯類間の違いは、ヒトがその化合物へのばく露量が少ないこと、または低ばく露レベルでの 発がん性を検出する疫学研究の相対的な感度の低さに起因している。しかしながら、このような 違いの理由は他にも生物学的考察に基づくものがある。例えば、DNA 反応性発がん物質が種特異 的な器官でのみ腫瘍を誘発する場合、MOA に関する利用可能なデータを考慮する必要があるが、 この key events に基づく動物 MOA はヒトには関連がない可能性もある。同様に、ヒトではげっ歯 類と比較して一般的に DNA 修復プロセスが発達している (Cortopassi & Wang, 1996 年; Hanawalt, 2001年)、あるいはげっ歯類に特有の生体内活性化の経路があることから、IPCS HRF における「実 験動物とヒトの間の key events の根本的、質的な違いに基づいて、MOA のヒトとの関連性は合理 的に排除できるか」及び/または「実験動物とヒトの間の動態的または薬力学的な要因のいずれ かの量的な違いに基づいて、MOAのヒトとの関連性は合理的に排除できるか」という問いに対し 「Yes」のステップへと進む可能性がある。あるいは、IPCS HRF はこれらのプロセスに関するそ の後のリスク判定の段階で利用可能な定量的な情報を提供しうる。

The need in applying the IPCS HRF for DNA-reactive carcinogens is to develop a set of key events that would clearly describe the cancer process and use these as the guide for establishing the human relevance of a rodent tumour MOA for any particular DNA-reactive carcinogen under consideration.

#### The IPCS HRF and risk assessment

Among the strengths of the framework are its flexibility, general applicability to carcinogens acting by any MOA, and the ability to explore the impact of each key event on the carcinogenic response. This includes determination of the nature of the dose–response curve, the identification and location of thresholds for individual key events, and their consequences for the overall tumour response curve. In addition, by considering the kinetic and dynamic factors involved in each key event, it may be possible to reach conclusions regarding the relevance or not of the carcinogenic response to specific subpopulations—for example, in early life, in those with particular diseases, or in those with specific polymorphisms. Alternatively, application of the framework can provide quantitative information on the differences between such groups. Application of the framework can also more generally inform the risk characterization of the chemical, even when it is concluded that the carcinogenic response per se is not relevant to humans.

As stated at the outset, MOA analysis and its human relevance counterpart are aspects of the hazard identification and characterization phases of risk assessment (National Research Council, 1983; Meek et al., 2003). Consistent with this paradigm, the human relevance case-studies referred to in the present report contribute to, but do not complete, a risk assessment for the chemicals under study. This is because a complete risk characterization requires not only evaluation of doses in the range of observations from experimental or occupational hygiene studies but also extrapolation to human exposure levels of interest in daily and lifetime activities.

Hazard characterization—and related MOA analysis—deals with the potential for harm in general terms, while the complete risk assessment puts this potential hazard into context with respect to exposure for decision-makers. Risk characterization seeks to describe the relationship between these effects and the doses to which humans are exposed in order to understand and estimate the nature and likelihood of effects in humans who are generally exposed at lower dose levels.

Understanding dose–response can have a profound effect on hazard characterization and therefore is an important component of the MOA analysis, particularly when non-linear processes or dose transitions are inherent in the relevant biology. Similarly, quantifying hazard in the context of dose informs the process of risk assessment by suggesting extrapolation models that are consistent with our understanding of the biology.

Estimating these generally lower human exposure levels is the task of the exposure analysis component of the risk assessment process. This usually involves extensive analysis of data collected from environmental media and plant and animal tissues, as well as those derived from pharmacokinetic models. This process also depends on analyses of human activity patterns and life stage and lifestyle factors that may bring about exposure. Ideally, based on

DNA 反応性発がん物質に IPCS HRF を適用する際に必要なことは、発がん過程を明確に説明する一連の key events を開発し、検討中の DNA 反応性発がん物質に対するげっ歯類における発がん MOA のヒトへの関連性を確立するための指針としてこれらを使用することである。

#### **IPCS HRF** 及びリスク評価

このフレームワークの強みは、柔軟性、あらゆる MOA によって作用する発がん物質への広範 な適用性及び発がん性反応における各 key event の影響を探索する能力といった点である。これに は、用量反応曲線の性質の決定、個々の key event の閾値の存在の特定やその値の特定及びそれら の腫瘍の反応曲線全体への影響の特定が含まれる。さらに、各 key event に関与する動態的及び薬 力学的な因子を考慮することで、特定の集団、例えば、幼若な人々、特定の疾患を持つ人々、ま たは特定の遺伝子多型を持つ人々に対する発がん性反応の関連性の有無に関する結論に到達する ことが可能になるかもしれない。あるいは、フレームワークを適用することで、そのようなグル ープ間の違いに関する定量的な情報を得ることができる。フレームワークの適用はまた、発がん 性反応自体がヒトには関連性がないと結論づけられた場合でも、化学物質のリスク評価に概ね有 益なものになりうる。

冒頭で述べたように、MOA 解析とヒトへの関連性の解析は、リスク評価におけるハザードの特定と特性評価の段階の一側面である(National Research Council、1983年; Meek ら、2003年)。このフレームワークに沿って、本文書で言及しているヒトへの関連性に関する事例研究は、完全なものではないが、研究対象の化学物質のリスク評価に貢献する。これは、完全なリスク評価を行うためには、実験的研究や職業衛生研究で設定された範囲の用量を評価するだけでなく、日常生活や生涯の活動における対象化学物質のヒトへのばく露レベルへの外挿を必要とするからである。

ハザードの特性評価及び関連する MOA 解析は、一般的な用語で危害の可能性を扱うのに対し、 完全なリスク評価は、意思決定者に対してこの潜在的なハザードをばく露に関する文脈で説明す るものである。リスク評価は、一般的に低用量レベルで被ばくしているヒトにおける影響の性質 と発生する可能性を理解し、推定するために、これらの影響とヒトへのばく露量との関係を記述 しようとするものである。

用量反応関係を理解することは、ハザードの特性評価に大きな影響を及ぼす可能性があるため、 MOA 解析の重要な要素であり、生物学的に非線形的なプロセスや種間における閾値の差が内在し ている場合には特に重要な要素である。同様に、用量に関連したハザードを定量化することは、 生物学的理解と整合性のある外部モデルを提示することで、リスク評価のプロセスに役立つ。

これらの一般的に低いヒトばく露レベルを推定することは、リスク評価におけるばく露解析の 課題である。これには通常、環境媒体、動植物組織、薬物動態モデルから得られたデータの広範 な解析が含まれる。このプロセスはまた、ヒトの活動パターン、ライフステージや生活様式とい ったばく露をもたらす可能性のある要因の解析にも依存する。

this information, a range of exposure scenarios is developed for different groups (men, women, children, infants, special groups, based, for example, on ethnicity or occupation) for use in identifying populations of concern. While hazard characterization, which is largely included in the framework analysis, involves quantification (dose–response analysis), estimating external exposures and contextualizing the hazard with respect to these estimates comprise subsequent steps in the risk assessment process. For example, in the case of melamine (Meek et al., 2003, case-study 7), it was concluded that the animal MOA was potentially relevant to humans. However, recognition that bladder carcinoma formation occurred only at very high doses carried forward to the subsequent stages of the risk assessment, exposure assessment, and risk characterization. The full risk assessment established that human exposures would not achieve levels necessary to produce bladder carcinomas, by a substantial margin.

#### CONCLUSIONS

This IPCS HRF has been developed based on experience gained from the original 2001 IPCS MOA Framework and consideration of the 2003 ILSI/RSI human cancer relevance framework. Many aspects of these frameworks have been adopted, but a number of changes have been made to improve clarity and to introduce some elements not previously considered (e.g. sensitive subpopulations). The utility and role of the framework as an analytical tool within hazard characterization and within the overall risk assessment/characterization paradigm—that is, informing human relevance and dose–response extrapolation—have been emphasized. A number of general points and conclusions follow from the development of this framework:

- 1. Prior to embarking on a framework analysis, there needs to be careful evaluation of the weight of evidence for a carcinogenic response in experimental animals.
- 2. Peer involvement and independent review are essential prerequisites for the general acceptance and scientific defensibility of a new MOA.
- 3. The framework is applicable to all MOAs for carcinogens, including DNA reactivity.
- 4. Although human relevance is likely to be assumed for most DNA-reactive carcinogens, the human relevance analysis is a valuable approach to enhance understanding, improve characterization of the hazard and risk, and identify exceptions.
- 5. When dealing with a chemical that may operate through a novel MOA, the analysis is focused on the chemical and entails a detailed evaluation via the HRF. However, when a specific chemical produces a tumour response consistent with an already established and peer-reviewed MOA through which other chemicals have been shown to operate, the analysis is then focused on the established MOA and a determination of whether the chemical produces its carcinogenic effect via the same key events established for the pathway.
- 6. When evaluating the human relevance of a tumour response found in experimental animals, the concordance analysis of key events is for the MOA and is not necessarily a chemical-specific evaluation. Chemical-specific and generic information relevant to the carcinogenic process can be valuable in the analysis. As knowledge advances, MOAs will become less chemical specific and will be based even more on the key biological

理想は、この情報に基づいて懸念される集団を特定するために、異なる集団(男性、女性、子供、 乳幼児、民族性や職業などに基づく特別な集団)を対象としたそれぞれのばく露量の範囲に関す るシナリオが作成されることである。その大部分がフレームワーク解析に含まれているハザード の特性評価には定量化(用量反応解析)が含まれているが、外部ばく露量の推定及びこれらの推 定ばく露量におけるハザードの説明は、リスク評価に含まれるその次のステップにおいて行われ る。例えば、メラミンの場合(事例研究 7、Meek ら、2003 年)では、動物の MOA はヒトとの関 連性が高いと結論づけられた。しかし、膀胱がんの形成は非常に高用量でしか起こらないという 認識により、リスク評価、ばく露評価、リスク判定の次の段階へと解析が進められた。リスク評 価全体として、メラミンのヒトへのばく露は膀胱がんの発生に必要なレベルを大きく下回るとい う評価が下された。

#### 結論

この IPCS HRF は、2001 年の IPCS MOA フレームワークから得られた経験と 2003 年の ILSI/RSI ヒト発がん性関連フレームワークの検討に基づいて作成された。これらのフレームワークにおけ る概念の多くが採用されているが、明確性を向上させ、これまで考慮されていなかった要素(例 えば、感受性の高い集団)を導入するために、多くの変更が加えられている。ハザードの特性評 価及び全体的なリスク評価/判定のフレームワークにおける解析ツールとしてのフレームワークの 有用性と役割、すなわちヒトへの関連性と用量反応性の外挿に役立つことが強調されている。こ のフレームワークの開発から得られた、多くの要点と結論は以下のとおりである。

- 1. フレームワーク解析に着手する前に、実験動物における発がん性反応のエビデンスの重み付け を慎重に評価する必要がある。
- 専門家の関与及び第三者によるレビューは、新しい MOA が一般に受け入れられるため、また、 科学的な正当性を得るための必須条件である。
- 3. フレームワークは、DNA 反応性を含むすべての発がん MOA に適用できる。
- 4. ほとんどの DNA 反応性発がん物質についてはヒトへの関連性が想定されると思われるが、ヒト関連性解析は、理解を深め、ハザードとリスクの特性評価/判定を改善し、例外を特定するための重要なアプローチである。
- 5. 新規の MOA を介して作用する可能性のある化学物質を扱う場合、解析はその化学物質に焦点を当て、HRF を介して詳細な評価を行うことが必要となる。しかし、ある化学物質が他の化学物質において既に確立され、専門家のピアレビューを経た MOA と一致した腫瘍反応をもたらす場合、解析は確立されている MOA と、その化学物質がその MOA の一連の流れにおいて確立されている key events を介して発がん作用を生み出すか否かに焦点を当てて行われる。
- 6. 実験動物で発見された腫瘍反応のヒトへの関連性を評価する場合、key eventsの一致解析は MOA に対するものであり、必ずしも化学物質に焦点を当てた評価ではない。発がんプロセス に関連する化学物質特有の情報や化学物質一般の情報は、解析において貴重なものとなり得る。 知識の進歩に伴い、MOA は化学物質特有のものではなくなり、関連する重要な生物学的プロ セスにより一層依拠することとなり、それにより、ある化合物におけるヒトとの関連性を別の 化合物へと一般化することが可能になるであろう。

processes involved, allowing greater generalization of human relevance from one compound to another.

- 7. The biological understanding and significance of the key events can inform the approach to dose–response extrapolation for cancer risk, and thus understanding of the MOA can have a profound effect on the hazard and risk characterization, particularly when nonlinear processes or dose transitions are inherent in the relevant biology.
- 8. It is recommended that a database of generally accepted MOAs and informative casestudies be established and maintained. It should provide examples that add to the existing case-studies developed by ILSI/RSI and IPCS and that are instructive in the application of the framework analysis. This database is particularly important as experience continues to evolve in the development of MOAs of carcinogens.
- 9. It is important to consider potentially susceptible subgroups and different life stages in the analysis.

In conclusion, the IPCS HRF provides a rigorous and transparent approach for judging whether data support a postulated mode of carcinogenic action for a chemical and for evaluating its relevance for humans. The scientific community is encouraged to use this approach as a means to increase the use of mechanistic information in cancer risk assessment and is encouraged to provide feedback, which may lead to additional refinements in the future. The framework is of value to both the risk assessment and research communities in furthering our understanding of carcinogenic processes, in identifying critical data gaps, and in informing the design of studies related to MOAs. When a carcinogenic response is considered potentially relevant to humans, information obtained on the key events during the analysis can prove invaluable in subsequent hazard quantification of the compound. It should be possible to extend the framework to non-cancer end-points, and further work on this is recommended. Thus, application of the IPCS HRF would be an invaluable tool for harmonization across end-points.

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#### REFERENCES

Cohen M, Meek ME, Klaunig JE, Patton DE, Fenner-Crisp PA (2003) The human relevance of information on carcinogenic modes of action: An overview. *Critical Reviews in Toxicology*, **33**:581–589.

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- 7. Key eventsの生物学的理解と生体における重要性は、発がんリスクの用量反応性の外挿性に関する解解析に情報を提供することができる。そのため MOA の理解はハザードとリスクの判定に大きな影響を与えうる。特に非線形性のプロセスや種間の閾値の差が、関連する生物学的性質に内在する場合に顕著である。
- 8. 一般的に受け入れられている MOA と有益な事例研究のデータベースを構築し、維持することが推奨される。このデータベースは、ILSI/RSI 及び IPCS によって行われた既存の事例研究に加え、フレームワーク解析の適用において有益な事例を提供するものでなければならない。このデータベースは、発がん物質の MOA の研究において知見が蓄積し続ける中で特に重要である。
- 解析において、潜在的に感受性の高い集団やさまざまなライフステージを考慮することが重要である。

結論として、IPCS HRF は、推定される化学物質の発がん MOA をデータが裏付けるかどうかを 判断し、ヒトへの関連性を評価するための厳格で透明性のあるアプローチを提供している。科学 界において発がんリスク評価における作用機序に関する情報の利用を増やす手段として、このア プローチを利用し、フィードバックを行うことが奨励されており、このことが将来的な更なる改 良につながるだろう。このフレームワークは、発がんプロセスの理解を深め、重要なデータギャ ップを特定し、MOA に関連した研究の設計に有益な情報をもたらすという点で、リスク評価と研 究の両方のコミュニティにとって価値がある。発がん反応がヒトに関連する可能性があると考え られる場合、解析中に key events について得られた情報は、その後の化合物のハザードの定量化 において極めて貴重なものとなりうる。このフレームワークは発がん以外のエンドポイントにま で拡張できるようにするべきであり、このことに関する更なる研究が推奨される。したがって、 IPCS HRF の適用は、エンドポイント間の調和のための貴重なツールとなるであろう。

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#### 参考文献

Cohen M, Meek ME, Klaunig JE, Patton DE, Fenner-Crisp PA (2003) The human relevance of information on carcinogenic modes of action: An overview. Critical Reviews in Toxicology, 33:581–589.

#### Harmonization Project Document No. 4

Committee on Carcinogenicity (2004) *Guidance on a strategy for the risk assessment of chemical carcinogens.* London, Department of Health.

Cortopassi GA, Wang E (1996) There is substantial agreement among interspecies estimates of DNA repair activity. *Mechanisms of Ageing and Development*, **91**:211–218.

Hanawalt PC (2001) Revisiting the rodent repairadox. *Environmental and Molecular Mutagenesis*, **38**:89–96.

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP (2006) Mode of action in relevance of rodent livers to human cancer risk. *Toxicological Sciences*, **89**:51–56.

IPCS (2000) Scoping meeting to address the human relevance of animal modes of action in assessing cancer risk, Carshalton, United Kingdom, 8–10 November 2000. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2004) Report of the first meeting of the Cancer Working Group, Arlington, Virginia, USA, 3–5 March 2004. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2005) *Record of the Cancer Framework Workshop, Bradford, United Kingdom, 21–23 April 2005.* Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

National Research Council (1983) *Risk assessment in the federal government. Managing the process.* Washington, DC, National Academy Press.

Preston JR, Williams GM (2005) DNA-reactive carcinogens: Mode of action and human cancer hazard. *Critical Reviews in Toxicology*, **35**:673–683.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity: Case studies. *Toxicology and Applied Pharmacology*, **20**:226–294.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

#### Harmonization Project Document No. 4

Committee on Carcinogenicity (2004) Guidance on a strategy for the risk assessment of chemical carcinogens. London, Department of Health.

Cortopassi GA, Wang E (1996) There is substantial agreement among interspecies estimates of DNArepair activity. Mechanisms of Ageing and Development, 91:211–218.

Hanawalt PC (2001) Revisiting the rodent repairadox. Environmental and Molecular Mutagenesis, 38:89–96.

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP (2006) Mode of action in relevance of rodent livers to human cancer risk. Toxicological Sciences, 89:51–56.

 IPCS (2000) Scoping meeting to address the human relevance of animal modes of action in assessing cancer risk, Carshalton, United Kingdom, 8–10 November 2000. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2004) Report of the first meeting of the Cancer Working Group, Arlington, Virginia, USA, 3– 5 March 2004. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

IPCS (2005) Record of the Cancer Framework Workshop, Bradford, United Kingdom, 21–23 April 2005. Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/cancer/en/index.html).

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. Critical Reviews in Toxicology, 33:591–653.

National Research Council (1983) Risk assessment in the federal government. Managing the process. Washington, DC, National Academy Press.

Preston JR, Williams GM (2005) DNA-reactive carcinogens: Mode of action and human cancer hazard. Critical Reviews in Toxicology, 35:673–683.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity: Case studies. Toxicology and Applied Pharmacology, 20:226–294.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regulatory Toxicology and Pharmacology, 34:146–152.

USEPA (1999) *Guidelines for carcinogen risk assessment (review draft)*. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (NCEA-F-0644).

USEPA (2005) *Guidelines for carcinogen risk assessment*. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (EPA/639/P-03/001F).

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

USEPA (1999) Guidelines for carcinogen risk assessment (review draft). Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (NCEA-F-0644).

USEPA (2005) Guidelines for carcinogen risk assessment. Washington, DC, United States Environmental Protection Agency, Risk Assessment Forum (EPA/639/P-03/001F).

## THIAZOPYR AND THYROID DISRUPTION: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Vicki L. Dellarco, Douglas McGregor, Sir Colin Berry, Samuel M. Cohen, & Alan R. Boobis

Thiazopyr increases the incidence of male rat thyroid follicular cell tumours; however, it is not carcinogenic in mice. Thiazopyr is not genotoxic. Thiazopyr exerts its carcinogenic effect on the rat thyroid gland secondary to enhanced metabolism of thyroxine leading to hormone imbalance. The relevance of these rat tumours to human health was assessed by using the 2006 International Programme on Chemical Safety Human Relevance Framework. The postulated rodent tumour mode of action (MOA) was tested against the Bradford Hill criteria and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well established MOA for thyroid follicular cell tumours. Although the postulated MOA could theoretically operate in humans, marked quantitative differences in the inherent susceptibility for neoplasia to thyroid hormone imbalance in rats allows for the conclusion that thiazopyr does not pose a carcinogenic hazard to humans.

A number of chemical substances have been shown to induce thyroid follicular cell tumours in rats through a mode of action (MOA) that involves perturbation of thyroid hormone homeostasis via reduction of circulating thyroid hormones (Hurley et al., 1998; Capen et al., 1999; IARC, 2001). Homeostatic responses to low thyroid hormone concentrations result in a compensatory increase in the release of thyroid stimulating hormone (TSH) from the pituitary gland, which in turn stimulates the thyroid gland to increase thyroid hormone synthesis and release. Persistent elevation of TSH levels leads to thyroid follicular cell hypertrophy and hyperplasia, which, if maintained (as a result of continuous exposure to the compound), can eventually lead to neoplasia. This neoplastic MOA in rats is well accepted by the scientific community, and both the International Agency for Research on Cancer (Capen et al., 1999; IARC, 2001) and the United States Environmental Protection Agency (USEPA, 1998) have established specific guidance or policies for evaluating the human relevance of rodent thyroid follicular cell tumours.

Thiazopyr, a herbicide that induces rat thyroid follicular cell tumours by its effect on thyroid homeostasis, was the case-study used to illustrate the original 2001 International Programme on Chemical Safety (IPCS) framework for mode of carcinogenic action analysis (Sonich-Mullin et al., 2001). Thiazopyr's MOA is revisited as a case-study here to illustrate the additional guidance provided in the 2006 IPCS Human Relevance Framework (HRF) for evaluation of a neoplastic MOA for humans. This updated case-study highlights how accumulating experience with a particular MOA can make subsequent analyses less difficult. Because this case-study is based on an established MOA in which the key events have been well defined, this analysis will focus on whether thiazopyr produces the biological effects

チアゾピルと甲状腺障害:

発がん MOA のヒトへの関連性を解析するための

## IPCS フレームワークを用いた事例研究2

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チアゾビルは雄ラットの甲状腺濾胞細胞腫瘍の発生率を増加させるが、マウスに対して発がん性 はないとされている。チアゾビルに遺伝毒性はない。チアゾビルは、チロキシンの代謝亢進により ホルモンバランスが崩れることにより二次的にラット甲状腺に発がん作用を及ぼす。これらのラ ットにおける腫瘍とヒトの健康への関連性は、2006年の国際化学物質安全性計画ヒト関連性フレ ームワーク(IPCS HRF)を用いて評価された。推定されたげっ歯類における発がん MOA を Bradford Hill 基準に照らして検証したところ、用量と時間の一致、生物学的妥当性、整合性、強度、一貫性、 特異性の条件を満たしており、甲状腺濾胞細胞腫瘍について確立されている MOA に適合している ことがわかった。推定された MOA はヒトでも理論的には作用する可能性はあるが、甲状腺ホルモ ンの不均衡に対するラットの発がん性の感受性がヒトとは量的に顕著に異なることから、チアゾ ビルはヒトに対する発がんに関するハザードを有さないという結論が導き出された。

多くの化学物質が、循環甲状腺ホルモンの減少を介した甲状腺ホルモンのホメオスタシスのか く乱を伴う作用機序(MOA)を介してラットの甲状腺濾胞細胞腫瘍を誘発することが示されてい る(Hurleyら、1998年; Capenら、1999年; IARC、2001年)。甲状腺ホルモンの低下に対しホメ オスタシスを保とうとする反応は、下垂体からの甲状腺刺激ホルモン(TSH)の放出の代償的な増 加をもたらし、その結果、甲状腺を刺激して甲状腺ホルモンの合成及び放出を増加させる。TSH レベルの持続的な上昇は、甲状腺濾胞細胞の肥大及び過形成へとつながり、(化合物への持続的な ばく露の結果)高いTSHレベルが維持された場合、最終的には腫瘍形成へとつながる。ラットに おけるこの腫瘍形成 MOA は、科学界では十分に受け入れられており、国際がん研究機関(Capen ら、1999年; IARC、2001年)及び米国環境保護庁(USEPA、1998年)は、げっ歯類における甲 状腺濾胞細胞腫瘍のヒトへの関連性を評価するための独自のガイダンスまたは方針を確立してい る。

除草剤であるチアゾビルは、甲状腺ホルモンのホメオスタシスへ影響することによりラット甲 状腺濾胞細胞腫瘍を誘発するが、これは発がん MOA 解析のための 2001 年の国際化学物質安全性 計画(International Programme on Chemical Safety: IPCS)のオリジナルフレームワークを示すため に用いられた事例である(Sonich-Mullin ら、2001 年)。ここではチアゾビルの MOA を、事例研究 としてここで再検討し、2006 年の IPCS ヒト関連性フレームワーク(HRF)で提供されたヒトの 腫瘍形成 MOA 評価のための追加ガイダンスを説明した。この更新された事例研究では、ある特 定の MOA での経験の蓄積が、その後の解析の難易度をどの程度下げられるのかについて強調し ている。この事例研究は、key events が十分に定義されている確立された MOA に基づいているた め、この解析では、チアゾビルがこの経路で期待されている生物学的効果をもたらすか否かに焦

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expected of this pathway. This case-study also emphasizes the importance of understanding the basic physiological processes underlying a toxicity pathway in animals and humans. For some compounds, chemical-specific data might be critical in evaluating the key events in humans. For others, the underlying biology is sufficient to allow interpretation of the human relevance of the carcinogenic MOA, both qualitatively and quantitatively. Thiazopyr is an example of the latter. Another MOA case-study of thyroid hormone disruption and the human relevance of rat thyroid follicular cell tumours is available for phenobarbital (Lehman-McKeeman & Hill, in Meek et al., 2003).

The present MOA analysis begins with a brief summary of the available information on the carcinogenicity of thiazopyr, followed by a discussion of the experimental biochemical and histopathological data considered for this thyroid disruption MOA. It is not intended to be a comprehensive assessment of the chemical per se.

## CARCINOGENICITY DATA

Human epidemiological data on the carcinogenicity of thiazopyr are not available. Thiazopyr produces effects on liver and thyroid in various laboratory species, including mice, rats, and dogs. Thiazopyr was found to induce thyroid tumours in male rats only and appears to do so by increasing the hepatic metabolism and excretion of thyroid hormones.

Chronic dietary administration of thiazopyr to mice and rats resulted primarily in thyroid follicular cell tumours in male rats but not in female rats (Naylor & McDonald, 1992; Naylor & Raju, 1992). There were no significant increases in the incidences of any tumours in either sex in the chronic study of mice treated with thiazopyr at up to 800 mg/kg in the diet (128.4 mg/kg body weight [bw] per day in males and 215.9 mg/kg bw per day in females) (Naylor & Raju, 1992). In the rat carcinogenicity study, thiazopyr (technical, 94.8% pure) was administered to male and female Sprague-Dawley (SD) rats (60 per sex per group) at dietary concentrations of 0, 1, 10, 100, 1000, or 3000 mg/kg, providing dose levels of 0, 0.04, 0.4, 4.4, 44.2, or 136.4 mg/kg bw per day for males and 0, 0.06, 0.6, 5.6, 56.3, or 177.1 mg/kg bw per day for females (Naylor & McDonald, 1992). The incidences of thyroid follicular cell adenomas and carcinomas were increased in male rats of the 1000 mg/kg (44.2 mg/kg bw per day) and 3000 mg/kg to male rats is primarily accounted for by benign tumours.

# POSTULATED MOA FOR THE INDUCTION OF THYROID FOLLICULAR CELL TUMOURS IN RATS

The postulated MOA for thiazopyr-induced thyroid follicular cell tumours involves the perturbation of homeostasis of the pituitary-thyroid axis by an extrathyroidal mechanism. Specifically, thiazopyr induces hepatic thyroxine (T4)-uridine diphosphate (UDP) glucurono-syltransferase (UGT) activity, leading to enhanced metabolism of T4 by conjugation and increased biliary excretion of the conjugated hormone. The result of this enhanced liver metabolism is a decrease in serum T4 (and sometimes triiodothyronine, or T3) half-life. The pituitary gland responds to a decrease in circulating serum levels of T4 by enhancing the output and serum level of TSH. Prolonged elevation of circulating TSH levels stimulates the

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点を当てている。この事例研究はまた、動物及びヒトにおける毒性発現の経路の基礎となる生理 学的プロセスを理解することの重要性を強調している。いくつかの化合物では、化学物質特有の データがヒトでの key events を評価する上で重要な場合があるかもしれないが、その他の化合物 については、基礎となる生物学的知見があれば、発がん MOA のヒトへの関連性を定性的にも定 量的にも解釈できる場合がある。チアゾピルは後者の例である。甲状腺ホルモンのかく乱とラッ ト甲状腺濾胞細胞腫瘍のヒトへの関連性に関する他の MOA 事例研究としてフェノバルビタール についての事例研究がある(Lehman-McKeeman & Hill, in Meek ら、2003 年)。

本 MOA 解析は、チアゾピルの発がん性に関する利用可能な情報の簡単な要約から始まり、続いて、この甲状腺ホルモンかく乱 MOA で考察された生化学的及び病理組織学的な実験データの 議論が行われる。本解析は化学物質そのものの包括的な評価を意図したものではない。

## 発がん性データ

チアゾビルの発がん性に関するヒトの疫学的データは得られていない。チアゾビルは、マウス、 ラット及びイヌを含む様々な実験動物種において、肝臓及び甲状腺に影響を及ぼす。チアゾビル は雄ラットにおいてのみ甲状腺腫瘍を誘発することが確認されており、甲状腺ホルモンの肝代謝 及び排泄を増加させることによってこれを引き起こすようである。

チアゾビルはマウス及びラットへの慢性的な混餌投与により、主に雄ラットに甲状腺濾胞細胞 腫瘍を誘発したが、雌ラットでは誘発しなかった(Naylor & McDonald、1992 年; Naylor & Raju、 1992 年)。チアゾビルを最大 800 mg/kg まで混餌投与したマウスの慢性試験(雄では 1 日あたり 128.4 mg/kg 体重[bw]、雌では 1 日あたり 215.9 mg/kg bw)では、いずれの性においても腫瘍の発 生率に有意な増加は認められなかった(Naylor & Raju、1992 年)。ラット発がん性試験では、チア ゾビル(原体、純度 94.8%)を雄及び雌の Sprague-Dawley ラット(SD ラット)(各群雌雄 60 匹ず つ)に 0、1、10、100、1000 または 3000 mg/kg の濃度で混餌投与し、雄では 1 日あたり 0、0.04、 0.4、4.4、44.2、136.4 mg/kg bw、雌では 1 日あたり 0、0.06、0.6、5.6、56.3 または 177.1 mg/kg bw の用量を与えた(Naylor & McDonald、1992)。甲状腺濾胞細胞腺腫及びがんの発生率は、1000 mg/kg (1 日あたり 44.2 mg/kg bw)及び 3000 mg/kg(1 日あたり 136.4 mg/kg bw)投与群の雄ラットで増 加した(表 1)。なお、雄ラットにおける腫瘍発生率の増加は主に良性腫瘍によるものであったこ とに留意すべきである。

## ラットにおける甲状腺濾胞細胞腫瘍の誘発において推定される MOA

チアゾビル誘発性甲状腺濾胞細胞腫瘍において推定される MOA は、甲状腺外における機序に よる下垂体-甲状腺軸のホメオスタシスの乱れを含むものであった。具体的な流れを以下に述べる。 チアゾビルは肝臓におけるチロキシン(T4)-ウリジン二リン酸(UDP)グルクロン酸転移酵素(UGT) 活性を誘導し、抱合による T4 の代謝を促進し、ホルモンの抱合体の胆汁排泄を増加させる。この 肝臓代謝の促進の結果として、血清 T4 及び時にはトリヨードサイロニン(T3)の半減期が減少す る。下垂体は、TSH の放出及び血清レベルを高めることにより、T4 の循環血清レベルの低下に対 応する。循環 TSH レベルの長期的な上昇は、甲状腺を刺激して甲状腺ホルモンの貯蔵を枯渇させ、 ホルモン産生を誘導し続ける。 thyroid gland to deplete its stores of thyroid hormone and continues to induce hormone production. Thus, the thyroid follicular cells enlarge (hypertrophy) and are induced to proliferate at an increased rate and to increase in number (hyperplasia). With chronic exposure, thyroid hyperplasia eventually progresses to neoplasia.

## Table 1. Thyroid follicular cell tumour incidence in Sprague-Dawley male rats (2-year chronic study).

		Dose (mg/kg bw per day) <sup>a</sup>				
	0	0.04	0.4	4.4	44.2	136.4 <sup>b</sup>
Adenomas	1/50	2/47	0/49	2/47	8/49	12/48
Carcinomas	1/50	1/47	0/49	0/47	1/49	4/48
Combined	2/50	3/47	0/49	2/47	9/49	14/48
%	(2)	(6)	(0)	(4)	(18)	(29)
Р	0.000 <sup>c</sup>	0.470	0.253	0.668	0.024*	0.001**

Note: Tumour incidences were extracted from data submitted to the USEPA Office of Pesticide Programs (Naylor & McDonald, 1992). Significance: \* P < 0.05; \*\* P < 0.01 (statistical analyses based on Fisher's exact test).

<sup>a</sup> Doses in mg/kg bw per day were estimated.

<sup>b</sup> Two animals in the 136.4 mg/kg bw per day or 3000 mg/kg diet dose group had both benign and malignant tumours.

<sup>c</sup> For trend with dose.

### **KEY EVENTS IN EXPERIMENTAL ANIMALS**

The sequence of key events in thiazopyr's mode of carcinogenic action includes:

- induction of hepatic UGT activity;
- increase in hepatic metabolism and biliary excretion of T4;
- decrease in serum T4 half-life and concentration;
- increase in circulating TSH concentration;
- cellular thyroid hypertrophy and follicular cell hyperplasia.

An evaluation follows to determine whether thiazopyr works via disruption of thyroid– pituitary status by increasing hepatic clearance of circulating thyroid hormone. Thus, based on the key events listed above, biological indicators of thiazopyr's MOA should include changes in liver metabolism, alterations in hormone levels, increases in thyroid growth, and lesion progression in the thyroid. These effects have been observed and measured in male rats in short-term and subchronic studies, and at interim and terminal sacrifices in a chronic study (Hotz et al., 1997). The dose–response and temporal analyses of the key events and tumour response are presented below.

#### **DOSE-RESPONSE RELATIONSHIP AND CONCORDANCE**

A summary of the no-observed-adverse-effect levels (NOAELs) and lowest-observedadverse-effect levels (LOAELs) for the key effects in thiazopyr's MOA are provided in Table 2. In the 56-day study by Hotz et al. (1997), male SD rats (20 per dose) were fed diets containing thiazopyr at 0, 10, 30, 100, 300, 1000, or 3000 mg/kg (doses not measured, but その結果、甲状腺濾胞細胞は大きくなり(肥大)、高い増殖率で細胞数を増やす(過形成)ように 誘導される。慢性的なチアゾビルのばく露により、甲状腺過形成は最終的には腫瘍へと進行する。

## 表 1. Sprague-Dawley 雄ラットにおける甲状腺濾胞細胞腫瘍の発生率(2年間の慢性試験)

		Dose (mg/kg bw per day) <sup>a</sup>				
	0	0.04	0.4	4.4	44.2	136.4 <sup>b</sup>
腺腫	1/50	2/47	0/49	2/47	8/49	12/48
がん腫	1/50	1/47	0/49	0/47	1/49	4/48
合計	2/50	3/47	0/49	2/47	9/49	14/48
%	(2)	(6)	(0)	(4)	(18)	(29)
Р	$0.000^{c}$	0.470	0.253	0.668	0.024*	0.001**

注: 腫瘍の発生率は、USEPA Office of Pesticide Programs (Naylor & McDonald, 1992 年) に提出されたデータから 抽出した: 有意差 \* P < 0.05; \*\* P < 0.01 (フィッシャーの正確確率検定に基づく統計解析)

\*1日あたりの投与量をmg/kg bw で推定した。

b 136.4mg/kg bw/日(3000mg/kg) 混餌投与群の2匹の動物には、良性腫瘍と悪性腫瘍の両方が認められた。
 \* 投与量に従ってP値が低下した。

#### 実験動物における key events

チアゾピルの発がん MOA における一連の key events には、以下のものが含まれる。

- T4の肝臓での代謝及び胆汁排泄の増加
- 血清 T4 の半減期及び濃度の低下
- 循環 TSH 濃度の上昇
- 甲状腺濾胞細胞肥大及び濾胞細胞過形成

次に、チアゾビルが、循環甲状腺ホルモンの肝クリアランスを増加させて甲状腺-下垂体軸を乱 すことによって作用するかどうかを評価する。上記の key events に基づき、チアゾビルの MOA の 生物学的指標は、肝臓代謝の変化、ホルモンレベルの変化、甲状腺の成長の増加及び甲状腺の病 変の進行が挙げられることになる。これらの影響は、短期及び亜慢性試験さらに慢性試験におけ る中間及び計画殺において、雄ラットで観察され、測定されている(Hotz ら、1997 年)。Key events 及び腫瘍反応の用量反応性を以下に示す。

## 用量反応関係及び一致性

チアゾビルの MOA における主要な影響について、無毒性量(NOAEL)及び最小毒性量(LOAEL) の概要を表2に示す。Hotz ら (1997)による56日間の試験では、雄SD ラット(各群20匹)に、 チアゾビルを0、10、30、100、300、1000、または3000 mg/kgの濃度で(測定されていないが、 チアゾビルの摂取量は1日あたり0、0.5、1.5、5、50及び150 mg/kg bwと推定される)を56 日間混餌投与し、肝臓(重量、肝T4-UGT活性、T4胆汁排泄)、甲状腺(重量、肥大/過形成)及 びホルモン(T4、T3、リバースT3(rT3)及びTSHの血清レベル)に対する影響を評価した。こ の試験では、チアゾビルの主要作用部位である肝臓への影響が、下垂体-甲状腺のホメオスタシス のかく乱の最も敏感な指標であると考えられる。50 mg/kg bw/day 投与群及び150 mg/kg bw/day 投

estimated to be 0, 0.5, 1.5, 5, 15, 50, and 150 mg/kg bw per day) for 56 days and evaluated for the effects on liver (weights, T4-hepatic UGT activity, T4 biliary elimination), thyroid (weights, hypertrophy/hyperplasia), and hormones (serum levels of T4, T3, reverse T3, or rT3, and TSH). In this study, the effects on liver, thiazopyr's primary site of action, appear to be the most sensitive indicator of pituitary-thyroid homeostasis perturbation. Statistically significant increases in hepatic T4-UGT activity in the 50 and 150 mg/kg bw per day groups (approximately 3- and 6-fold increases in activity over controls when adjusted for liver weight, respectively) were found at the end of the 56-day treatment period. Consistent with the increase in T4-UGT activity, clearance of T4 from the blood and elimination in bile (40% increase in excretion of <sup>125</sup>I-labelled T4) were increased after 150 mg/kg bw per day of thiazopyr (only dose evaluated). Statistically significant increases in liver weight were found at 15, 50, and 150 mg/kg bw per day of thiazopyr in the 56-day study in male rats by Hotz et al. (1997). In the 2-year rat study (Naylor & McDonald, 1992), absolute liver weights were increased by 122% at 44.2 mg/kg bw per day and by 178% at 136.4 mg/kg bw per day relative to controls. There were also statistically significant increases in the incidence of liver hypertrophy at 44.2 and 136.4 mg/kg bw per day (47/61 and 52/60 versus 0/60 in controls, respectively) in the 2-year rat study.

## Table 2. Summary of effects on liver, hormones, and thyroid from a 56-day study (Hotz et al., 1997) and the 2-year chronic study (Naylor & McDonald, 1992) in male rats.

Effect	NOAEL/LOAEL
Liver	
Induction of UGT	15/50 mg/kg bw per day (56-day study)
Increase in T4 biliary elimination	<150/150 mg/kg bw per day (only dose tested in 56-day study)
Increase in liver weight	5/15 mg/kg bw per day (56-day study)
	44.2/136.4 mg/kg bw per day (2-year study)
Hepatocellular hypertrophy	4.4/44.2 mg/kg bw per day (2-year study)
Hormones	
Decrease in serum T4	50/150 mg/kg bw per day (56-day study)
Increase in serum TSH	50/150 mg/kg bw per day (56-day study
Thyroid	
Increase in thyroid weight	15/50 mg/kg bw per day (56-day study)
	44.2/136.4 mg/kg bw per day (2-year study)
Increase in thyroid hyperplasia	44.2/136.4 mg/kg bw per day (2-year study)
Increase in thyroid tumours	4.4/44.2 mg/kg bw per day (2-year study)

Consistent with the enhanced hepatic clearance of T4 described above, when Hotz et al. (1997) treated male SD rats with doses of thiazopyr, statistically significant ( $P \le 0.05$ ) decreases in serum T4 levels (by 30%) and increases in TSH (by 60%) were found after 56 days of treatment at the highest dose tested (Table 3). T3 serum levels were non-significantly lower at 1.5 mg/kg bw per day and statistically significantly higher at 150 mg/kg bw per day after 56 days of treatment. In general, hepatic microsomal enzyme inducers appear to affect T3 less than T4; thus, T4 and TSH tend to be more reliable indicators of altered pituitary–

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与群では、肝 T4-UGT 活性が 56 日間の投与期間終了時に統計学的に有意に増加した(肝重量で標準化したところ、対照群と比較してそれぞれ約3倍と6倍に活性が増加した)。T4-UGT 活性の増加と一致して、血中からのT4のクリアランス及び胆汁中への排泄(125I標識T4の排泄量)は、 チアゾピルを150 mg/kg bw/day 投与後に40%増加した(150 mg/kg bw/day が評価された唯一の投 与量)。Hotz ら(1997年)による雄ラットを対象とした 56 日間の試験では、チアゾピルを1日あ たり15、50 及び150 mg/kg bw 投与した場合に、肝重量の統計学的に有意な増加が認められた。 ラット2年間の試験(Naylor & McDonald、1992年)では、絶対肝重量は対照群と比較して、44.2 mg/kg bw/day 投与群で122%、136.4 mg/kg bw/day 投与群で178%増加した。また、このラットの2 年間試験では、44.2 及び136.4 mg/kg bw/day 投与群で肝肥大の発生率が統計的に有意に増加した (対照群の0/60に対してそれぞれ 47/61 及び 52/60)。

## 表 2. 雄ラットを対象とした 56 日間の試験(Hotz ら、1997)及び 2 年間の慢性試験(Naylor & McDonald、1992)から得られた肝臓、ホルモン及び甲状腺に対する影響の概要

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	影響	NOAEL/LOAEL
	肝臓	
	UGT の誘導	15/50 mg/kg bw/day (56 日間試験)
	T4 の胆汁排泄量の増加	<150/150 mg/kg bw/day (56 日間試験において 150 mg/kg bw/day のみ
	身	厚施された)
	肝臓重量の増加	5/15 mg/kg bw/day (56 日間試験)
		44.2/136.4 mg/kg bw/day (2 年間試験)
	肝細胞肥大	4.4/44.2 mg/kg bw/day (2 年間試験)
	ホルモン	
	血清 T4 の減少	50/150 mg/kg bw/day (56 日間試驗)
	血清 TSH の上昇	50/150 mg/kg bw/day (56 日間試験)
	甲状腺	
	甲状腺重量の増加	15/50 mg/kg bw/day (56 日間試験)
		44.2/136.4 mg/kg bw/day (2 年間試験)
	甲状腺過形成の増加	44.2/136.4 mg/kg bw/day (2 年間試験)
	甲状腺腫瘍の増加	4.4/44.2 mg/kg bw/day (2 年間試驗)

Hotz ら (1997) が雄 SD ラットにチアゾビルを投与したとき、上述した T4 の肝クリアランスの 亢進と同様に、最高用量の投与 56 日後に血清 T4 濃度の統計学的に有意な (P $\leq$ 0.05) 減少 (30%減 少) と TSH の増加 (60%増加) が認められた (表 3)。T3 の血清レベルは、1.5 mg/kg bw/day では 56 日投与後に統計学的に有意ではないものの低下し、150 mg/kg bw/day では有意に上昇した。一 般に、肝ミクロソーム酵素誘導剤による影響は T4 よりも T3 に影響を与えにくいようである。し たがって、T4 及び TSH は、下垂体-甲状腺のホメオスタシスの変化の指標としてはより信頼性の 高い傾向がある (Liu ら、1995 年、Hurley ら、1998 年、Hood ら、1999 年)。

thyroid homeostasis (Liu et al., 1995; Hurley et al., 1998; Hood et al., 1999). In the case of thiazopyr, there appears to be a poor correlation between the doses causing the T4 and TSH effects and those causing an increased incidence of thyroid follicular cell tumours. The lowest dose of thiazopyr producing a statistically significant (P < 0.05) increase in thyroid follicular cell tumours in male SD rats was 44.2 mg/kg bw per day in the 2-year study, whereas the NOAEL for effects on T4 and TSH was 50 mg/kg bw per day in the 56-day study (Table 2). Generally, effects on liver enzymes/weight and pituitary-thyroid hormone concentrations would be anticipated to occur at doses at least as low as those that produce thyroid weight changes and increases in thyroid tumour incidence, given that this thyroid disruption MOA is a threshold phenomenon. This apparent discrepancy is probably not real, because neither of the doses quoted is accurate. In the 2-year study, the milligrams per kilogram body weight doses were averaged estimates for the entire study, whereas the relevant doses for comparison with the 56-day mechanistic study are those for rats of 12–20 weeks of age. These doses would have been at least 2-fold higher than those that were readily available (so the real LOAEL for neoplasia would have been about 90 mg/kg bw per day). They would also have been more relevant for neoplasia, because the critical period for hormonal perturbations (e.g. prolonged elevation of TSH) to initiate pathological changes would be early, not late, in the 2-year study. The doses calculated for the 56-day study are also likely to be inaccurate, because food intake information was not available in the publication; the doses are estimates based on assumed intakes. Having acknowledged this uncertainty, it is observed that thyroid weights were increased significantly at 50 mg/kg bw per day and liver weights were increased at 15 mg/kg bw per day, which is consistent with the liver being the initial target in thiazopyr's MOA.

#### Table 3. Fifty-six-day study in male rats: Hormonal effects (Hotz et al., 1997).

	Dose (mg/kg bw per day) <sup>a</sup>						
	0	0.5	1.5	5	15	50	150
T4 (µg/dl)	4.1 ± 0.2	4.3 ± 0.3	3.9 ± 0.2	4.1 ± 0.2	4.0 ± 0.2	4.0 ± 0.2	2.9 ± 0.1 <sup>a</sup>
T3 (ng/dl)	84 ± 3	82 ± 4	68 ± 2	84 ± 3	82 ± 3	91 ± 4	$110 \pm 6^{a}$
TSH (ng/ml)	2.7 ± 0.2	$3.5 \pm 0.4$	2.7 ± 0.1	3.1 ± 0.4	2.9 ± 0.3	3.1 ± 0.2	$4.3 \pm 0.4^{a}$

Note: The mg/kg bw per day doses were estimated. Values are mean  $\pm$  standard error of the mean; 19 or 20 animals per group.

<sup>a</sup> Significantly different from control with Dunnett's test after analysis of variance (ANOVA) ( $P \le 0.05$ ).

As stated above, prolonged TSH stimulation leads to both hypertrophy and hyperplasia of the thyroid. In the 2-year rat study, there was a poor dose correlation between thyroid hyperplasia alone and tumour incidence. While tumour incidence was increased at 44.2 mg/kg bw per day, a statistically significant increase in the incidence of hyperplasia (8/58 versus 1/60 in controls) was found only at 136.4 mg/kg bw per day. Furthermore, in the 56-day rat study, where thyroid histology was reported as follicular cell hypertrophy and hyperplasia combined, there was a significant increase in the incidence of this diagnosis at 150 mg/kg bw per day but not at lower doses (Hotz et al., 1997). There was, however, a good dose correlation between increases in thyroid weights in the 56-day study and tumour incidence in the 2-year study. Statistically significant increases in thyroid weights of 46% were found at 150 mg/kg bw per day and 25% at 50 mg/kg bw per day (Hotz et al., 1997).

チアゾピルの場合、T4 及び TSH に影響を及ぼす用量と、甲状腺濾胞細胞腫瘍の発生率の増加を 引き起こす用量との間には、相関関係が乏しいようである。雄 SD ラットにおいて甲状腺濾胞細 胞腫瘍の統計学的に有意(P<0.05)な増加をもたらしたチアゾピルの最低用量は、2年間の試験で は 1 日当たり 44.2 mg/kg bw であったが、T4 及び TSH に対する影響の NOAEL は 56 日間の試験 では1日当たり50mg/kg bwであった(表2)。一般的に、肝酵素/肝重量及び下垂体-甲状腺ホル モン濃度への影響は、この甲状腺ホルモンかく乱 MOA が閾値を有する現象であることを考える と、少なくとも甲状腺の重量変化及び甲状腺腫瘍発生率の増加をもたらす用量と同程度の低用量 で起こると予想される。引用された投与量のどちらも正確ではないので、この見かけの不一致は おそらく真ではない。2年間の試験における体重1kg当たりの投与量は、試験期間全体の平均的 な推定値であったのに対し、比較に用いられた56日間のメカニズム試験での投与量は、12~20週 齢のラットにおけるものである。これらの投与量は、容易に算出可能な投与量よりも少なくとも 2 倍高かったと考えられる(したがって、腫瘍に対する実際の LOAEL は 1 日あたり約 90 mg/kg bw であったはずである)。また、ホルモンのかく乱(例えば TSH の長期上昇)が病理学的変化を もたらし始める臨界期は、2 年間の試験において後期ではなく早期であったため、これらの投与 量は腫瘍に対する関連性も高かったと考えられる。56日間の試験で計算された投与量は公表され た論文からは摂餌量の情報が入手できなかったため、摂餌量の推定値に基づいて計算された。そ のためこれについても不正確である可能性が高い。この不確実性を認識した上で、1 日あたり 50 mg/kg bw で甲状腺重量が有意に増加し、1日あたり 15 mg/kg bw で肝臓重量の増加が認められた が、これはチアゾピルの MOA では最初のターゲットが肝臓であることと一致している。

表 3. 雄ラットにおける 56 日間の試験:ホルモン作用(Hotz ら、1997年)

	Dose (mg/kg bw per day) <sup>a</sup>						
	0	0.5	1.5	5	15	50	150
T4 (µg/dL)	$4.1\pm0.2$	$4.3\pm0.3$	$3.9\pm 0.2$	$4.1\pm0.2$	$4.0\pm0.2$	$4.0\pm0.2$	$2.9\pm0.1^a$
T3 (ng/dL)	$84\pm3$	$82\pm4$	$68\pm2$	$84\pm3$	$82\pm3$	$91\pm 4$	$110\pm 6^a$
TSH (ng/mL)	$2.7\pm0.2$	$3.5\pm 0.4$	$2.7\pm0.1$	$3.1\pm 0.4$	$2.9\pm0.3$	$3.1\pm 0.2$	$4.3\pm0.4^a$
注:投与量 mg/kg							は20匹。

<sup>a</sup>分散分析 (ANOVA)後のDunnettの検定で対照群と統計学的に有意に異なる (P≦0.05)。

上述のように、長期のTSH 刺激は甲状腺の肥大と過形成の両方を引き起こす。ラットの2年間の試験では、甲状腺肥大のみと腫瘍発生率との間には用量相関性は見られなかった。腫瘍発生率は44.2 mg/kg bw/day 投与群で増加したが、過形成の発生率の統計学的に有意な増加(対照群 1/60 に対し 8/58)は136.4 mg/kg bw/day 投与群でのみ認められた。さらに、ラットの56日間の試験では、組織学的に甲状腺濾胞細胞肥大と過形成が併発しており、150 mg/kg bw/day 投与群での発生率は統計学的に有意に増加したが、それ以下の用量では増加しなかったと報告されている(Hotz ら、1997 年)。しかし、56日間の試験での甲状腺重量の増加と2年間の試験での腫瘍発生率との間には良好な用量相関性があった。150 mg/kg bw/day 投与群で 46%、50 mg/kg bw/day 投与群で 25%の統計学的に有意な甲状腺重量の増加が認められた(Hotz ら、1997 年)。

#### **TEMPORAL RELATIONSHIP**

If an event (or events) is an essential element of tumorigenesis, it must precede tumour appearance. Multiple exposure time data at 7, 14, 28, 56, and 90 days are available in which male SD rats were offered diets containing thiazopyr at 3000 mg/kg (150 mg/kg bw per day) (Hotz et al., 1997). Liver weights and hepatic T4-UGT activity were increased at all observation times from the earliest time of assessment on day 7. Biliary excretion of conjugated T4 was not measured in this experiment; however, serum T4 was reduced at all observation times. Increases in circulating TSH were observed at all sampling times, although the increase was not significant at 14 days after treatment began. Increases in thyroid weight were also observed at all sampling times. Histologically, there was a time-related increase in hypertrophy/hyperplasia beginning at 14 days. In the 2-year rat study, the first thyroid adenoma was observed at week 69 at a dose of 136.4 mg/kg bw per day. Thus, there is a logical temporal response for the key events in thiazopyr-induced thyroid follicular cell tumour formation in which all key events precede tumour formation.

# STRENGTH, CONSISTENCY, AND SPECIFICITY OF ASSOCIATION OF THE TUMOUR RESPONSE WITH KEY EVENTS

Strength, consistency, and specificity of the association can be established from the studies described above. The quantifiable precursor events, fundamental to the proposed MOA, are relatively consistent with the emergence of thyroid follicular cell tumours. Observation of liver weight increase and induction of hepatic T4-UGT in rats receiving the thiazopyr in the diet would be consistent with perturbation of homeostasis of the pituitary-thyroid axis by an extrathyroidal mechanism. An increase in hepatic T4-UGT activity is a step occurring before the other key biochemical changes and before thyroid follicular cell hypertrophy and hyperplasia. Thiazopyr treatment clearly results in a decrease in circulating T4 and an increase in TSH following enhanced liver metabolism of T4. Furthermore, in subchronic studies, the increases in thyroid weight and the development of hypertrophy/hyperplasia were shown to appear to a statistically significant degree under the same conditions of dose and time as the appearance and reversal of changes in thyroid hormone levels and thyroid hormone metabolism. Stop/recovery studies (Hotz et al., 1997) showed that cessation of thiazopyr dosing was followed by a return of hormone levels to control values, as well as a reduction in liver and thyroid weights and reversal of hyperplasia of thyroid follicular cells. Early dosing withdrawal would be expected to result in a reversal of hypothyroidism and of lesion progression for this non-genotoxic MOA. The only sign that was slow to reverse was the increase in thyroid weight after the longest dosing period.

#### **BIOLOGICAL PLAUSIBILITY AND COHERENCE**

There are considerable data from studies in laboratory rodents demonstrating the relationship between sustained perturbation of the hypothalamic–pituitary–thyroid axis, prolonged stimulation of the thyroid gland by TSH, and the progression of thyroid follicular cells to hypertrophy, hyperplasia, and eventually neoplasia (McClain, 1995; Hard, 1998; Hurley et al., 1998; Capen et al., 1999; IARC, 2001). Increased secretion of TSH may result via several mechanisms, including increased hepatic clearance of T4, as is the case with thiazopyr.

### 時間的関連性

ある事象が腫瘍形成の必須要素である場合、それは腫瘍の発生に先行して発現しなければなら ない。雄 SD ラットに 3000 mg/kg (1日あたり 150 mg/kg bw)のチアゾピルを混餌投与した 7、14、 28、56 及び 90 日後といった複数のタイミングにおけるデータが利用可能である(Hotz ら、1997 年)。肝重量増加及び肝 T4-UGT 活性上昇は、最も早く測定した 7 日目から全ての測定時期におい て認められた。この実験では抱合化 T4 の胆汁排泄は測定されなかったが、血清 T4 はすべての観 察時期において減少した。循環 TSH の増加は、投与開始後 14 日目においては有意ではなかった が、すべてのサンプリング時期で観察された。甲状腺重量の増加もすべてのサンプリング時期で 観察された。組織学的には、14 日目から肥大/過形成の経時的な増加が認められた。2 年間のラッ ト試験では、1 日あたり 136.4 mg/kg bw を投与した 69 週目に最初の甲状腺腺腫が観察された。こ のように、チアゾピル誘発性甲状腺濾胞細胞腫瘍形成においては、すべての key events が腫瘍形 成に先行するという論理的な経時的反応が認められた。

#### 腫瘍反応と key events との関連性の強さ、一貫性及び特異性

この関連性の強さ、一貫性、特異性は、上述の試験から確立できる。推定される MOA の基礎と なる定量可能な前駆事象は、甲状腺濾胞細胞腫瘍の発現と比較的一致している。チアゾピルを混 餌投与されたラットにおいて、肝重量の増加及び肝 T4-UGT の誘導が観察されることから、甲状 腺外の機序による下垂体-甲状腺軸のホメオスタシスのかく乱と一致しているようである。肝 T4-UGT 活性の上昇は、他の重要な生化学的変化や甲状腺濾胞細胞の肥大及び過形成の前に起こる段 階である。チアゾピルの投与は肝臓における T4 の代謝が促進されるため、循環 T4 の減少及び TSH の増加が明らかである。さらに、亜慢性試験では、甲状腺重量の増加及び肥大・過形成の発 生は、甲状腺ホルモン濃度及び甲状腺ホルモン代謝の変化の出現や回復と同じ用量及び時間の条 件下で統計学的に有意な程度で現れることが示された。中止/回復試験(Hotz ら、1997 年)では、 チアゾピルの投与中止後、ホルモン値がコントロール値に戻り、肝臓と甲状腺の重量が減少し、 甲状腺濾胞細胞の過形成が回復することが示された。早期に投与を中止することで、この非遺伝 毒性 MOA における甲状腺ホルモンの低下と病変の進行が回復することが期待された。唯一回復 が遅くなったのは、最も長い投与期間の後の甲状腺重量の増加であった。

#### 生物学的妥当性及び整合性

実験用のげっ歯類を用いた研究から、視床下部-下垂体-甲状腺軸の持続的なかく乱、TSH による甲状腺への長期的な刺激及び甲状腺濾胞細胞の肥大、過形成、最終的な腫瘍への進行との関係を示すデータが数多く存在する(McClain、1995年; Hard、1998年; Hurley ら、1998年; Capen ら、1999年; IARC、2001年)。チアゾピルの場合と同様に、TSH の分泌増加は、T4 の肝クリア ランスの増加を含むいくつかのメカニズムを介して生じる可能性がある。

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Circulating levels of T4 are monitored by the thyrotropic cells of the pituitary gland that are responsible for the synthesis of TSH. In the pituitary gland, T4 is metabolized by 5'-deiodinase type II to T3, which then binds to specific receptors in the cell nucleus. A decrease in T3 receptor occupancy results in stimulation of TSH synthesis and secretion. Studies in vivo have shown that injection of rats with TSH leads to reductions in thyroid follicular cell nuclear statin, a non-proliferation-specific nuclear antigen, indicating that these cells were leaving the non-dividing state to resume the cell cycle (Bayer et al., 1992). This study showed that low, repeated doses of TSH (0.25 IU per rat twice daily) produced a cumulative response in nuclear statin levels over 10 days, which returned to normal resting levels within 5 days of cessation of TSH injections. Reduction in nuclear statin is also an early event that parallels the earliest known pinocytotic response to TSH. These data are consistent with increased TSH concentrations alone causing thyroid follicular cells of rats to enter a state of preproliferation. Therefore, the suggestion that thiazopyr causes thyroid follicular cell neoplasms in rats by initially inducing hepatic T4-UGT is coherent with the known physiology of the hypothalamus-pituitary-thyroid dynamic control system, at least to the stage of hypertrophy and hyperplasia.

Lastly, the tumour response elicited by thiazopyr is typical of a rodent thyroid carcinogen, in that thyroid follicular cell tumours are found in male rats but not in female rats or mice. Rats tend to be more sensitive to thyroid carcinogenesis than mice, and male rats are frequently found to be more sensitive than female rats with respect to the proportion of chemicals that induce thyroid tumours (Hurley et al., 1998). In keeping with this, TSH levels are typically higher in male rats than in females (Hill et al., 1989). In addition, male rats are sometimes more prone to hepatic enzyme induction than females of the same strain, but this depends on the enzyme in question, the dose of the inducing compound, and the age of the animals (Sundseth & Waxman, 1992; Agrawal & Shapiro, 1996; Oropeza-Hernandez et al., 2003).

### **OTHER MODES OF ACTION**

Mutagenesis is always one possible MOA to consider, but no genetic toxicity has been demonstrated for thiazopyr in the following tests:

- mutation in four strains of *Salmonella typhimurium* (Bakke, 1989a);
- mutation at the *hgpt* locus of Chinese hamster ovary cells (Li & Myers, 1989);
- micronucleus induction in bone marrow cells of mice treated in vivo (Flowers, 1990);
- unscheduled DNA synthesis induction in hepatocytes of rats treated in vivo (Bakke, 1989b).

Therefore, the available evidence indicates that mutagenesis is not an alternative MOA for thiazopyr.

Additional effects on the hypothalamic–pituitary–thyroid axis and disruption of other pathways of thyroid hormone metabolism are other possibilities for altering thyroid homeostasis. These variations would not differ in any fundamental way from the one that has been proposed for thiazopyr, in that all would lead to prolonged TSH stimulation with continuous exposure.

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T4 の循環レベルは、TSH の合成を担う下垂体の甲状腺刺激細胞によってモニターされている。 下垂体では、T4 は、5-dried-deio-dinase(ヨウ素ペルオキシダーゼ)II 型によりT3 に代謝され、T3 は細胞核内の特定の受容体に結合する。T3 受容体の占有率が低下すると、TSH 合成と分泌が刺激 される。ラットへのTSH の注射が、非増殖細胞特異的核抗原である甲状腺濾胞細胞核スタチンが 減少し、これらの細胞が非分裂状態を脱して細胞周期を再開していることが生体内での研究で示 されている(Bayer ら、1992 年)。この試験では、低用量のTSH を反復投与(ラット1匹あたり 0.25 IUを1日2回)すると、核内スタチンレベルが10日間にわたって累積反応が生じ、TSHの 注射を中止してから5日以内に正常な安静時のレベルに戻ることが示された。核内スタチンの減 少もまた、TSH に対する最も早い反応として知られているピノサイトーシスと類似した初期の事 象である。これらのデータは、TSH 濃度の増加だけでラットの甲状腺濾胞細胞を増殖前の状態に なることと矛盾しない。したがって、チアゾビルが最初に肝T4-UGTを誘導することでラットの 甲状腺濾胞細胞腫瘍を引き起こすという指摘は、少なくとも肥大と過形成の段階までは視床下部 下垂体-甲状腺の動的な制御システムに関する既知の生理学的知見と一致している。

最後に、チアゾビルにより誘発される腫瘍反応はげっ歯類の甲状腺発がん物質の典型的なもの であり、甲状腺濾胞細胞腫瘍は雄ラットにはみられるが、雌のラットやマウスには認められない。 ラットはマウスよりも甲状腺発がん物質に対し感受性が高い傾向があり、さらに、雄ラットは雌 ラットよりも甲状腺腫瘍を誘発する化学物質の割合が大きいことがよくみられている (Hurley ら、 1998年)。このことと一致して、TSH レベルは通常雄ラットの方が雌よりも高い (Hill ら、1989 年)。さらに、雄ラットは時に同系統の雌よりも肝酵素が誘導されやすいことがあるが、これは酵 素の種類、酵素誘導を起こす化合物の用量や動物の年齢に依存する (Sundseth & Waxman、1992年; Agrawal & Shapiro、1996年; Oropeza-Hernandez ら、2003年)。

#### その他の MOA

変異原性は常に考慮すべき可能性のある MOA の 1 つであるが、以下の試験においてチアゾピルの遺伝毒性は示されていない。

- ネズミチフス菌 (Salmonella typhimurium) の4つの菌株を用いた突然変異試験 (Bakke、1989 年 a)。
- チャイニーズハムスター卵巣細胞を用いたの突然変異試験(hgpt 座位)(Li & Myers、1989 年)。
- マウスの骨髄細胞を用いた In vivo 小核試験(Flowers、1990年)。
- ラットの肝細胞を用いた In vivo 不定期 DNA 合成試験(Backke、1989 年 b)。

したがって、利用可能なエビデンスは、突然変異誘発がチアゾピルの代替 MOA ではないこと を示している。

その他の視床下部-下垂体-甲状腺軸への影響及び甲状腺ホルモン代謝における他の経路のかく 乱は、甲状腺のホメオスタシスを変化させる可能性がある。しかし、これらの変化は、チアゾピ ルについて推定されているものと根本的には変わらず、すべて継続的なばく露による TSH 刺激の 長期化につながっているであろう。

#### UNCERTAINTIES, INCONSISTENCIES, AND DATA GAPS

There appears to be a lack of dose concordance for thyroid tumours and hormone changes, but this is likely to be due to inaccuracies in the milligrams per kilogram body weight doses compared—which either were estimated (versus calculated on the basis of food consumption and body weight data) and cover an early period in the life of rats or were averages for the whole duration of the experiment—as well as experimental variability.

## **ASSESSMENT OF POSTULATED MODE OF ACTION**

The data presented are judged, with a moderately high degree of confidence, to be adequate to explain the development of thyroid follicular cell tumours in male rats following chronic dietary exposure to thiazopyr. Thiazopyr clearly increased liver weights (i.e. the initial target organ) at doses lower than those causing tumours and enhanced thyroid growth (i.e. increased thyroid weights) at the lowest tumorigenic dose.

### Human applicability of the proposed MOA

The IPCS HRF, which was developed from the Risk Science Institute/International Life Sciences Institute "Human Relevance Framework" (Meek et al., 2003) and modified based on discussions by the IPCS Cancer Working Group (Boobis et al., current document), presents a four-part approach to addressing a series of three questions and leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumours.

*1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?* As described in detail above, there is clear evidence that thiazopyr alters thyroid homeostasis by UGT induction, by reducing serum T4 levels and consequently elevating serum TSH.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? The current understanding of the regulation of thyroid hormone homeostasis in humans and of the role of increased TSH levels (as a result of altered thyroid homeostasis) as a risk factor for thyroid cancer was considered in order to assess the human relevance of the key events in thiazopyr's animal mode of carcinogenic action. Although there are substantial quantitative dynamic differences (discussed below), the fundamental mechanisms involved in the function and regulation of the hypothalamic-pituitary-thyroid axis in rats are qualitatively similar to those in humans (Bianco et al., 2002). Therefore, an agent that decreases T4 levels in rats could likewise reduce T4 in humans; this, in turn, could potentially lead to an increase in TSH levels. There are data showing that rodents and humans respond in a similar fashion to perturbations of pituitary-thyroid function. For example, it is well known that iodine deficiency, which readily leads to decreased thyroid hormone levels, stimulates thyroid cell proliferation in humans, leading to goitre. If left untreated, iodine deficiency may lead to tumour formation, albeit rarely (Thomas & Williams, 1999). Although there is no evidence of increased susceptibility to thyroid cancer, a number of pharmaceuticals (e.g. propylthiouracil, lithium, amiodarone, iopanoic acid) that disrupt thyroid homeostasis by acting directly on the thyroid gland (e.g. by inhibiting hormone synthesis or release or by blocking the conversion

## 不確実性、矛盾、データギャップ

甲状腺腫瘍とホルモンの変化には用量の一致がみられないが、これは比較に用いられた体重 1 kg 当たりのミリグラム投与量(摂餌量と体重値から算出された)が不正確であること、比較した 投与量が一方は若齢期のラットにおける平均値であり、もう一方が試験全体の平均値であったこ と、また、試験間のばらつきなどに起因している可能性がある。

## 推定される MOA の評価

提示されたデータは、中程度に高い信頼度で、チアゾビルの混餌投与による慢性ばく露後の雄 ラットにおける甲状腺濾胞細胞腫瘍の発生を説明するのに十分であると判断される。チアゾビル は、腫瘍を引き起こす用量よりも低い用量で明らかに肝臓重量を増加させ(すなわち、肝臓が最 初の標的臓器である)、腫瘍を誘発させる中で最も低い用量で甲状腺の成長(すなわち、甲状腺重 量の増加)を促進させた。

## 推定される MOA のヒトへの適用可能性

IPCS HRF はリスクサイエンス研究所/国際生命科学研究機構の「ヒト関連性フレームワーク」 (Meek ら、2003 年)をもとに開発され、「IPCS がんワーキンググループ」の議論をもとに修正さ れたものであり(Boobis ら,本書)、一連の3つの問いかけに対処し、動物の腫瘍における MOA のヒトへの関連性に関する文書化された論理的な結論を導くための4つの部分からなるアプロー チを提示している。

1. 動物 MOA を確立するのにエビデンスの重み付けは十分か?上で詳述したように、チアゾピルは UGT 誘導により血清 T4 レベルを低下させ、その結果として血清 TSH を上昇させることで、 甲状腺ホルモンのホメオスタシスを変化させるという明確なエビデンスがある。

2. 実験動物とヒトとの間のkey events の根本的、質的な違いに基づいて、MOA のヒトとの関連 *性を合理的に排除することができるか*?ヒトにおける甲状腺ホルモンのホメオスタシスの調節に 関する現在の理解と、(甲状腺ホルモンのホメオスタシスの変化の結果としての) TSH レベルの上 昇が甲状腺がんのリスク因子として果たす役割について、チアゾピルの動物における発がん MOA の key events のヒトへの関連性を評価するために検討がなされた。ラットにおける視床下部-下垂 体-甲状腺軸の機能と調節に関与する基本的なメカニズムは、量的な薬力学的差異(後述)はある が、定性的にはヒトのそれと類似している(Biancoら、2002 年)。したがって、ラットで T4 レ ベルを低下させる薬剤は、ヒトでも同様にT4を低下させる可能性があり、その結果、TSH レベル の上昇につながる可能性がある。げっ歯類とヒトは、下垂体-甲状腺機能のかく乱に対して同様の 反応を示すデータがある。例えば、甲状腺ホルモンのレベルを容易に低下させるヨウ素欠乏症は、 ヒトでは甲状腺細胞の増殖を刺激して甲状腺腫を引き起こすことが知られている。ヨウ素欠乏症 を放置すると、まれではあるが腫瘍形成につながることがある(Thomas & Williams、1999年)。甲 状腺がんになりやすいというエビデンスはないが、甲状腺に直接作用して(例えば、ホルモン合 成や放出を阻害する、T4からT3への変換を阻害する)甲状腺ホルモンのホメオスタシスを乱す 多くの医薬品(プロピルチオウラシル、リチウム、アミオダロン、イオパノ酸など)は、ヒトでは 甲状腺ホルモンの低下及び TSH の上昇につながることが知られている(Ron ら、1987年)。

of T4 to T3) are known to lead to hypothyroidism and increases in TSH in humans (Ron et al., 1987).

In contrast to rats, no increases in TSH levels have been found in humans following exposure to agents that induce hepatic microsomal enzymes and reduce circulating T4 levels (discussed in Lehman-McKeeman & Hill, in Meek et al., 2003). For example, the pharmaceutical compounds phenytoin, rifampin, and carbamazepine induce hepatic microsomal enzymes, including UGT, and reduce circulating T4 levels, but TSH levels are unchanged (Curran & DeGroot, 1991); agents that produce thyroid tumours in rats by increasing glucuronidation and biliary excretion of T4 at high experimental doses (e.g. omeprazole, lansoprazole, and pantoprazole) produce no changes in thyroid hormones at clinical doses in humans (Masubuchi et al., 1997). Thus, there appears to be a substantial difference in the dose–response relationship for altered homeostasis of the pituitary–thyroid axis in rats compared with humans. As discussed below, this observation is due to quantitative dynamic differences between rats and humans in the basic physiological processes underlying pituitary–thyroid function.

3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans? Thiazopyr does not target the thyroid directly. Rather, its primary effect is on hepatic metabolizing enzymes, and the increase in metabolic activity indirectly increases the systemic clearance of T4, leading to the hypothyroid state and the compensatory increase in TSH found in rats. Although there are no chemical-specific data on the potential for thiazopyr to disrupt thyroid hormone homeostasis in humans, a number of other microsomal enzyme inducers have been extensively studied, such as phenobarbital (Lehman-McKeeman & Hill, in Meek et al., 2003). As discussed above, agents that produce hypothyroidism by altering hepatic clearance of T4 do not appear to result in elevated TSH levels in humans. Presumably, TSH is not increased because a critical reduction of T4 is not reached.

There are several important physiological and biochemical differences between rats and humans related to thyroid function. Rats have a smaller reserve capacity of thyroid hormones when compared with humans. The rat has a much shorter thyroid hormone half-life than humans. The half-life of T4 is about 12 h in rats compared with 5–9 days in humans (Dohler et al., 1979). The shorter half-life in rats is likely related to the absence of a high affinity binding globulin for T4 that is present in humans (Hill et al., 1989). In rats, the increased clearance contributes to the need for a higher rate of production of T4 (per unit of body weight) to maintain normal levels of T4. In contrast, in humans, the binding of thyroid hormone to this globulin accounts for a slower metabolic degradation and clearance, which in turn result in the thyroid gland being less active than in rats. The constitutive TSH levels are approximately 25 times higher in rats than in humans, reflecting the higher activity of the pituitary–thyroid axis in rats (Dohler et al., 1979; McClain, 1992). Therefore, humans are quantitatively less sensitive than rats to agents that reduce T4 and lead to elevated TSH. There is no increased risk of thyroid tumour development if TSH is not elevated.

Another difference of rats compared with humans is the histological appearance of the thyroid. This histological difference is related to the higher rate of production of T4 to

ラットとは対照的に、ヒトでは、肝ミクロソーム酵素が誘導され、循環 T4 レベルを低下させる 薬剤にばく露されても TSH レベルの上昇は認められていない(Lehman-McKeeman & Hill, in Meek ら、2003 年)。例えば、医薬品のフェニトイン、リファンピン、カルバマゼピンは、UGT を含む 肝ミクロソーム酵素を誘導し、血中の T4 レベルを低下させるが、TSH レベルは変化しない(Curran & DeGroot、1991 年)。同様に、実験的に高用量で T4 のグルクロン酸抱合及び胆汁排泄を増加さ せることによりラットに甲状腺腫瘍を発生させる薬剤(例えば、オメプラゾール、ランソプラゾ ール、パントプラゾール)は、ヒトにおいて臨床用量では甲状腺ホルモンに変化をもたらさない (Masu-buchi ら、1997 年)。このように、ラットにおける下垂体-甲状腺軸のホメオスタシスの変 化の用量反応関係には、ヒトと比較してかなりの違いがあるように思われる。後述するように、 この現象は、ラットとヒトでは下垂体-甲状腺機能の基礎となる生理学的プロセスの量的な薬力学 的差異に起因している。

3. 実験動物とヒトとの間の薬力学的または動態的要因のいずれかの量的差異に基づいて、MOA のヒトへの関連性を合理的に排除することができるか?チアゾビルは甲状腺を直接標的とするも のではない。むしろ、その主な作用は肝代謝酵素に対するものであり、代謝活性の亢進は間接的 に T4 の全身クリアランスを増加させ、ラットでみられるような甲状腺機能低下状態と TSH の代 償性の増加をもたらす。チアゾビルがヒトにおける甲状腺ホルモンの恒常性を乱す可能性につい ては、チアゾビル特有のデータはないが、フェノバルビタール(Lehman-McKeeman & Hill, in Meek ら、2003 年)のような他の多くのミクロソーム酵素誘導剤で広く研究されている。上述したよう に、T4 の肝クリアランスを変化させることで甲状腺機能減退症下を引き起こす薬剤は、ヒトでは TSH レベルの上昇をもたらさないようである。おそらく、T4 の減少が閾値まで達していないので、 TSH は上昇しないのではないかと考えられる。

甲状腺機能に関連して、ラットとヒトの間にはいくつかの重要な生理学的及び生化学的相違が ある。ラットはヒトに比べて甲状腺ホルモンの予備容量が小さい。ラットはヒトに比べて甲状腺 ホルモンの半減期が非常に短く、T4の半減期は、ヒトの5~9日に対し、ラットでは約12時間で ある(Dohler ら、1979年)。ラットの半減期が短いのは、ヒトに存在するT4の高親和性結合グロ ブリンが存在しないことに関係していると考えられる(Hill ら、1989年)。ラットでは、クリアラ ンスの増加によって正常なT4レベルを維持するために、より高い体重あたりのT4産生率を必要 とする。対照的に、ヒトではこのグロブリンへ甲状腺ホルモンが結合することによって、ラット と比較して代謝的分解及びクリアランスが遅く、その結果、甲状腺はラットよりも活性が低い。 恒常的なTSHレベルは、ラットではヒトよりも約25倍高く、ラットでは下垂体-甲状腺軸の活性 が高いことを反映している(Dohler ら、1979年; McClain、1992年)。したがって、ヒトは、T4を 減少させTSHの上昇をもたらす薬剤に対して、ラットよりも定量的に感受性が低い。TSH が上昇 していなければ、甲状腺腫瘍発生のリスクが高まることはない。

ヒトとの比較においてラットのもう一つの違いは、甲状腺の組織像にある。この組織学的な相 違は、ラットでは安定した一定の血清 T4 濃度を維持するための T4 のより高い産生速度と関連し ており、これによりラットの甲状腺は、人間を含む霊長類のそれよりも"機能的に活性化"した状 態となっている(McClain、1995 年)。

maintain a consistent serum concentration, thus making the rat thyroid more "functionally active" than that of primates, including humans (McClain, 1995). More of the follicular epithelium in the rat is stimulated to synthesize thyroglobulin, and therefore more of the follicular cells are tall cuboidal and appear to be active in synthesis. In contrast, more of the follicular cells in humans tend to be short cuboidal or almost squamous in appearance, suggesting they are quiescent. Because rat follicular cells are already generally active, under stimulation from TSH, they will respond with hyperplasia more readily than human follicular cells. Because of the greater storage capability of the human thyroid and the greater numbers of cells in a quiescent state, human thyroid follicular cells will be roused from their quiescent state to synthesize and secrete additional thyroid hormone without the need for a hyperplastic response to re-establish homeostasis. Therefore, the primary response in the human thyroid gland would be thyroglobulin reabsorption and cellular hypertrophy rather than hyperplasia. In short, there is much greater buffering capacity in the biochemistry of the human than the rat thyroid.

Even though certain agents can cause a reduction in thyroid hormone levels in humans, there is no clear evidence that these agents increase susceptibility to thyroid cancer (Ron et al., 1987). For example, epidemiological studies with phenobarbital do not show any increased risk of thyroid cancer (Olsen et al., 1993). Studies of individuals with conditions that would lead to elevated TSH (patients with Graves disease or goitre) indicate that the occurrence of thyroid cancer is rare in these circumstances (e.g. Mazzaferri, 2000; Gabriele et al., 2003). A study of environmental and heritable causes of cancer among 9.6 million individuals, using the nationwide Swedish Family-Cancer Database, found that the environment did not appear to play a principal causative role in thyroid cancer (Lichtenstein & Hemminki, 2002). The only known human thyroid carcinogen is radiation, a mutagenic exposure.

As summarized in Table 4, there is sufficient evidence in the general literature on the biochemical and physiological differences in thyroid function to indicate differences in tumour susceptibility between rats and humans. In contrast to humans, rats are very susceptible to thyroid neoplasia secondary to hypothyroidism. In particular, modest changes in thyroid hormone homeostasis will promote tumour formation in rats. Thus, thyroid tumours induced by thiazopyr involving increased hepatic clearance of hormone and altered homeostasis of the pituitary–thyroid axis in rodents are considered not relevant to humans, based on quantitative dynamic differences.

4. Conclusion: statement of confidence, analysis, and implications. There is sufficient experimental evidence to establish a thyroid disruption MOA for thiazopyr-induced thyroid follicular cell tumours in rats. Although thiazopyr may potentially result in hypothyroidism in humans, there is sufficient quantitative evidence on the basic physiological processes in the general literature to conclude that thyroid tumours induced by a process involving increased hepatic clearance of thyroid hormone and altered homeostasis of the pituitary–thyroid axis in rodents is not likely to lead to an increase in susceptibility to tumour development in humans. Although there are no human data on thiazopyr, clinical data on other hepatic microsomal enzyme inducers were critical to this human relevance analysis. The general literature provided sufficient evidence to show that unlike in the rat, decreased T4 levels typically show no evidence of compensatory increases in TSH levels in humans. There is also cellular and

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

ラットの濾胞上皮の多くは、刺激を受けてサイログロブリンを合成するため、濾胞細胞の多くは 背の高い立方体状をしており、活発にホルモン合成しているように見える。対照的に、ヒトの濾 胞細胞の多くは、短い立方体状またはほぼ扁平な外観を呈し、静止状態のように見える傾向があ る。ラットの濾胞細胞はすでに全体的に活性化しているので、TSHによる刺激を受けると、ヒト の濾胞細胞よりも容易に反応し過形成を生じる。ヒトの甲状腺は貯蔵能力が高く、休止状態にあ る細胞の数が多い。そのため、ヒトの甲状腺濾胞細胞は恒常性を回復するための過形成反応を必 要とせずに、休止状態から覚醒して追加の甲状腺ホルモンを合成・分泌する。したがって、ヒト 甲状腺における主要な反応は、過形成ではなくサイログロブリンの再吸収と細胞の肥大であろう。 要するに、ラット甲状腺よりもヒト甲状腺の方が、生化学的な面においてははるかに大きな緩衝 能力を持っているということである。

ある薬剤がヒトにおいて甲状腺ホルモンレベルの低下を引き起こす可能性があるとしても、こ れらの薬剤が甲状腺がんの感受性を高めるという明確なエビデンスはない (Ron ら、1987 年)。例 えば、フェノバルビタールを用いた疫学研究では、甲状腺がんのリスクの増加は示されていない (Olsen ら、1993 年)。TSH が上昇するような状態にある人々 (バセドウ病または甲状腺腫の患者) を対象とする研究は、このような状況においても甲状腺がんの発生はまれであることを示してい る (例えば、Mazzaferri、2000 年;Gabriele ら、2003 年)。全国規模のスウェーデンの家族がんデ ータベースを用いた 960 万人のがんの環境的及び遺伝的原因を調査した結果、環境が甲状腺がん の主な原因となる役割を果たしているようには見えないことが明らかにされた (Lichtenstein & Hemminki、2002 年)。唯一知られているヒト甲状腺の発がん物質は放射線であり、すなわち変異 原性物質のばく露である。

表4に要約されているように、甲状腺機能の生化学的及び生理学的差異に関する一般的な文献 には、ラットとヒトの間で腫瘍に対する感受性に違いがあることを示す十分なエビデンスがある。 ヒトとは対照的に、ラットは甲状腺ホルモンの低下症に続発する甲状腺腫瘍に対して非常に感受 性が高い。特に、甲状腺ホルモンの恒常性にわずかでも変化があるとラットでは腫瘍形成が促進 される。したがって、げっ歯類におけるホルモンの肝クリアランスの増加と下垂体-甲状腺軸のホ メオスタシスの変化を伴うチアゾピルによって誘発される甲状腺腫瘍は、量的な動態の違いから ヒトへの関連性はないと考えられる。

4. 結論:信頼性、解析及び帰結の記述。ラットにおけるチアゾビル誘発性甲状腺濾胞細胞腫瘍に関 して、甲状腺ホルモンかく乱 MOA を確立するのに十分な実験的エビデンスが存在する。チアゾビル はヒトにおいて甲状腺機能減退症を引き起こす可能性があるが、一般的な文献には、げっ歯類におけ る甲状腺ホルモンの肝クリアランスの増加と下垂体-甲状腺軸のホメオスタシスの変化を伴うプロセ スによって誘発される甲状腺腫瘍は、ヒトの腫瘍発生に対する感受性の増加にはつながらないと結論 づけるのに十分な基本的な生理学的プロセスに関する定量的エビデンスがある。チアゾビルに関する ヒトでのデータはないが、他の肝ミクロソーム酵素誘導剤に関する臨床データは、チアゾビルのヒト への関連性の解析には不可欠であった。一般的な文献からは、ラットとは異なりヒトでは T4 レベルの 低下が代償的に TSH レベルを上昇させることを示すエビデンスはないことを示すのに十分なエビデ ンスが得られた。また、ラットの下垂体-甲状腺軸がこのようなホルモンのかく乱に対してヒトよりも はるかに感受性が高いという細胞学的及び生化学的エビデンスもある。

biochemical evidence that the rat pituitary–thyroid axis is much more sensitive than that in humans to such perturbations. This sensitivity is likely the result of the rapid turnover of T4 in rats coupled with the higher demand for TSH to maintain thyroid activity.

#### Table 4. A comparison of key events in rats and humans.

Key event	Evidence in rats	Evidence in humans
Increased hepatic clearance of T4	In short-term and chronic rat studies, the liver is found to be the most sensitive target, and evidence of increased T4 hepatic clearance is provided by studies on T4-hepatic UGT activity, T4 half-life, T4 biliary elimination, liver weights, and hypertrophy.	No data available for thiazopyr, but microsomal enzyme induction is plausible.
Decreased serum T4	Direct experimental evidence.	No data available for thiazopyr, but plausible given that other microsomal enzyme inducers have been shown to reduce T4 in humans.
Increased TSH levels	Direct experimental evidence.	No data available for thiazopyr, but other microsomal enzyme inducers have not been shown to increase TSH levels even when T4 is decreased.
Increased TSH increases thyroid cell proliferation and tumour formation	Direct experimental evidence.	Induction of thyroid follicular cell tumours secondary to hypothyroidism is remote in humans, given the quantitative differ- ences in thyroid function/homeostasis. Occurrence of thyroid cancer is rare even in severely hypothyroid individuals.

#### **IMPLICATIONS OF THE IPCS HRF**

The thiazopyr example is an illustration of an induced tumour response consistent with an MOA that has been previously defined and established. Thus, addressing the first question in the framework analysis, "Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?", became a determination of whether the data set on the chemical conforms to the same key events defined for the pathway of interest. This example further demonstrates how data on the basic understanding of the biological processes involved in the MOA provide an important means to compare the rodent and human key events. Thus, this generic human information was essential to evaluating the qualitative and quantitative differences between experimental animals and humans in addressing the plausibility of the cancer MOA for humans (i.e. questions 2 and 3 in the HRF).

#### Harmonization Project Document No. 4

この感受性の高さは、甲状腺の活動を維持するための TSH の需要が高いことと相まって、ラット における T4 の急速なターンオーバーが早いことに起因すると思われる。

#### 表 4. ラットとヒトにおける key events の比較

Key events	ラットにおけるエビデンス	ヒトにおけるエビデンス
T4 の肝クリアラ	ラットの短期及び慢性試験で、肝臓が	チアゾピルについてのデータはない
ンスの増加	最も感受性の高い標的であることが	が、ミクロソーム酵素の誘導が起こっ
	判明しており、T4 肝 UGT 活性、T4 半 減期、T4 胆汁排泄、肝重量及び肥大に 関する研究により、T4 肝クリアランス	ていると思われる。
	の増加のエビデンスが得られている。	
血清 T4 の減少	直接的な実験的エビデンス。	チアゾピルのデータはないが、他のミ
		クロソーム酵素誘導剤がヒトで T4 を
		減少させることが示されていること
		を考えると、チアゾピルについてもそ
		うであると思われる。
TSH レベルの上昇	直接的な実験的エビデンス。	チアゾピルのデータはないが、他のミ
		クロソーム酵素誘導剤において T4 が
		減少しても TSH レベルが上昇するこ
		とは示されていない。
TSH の増加は甲状	直接的な実験的エビデンス。	甲状腺ホルモンの低下に続発する甲
腺細胞の増殖と腫		状腺濾胞細胞腫瘍の誘発は、甲状腺機
瘍形成を促進させ		能/ホメオスタシスの齧歯類との量的
る。		な違いを考えると、ヒトでは起こりそ
		うにない。
		甲状腺ホルモンレベルがかなり低い
		ヒトにおいても甲状腺がんの発生は
		まれである。

## IPCS HRF の結果

チアゾピルの例は、過去に明確にされ、確立された MOA と一致する誘発された腫瘍反応につ いて示したものである。したがって、フレームワーク解析の最初の質問である「動物 MOA を確 立するのにエビデンスの重み付けは十分か」に対応することは、その化学物質に関するデータセ ットが対象となる経路に対して定義にされた同じ key events に適合しているか否かを判断する必 要があった。この例は、MOA に関与する生物学的プロセスの基本的な理解に関するデータが、ど のようにげっ歯類とヒトの key events を比較するための重要な手段となるかを示している。この ように、実験動物とヒトの間の質的及び量的な差異を評価し、ヒトに対する発がん MOA の妥当 性(すなわち、HRF の問 2 と 3)評価するためには、このヒトに関する一般的な情報は、不可欠 であった。

#### REFERENCES

Agrawal AK, Shapiro BH (1996) Phenobarbital induction of hepatic CYP2B1 and CYP2B2: Pretranscriptional and post-transcriptional effects of gender, adult age, and phenobarbital dose. *Molecular Pharmacology*, **49**(3):523–531.

Bakke JP (1989a) Ames/Salmonella mutagenicity assay with MON 13200: Study No. ML-88-191/EHL No. 88124. Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275535).

Bakke JP (1989b) *Evaluation of MON 13200 to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay in the male F-344 rat: Study No. SR-88-204/SRI No. LSC 6327.* Testing facility: SRI International, Menlo Park, CA. Submitted by Monsanto, St. Louis, MO (MRID No. 42275538).

Bayer I, Mitmaker B, Gordon PH, Wang E (1992) Modulation of nuclear statin expression in rat thyroid follicle cell following administration of thyroid stimulating hormone. *Journal of Cellular Physiology*, **150**:276–282.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, **23**(1):38–89.

Capen CC, Dybing E, Rice JM, Wilbourn JD, eds (1999) *Species differences in thyroid, kidney and urinary bladder carcinogenesis.* Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 147).

Curran PG, DeGroot LJ (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Reviews*, **12**:135–150.

Dohler KD, Wong CC, Von Zur Muhlen A (1979) The rat as a model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacology and Therapeutics*, **5**:305–318.

Flowers LJ (1990) *Micronucleus assay with MON 13200: ML-88-390/EHL Study No. 88230.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275537).

Gabriele R, Letizia C, Borghese M, De Toma G, Celia M, Izzo L, Cavalla A (2003) Thyroid cancer in patients with hyperthyroidism. *Hormone Research*, **60**(2):79–83.

Hard GC (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. *Environmental Health Perspectives*, **106**(8):1–21.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF (1989) Thyroid follicular cell carcinogenesis. *Fundamental and Applied Toxicology*, **12**(4):629–697.

#### 参考文献

Agrawal AK, Shapiro BH (1996) Phenobarbital induction of hepatic CYP2B1 and CYP2B2: Pretranscriptional and post-transcriptional effects of gender, adult age, and phenobarbital dose. *Molecular Pharmacology*, **49**(3):523–531.

Bakke JP (1989a) Ames/Salmonella mutagenicity assay with MON 13200: Study No. ML-88- 191/EHL No. 88124. Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275535).

Bakke JP (1989b) Evaluation of MON 13200 to induce unscheduledDNAsynthesis in the in vitro hepatocyteDNArepair assay in the male F-344 rat: Study No. SR-88-204/SRI No. LSC 6327. Testing facility: SRI International, Menlo Park, CA. Submitted by Monsanto, St. Louis, MO (MRID No. 42275538).

Bayer I, Mitmaker B, Gordon PH, Wang E (1992) Modulation of nuclear statin expression in rat thyroid follicle cell following administration of thyroid stimulating hormone. *Journal of Cellular Physiology*, **150**:276–282.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, **23**(1):38–89.

Capen CC, Dybing E, Rice JM, Wilbourn JD, eds (1999) *Species differences in thyroid, kidney and urinary bladder carcinogenesis*. Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 147).

Curran PG, DeGroot LJ (1991) The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrine Reviews*, **12**:135–150.

Dohler KD, Wong CC, Von Zur Muhlen A (1979) The rat as a model for the study of drug effects on thyroid function: Consideration of methodological problems. *Pharmacology and Therapeutics*, **5**:305–318.

Flowers LJ (1990) *Micronucleus assay with MON 13200: ML-88-390/EHL Study No. 88230.* Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275537).

Gabriele R, Letizia C, Borghese M, De Toma G, Celia M, Izzo L, Cavalla A (2003) Thyroid cancer in patients with hyperthyroidism. *Hormone Research*, **60**(2):79–83.

Hard GC (1998) Recent developments in the investigation of thyroid regulation and thyroid carcinogenesis. *Environmental Health Perspectives*, **106**(8):1–21.

Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF (1989) Thyroid follicular cell carcinogenesis. *Fundamental and Applied Toxicology*, **12**(4):629–697.

Hood A, Liu YP, Gattone VH 2nd, Klaassen CD (1999) Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicological Sciences*, **49**:263–271.

Hotz KJ, Wilson AG, Thake DC, Roloff MV, Capen CC, Kronenberg JM, Brewster DW (1997) Mechanism of thiazopyr-induced effects on thyroid hormone homeostasis in male Sprague-Dawley rats. *Toxicology and Applied Pharmacology*, **142**:133–142.

Hurley PM, Hill RN, Whiting RJ (1998) Mode of carcinogenic action of pesticides inducing thyroid follicular-cell tumors in rodents. *Environmental Health Perspectives*, **106**(8):437–445.

IARC (2001) *Some thyrotropic agents*. Lyon, International Agency for Research on Cancer, 763 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 79).

Li AP, Myers CA (1989) CHO/HGPRST gene mutation assay with MON 13200: Study No. ML-88-382/EHL No. 88071. Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275536).

Lichtenstein CK, Hemminki K (2002) Environmental and heritable cause of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. *International Journal of Cancer*, **1099**(2):260–266.

Liu J, Liu Y, Barter RA, Klaassen CD (1995) Alteration of thyroid homeostasis by UDPglucuronosyltransferase inducers in rats: A dose–response study. *Journal of Pharmacology and Experimental Therapeutics*, **273**:977–985.

Masubuchi N, Hakusui H, Okazaki O (1997) Effects of proton pump inhibitors on thyroid hormone metabolism in rats: A comparison of UDP-glucuronyltransferase induction. *Biochemical Pharmacology*, **54**(11):1225–1231.

Mazzaferri EL (2000) Thyroid cancer and Graves' disease: The controversy ten years later. *Endocrine Practice*, **6**:221–225.

McClain RM (1992) Thyroid gland neoplasia: Non-genotoxic mechanisms. *Toxicology Letters*, **64/65**:397–408.

McClain RM (1995) Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research*, **333**(1–2):131–142.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**(6):591–654.

#### Harmonization Project Document No. 4

Hood A, Liu YP, Gattone VH 2nd, Klaassen CD (1999) Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicological Sciences*, **49**:263–271.

#### HoodAらの文献が欠落

Hotz KJ, Wilson AG, Thake DC, Roloff MV, Capen CC, Kronenberg JM, Brewster DW (1997) Mechanism of thiazopyr-induced effects on thyroid hormone homeostasis in male Sprague-Dawley rats. *Toxicology and Applied Pharmacology*, **142**:133–142.

Hurley PM, Hill RN, Whiting RJ (1998) Mode of carcinogenic action of pesticides inducing thyroid follicular-cell tumors in rodents. *Environmental Health Perspectives*, **106**(8):437–445.

IARC (2001) *Some thyrotropic agents*. Lyon, International Agency for Research on Cancer, 763 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 79).

Li AP, Myers CA (1989) CHO/HGPRST gene mutation assay with MON 13200: Study No. ML-88-382/EHL No. 88071. Testing facility: Monsanto's Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto, St. Louis, MO (MRID No. 42275536).

Lichtenstein CK, Hemminki K (2002) Environmental and heritable cause of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. *International Journal of Cancer*, **1099**(2):260–266.

Liu J, Liu Y, Barter RA, Klaassen CD (1995) Alteration of thyroid homeostasis by UDPglucuronosyltransferase inducers in rats: A dose–response study. *Journal of Pharmacology and Experimental Therapeutics*, **273**:977–985.

Masubuchi N, Hakusui H, Okazaki O (1997) Effects of proton pump inhibitors on thyroid hormone metabolism in rats: A comparison of UDP-glucuronyltransferase induction. *Biochemical Pharmacology*, **54**(11):1225–1231.

Mazzaferri EL (2000) Thyroid cancer and Graves' disease: The controversy ten years later. *Endocrine Practice*, 6:221–225.

McClain RM (1992) Thyroid gland neoplasia: Non-genotoxic mechanisms. *Toxicology Letters*, 64/65:397–408.

McClain RM (1995) Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutation Research*, **333**(1–2):131–142.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**(6):591–654.

Naylor MW, McDonald MM (1992) *Chronic study of MON 13200 administered in feed to albino rats. Project No. ML-88-247/EHL 88148.* Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-24).

Naylor MW, Raju NR (1992) Chronic study of MON 13200 administered in feed to albino mice. Project No. ML-88-248/EHL 88147. Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-23).

Olsen JH, Wallin H, Boice JD, Rask K, Schulgen G, Fraumaen FF Jr (1993) Phenobarbital, drug metabolism and human cancer. *Cancer Epidemiology, Biomarkers and Prevention*, **5**:449–452.

Oropeza-Hernandez LF, Lopez-Romero R, Albores A (2003) Hepatic CYP1A, 2B, 2C, 2E and 3A regulation by methoxychlor in male and female rats. *Toxicology Letters*, **144**(1):93–103.

Ron E, Kleinerman RA, Boice JD, LiVolsi VA, Flannery JT, Fraumeni JF Jr (1987) A population-based case–control study of thyroid cancer. *Journal of the National Cancer Institute*, **79**:1–12.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Sundseth SS, Waxman DJ (1992) Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid omega-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. *Journal of Biological Chemistry*, **267**(6):3915–3921.

Thomas GA, Williams ED (1999) Thyroid stimulating hormone (TSH)-associated follicular hypertrophy and hyperplasia as a mechanism of thyroid carcinogenesis in mice and rats. In: Capen CC, Dybing E, Rice JM, Wilbourn JD, eds. *Species differences in thyroid gland, kidney and urinary bladder carcinogenesis*. Lyon, International Agency for Research on Cancer, pp. 45–59 (IARC Scientific Publications No. 147).

USEPA (1998) *Assessment of thyroid follicular cell tumors*. Washington, DC, United States Environmental Protection Agency, Office of Research and Development, Risk Assessment Forum (EPA/630/R-97/002; http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=13102; accessed 22 November 2004).

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Naylor MW, McDonald MM (1992) Chronic study of MON 13200 administered in feed to albino rats. Project No. ML-88-247/EHL 88148.

Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-24).

Naylor MW, Raju NR (1992) Chronic study of MON 13200 administered in feed to albino mice. Project No. ML-88-248/EHL 88147.

Testing facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, MO. Submitted by Monsanto Agricultural Company, St. Louis, MO (MRID No. 426197-23).

Olsen JH, Wallin H, Boice JD, Rask K, Schulgen G, Fraumaen FF Jr (1993) Phenobarbital, drug metabolism and human cancer. *Cancer Epidemiology, Biomarkers and Prevention*, **5**:449–452.

Oropeza-Hernandez LF, Lopez-Romero R, Albores A (2003) Hepatic CYP1A, 2B, 2C, 2E and 3A regulation by methoxychlor in male and female rats. *Toxicology Letters*, **144**(1):93–103.

Ron E, Kleinerman RA, Boice JD, LiVolsi VA, Flannery JT, Fraumeni JF Jr (1987) A population-based case–control study of thyroid cancer. *Journal of the National Cancer Institute*, **79**:1–12.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Sundseth SS, Waxman DJ (1992) Sex-dependent expression and clofibrate inducibility of cytochrome P450 4A fatty acid omega-hydroxylases. Male specificity of liver and kidney CYP4A2 mRNA and tissue-specific regulation by growth hormone and testosterone. *Journal of Biological Chemistry*, **267**(6):3915–3921.

Thomas GA, Williams ED (1999) Thyroid stimulating hormone (TSH)-associated follicular hypertrophy and hyperplasia as a mechanism of thyroid carcinogenesis in mice and rats. In: Capen CC, Dybing E, Rice JM, Wilbourn JD, eds. *Species differences in thyroid gland, kidney and urinary bladder carcinogenesis.* Lyon, International Agency for Research on Cancer, pp. 45–59 (IARC Scientific Publications No. 147).

USEPA (1998) Assessment of thyroid follicular cell tumors. Washington, DC, United States Environmental Protection Agency, Office of Research and Development, Risk Assessment Forum (EPA/630/R-97/002; http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=13102; accessed 22 November 2004).

## 4-AMINOBIPHENYL AND DNA REACTIVITY: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Samuel M. Cohen, Alan R. Boobis, M.E. (Bette) Meek, R. Julian Preston, & Douglas B. McGregor

The International Programme on Chemical Safety (IPCS) Human Relevance Framework (HRF) was evaluated for a DNA-reactive (genotoxic) carcinogen, 4-aminobiphenyl, based on a wealth of data in animals and humans. The mode of action (MOA) involves metabolic activation by *N*-hydroxylation, followed by *N*-esterification leading to the formation of a reactive electrophile, which binds covalently to DNA, principally to deoxyguanosine, leading to an increased rate of DNA mutations and ultimately to the development of cancer. In humans and dogs, the urinary bladder urothelium is the target organ, whereas in mice, it is the bladder and liver; in other species, other tissues can be involved. Differences in organ specificity are thought to be due to differences in metabolic activation versus inactivation. Based on qualitative and quantitative considerations, the MOA is possible in humans. Other biological processes, such as toxicity and regenerative proliferation, can significantly influence the dose–response of 4-aminobiphenyl-induced tumours. Based on the IPCS HRF, 4-aminobiphenyl would be predicted to be a carcinogen in humans, and this is corroborated by extensive epidemiological evidence. The IPCS HRF is useful in evaluating DNA-reactive carcinogens.

4-Aminobiphenyl is carcinogenic when administered to several species by a variety of routes (IARC, 1972, 1986, 1987). It was selected as a chemical for a case-study for the International Programme on Chemical Safety (IPCS) Human Relevance Framework (HRF) as a representative DNA-reactive carcinogen because of its established mode of action (MOA) in animal models, based on substantial data available evaluating its metabolic activation, DNA reactivity, genotoxicity, and carcinogenicity. It is also similar to numerous known animal and human carcinogens belonging to the chemical class of aromatic amines (structure–activity relationships), and there are extensive epidemiological, metabolic, and biochemical data in humans. This case-study illustrates the nature of data that are helpful in delineating MOAs for DNA-reactive carcinogens. Distinction between modulating factors and key events in an MOA analysis is also presented.

Based on the strong animal evidence and extensive epidemiological data, the International Agency for Research on Cancer (IARC) has classified 4-aminobiphenyl as a known human carcinogen (IARC, 1972, 1987). Although initially identified as a human urinary bladder carcinogen in individuals exposed to high levels occupationally, it has subsequently been demonstrated as a major component of cigarette smoke, leading to an increased risk of urinary bladder cancer in cigarette smokers (Del Santo et al., 1991; Curigliano et al., 1996). Additional research has shown that it is a ubiquitous environmental chemical occurring naturally when organic material containing nitrogen undergoes combustion.

4-AminobiphenylとDNAの反応性: 発がん MOA のヒトへの関連性を解析するための IPCS フレームワークを用いた事例研究1

## Samuel M. Cohen, Alan R. Boobis, M.E. (Bette) Meek, R. Julian Preston, & Douglas B. McGregor

DNA 反応性(遺伝毒性)発がん物質である 4-Aminobiphenyl を例に、動物とヒトにおける豊富なデ ータに基づいて国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒト 関連性フレームワーク(Human Relevance Framework: HRF)を評価した。この Mode of action (MOA) は、N-水酸化による代謝的活性化、N-エステル化による反応性親電子物質の生成を経て、この親電 子物質が DNA(主にデオキシグアノシン)と共有結合し、DNAの突然変異を促進させ、最終的に はがんの発生につながると考えられている。ヒトとイヌでは、膀胱が標的臓器であるのに対し、マ ウスでは膀胱と肝臓が標的臓器であり、他の種では他の組織が関与することもある。臓器特異性の 違いは、代謝的活性化と不活性化の違いによるものと考えられている。定性的及び定量的な考察か ら、ヒトの MOA が可能である。毒性や再生性増殖などの他の生物学的プロセスは、4-Aminobiphenyl 誘発性腫瘍の用量反応に大きく影響する可能性がある。IPCS HRF によれば、4-Aminobiphenyl はヒ トにおいて発がん物質であると予測され、これは広範な疫学的エビデンスによって裏付けられて いる。IPCS HRF は DNA 反応性発がん物質の評価に有用である。

4-Aminobiphenyl は、様々な経路で複数の種において発がん性が確認されている(IARC、1972 年、1986年、1987年)。4-Aminobiphenyl は、代謝的活性化、DNA 反応性、遺伝毒性、発がん性を 評価した多くのデータに基づき、動物モデルにおける MOA が確立されていることから、代表的 な DNA 反応性発がん物質として、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒト関連性フレームワーク(Human Relevance Framework: HRF)の事例研究対象 化学物質に選定された。また、芳香族アミンに属する既知の動物及びヒト発がん物質と類似して おり(構造活性相関)、ヒトにおける疫学的、代謝学的、生化学的データが豊富に存在している。 この事例研究は、DNA 反応性発がん物質の MOA を明確にするのに役立つデータの性質を示して いる。MOA 解析における調節因子と key eventsの区別についても示されている。

動物における有力なエビデンスと広範な疫学的データに基づいて、国際がん研究機関(IARC) は 4-Aminobiphenyl を既知のヒト発がん物質として分類した(IARC、1972 年、1987 年)。4-Aminobiphenyl は最初、職業的に高レベルにばく露されたヒトの膀胱発がん物質として同定された。 その後、タバコの煙の主成分として実証され、喫煙者の膀胱がんのリスク増加につながるといわ れている(Del Santo ら、1991 年; Curigliano ら、1996 年)。追加研究では、窒素を含む有機物が燃 焼する際に自然に発生し、至る所に存在する環境化学物質であることが示されている。

<sup>&</sup>lt;sup>1</sup> This article, to which WHO owns copyright, was originally published in 2006 in *Critical Reviews in Toxicology*, Volume 36, pages 803–819. It has been edited for this WHO publication and includes corrigenda.

<sup>&</sup>lt;sup>1</sup> この論文は、WHO が著作権を有するものであり、元々は 2006 年に Critical Reviews in Toxicology, Volume 36, pages 803-819 に掲載 されたものである。この論文は WHO の出版物のために編集されたもので、正誤表が含まれている。 44

#### **CARCINOGENICITY OF 4-AMINOBIPHENYL IN ANIMALS**

Experimental studies indicate that 4-aminobiphenyl is carcinogenic in mice, rats, rabbits, and dogs, although significant target tissue differences and susceptibility have been observed (IARC, 1972). By most routes of exposure, 4-aminobiphenyl is primarily a carcinogen of the liver and, to a lesser extent, the urinary bladder in mice, whereas in dogs (and humans), the urinary bladder appears to be the target organ. Many of the studies were conducted a number of years ago, and published accounts include only limited details. In addition, potential precursor lesions at interim periods were rarely documented, and none of the studies included protocols, such as stop/recovery, which might be informative in the context of MOA. Nonetheless, results indicate clear species and individual differences in response (e.g. Block et al., 1978), characteristic of MOAs entailing competing metabolic activation and deactivation processes (Table 1).

#### Table 1. Carcinogenicity studies of 4-aminobiphenyl in various species.

Species	Route/dose	Incidence	Comment	Reference
Mice	Gavage; 1 mg/week for 38 weeks	Bladder carcinomas in 2/12 mice surviving to 90 weeks		Clayson et al. (1965)
Mice	Gavage; 0 or 1.5 mg/week for 52 weeks	Bladder carcinomas in 1/21 exposed males vs 0/19 in controls; increased incidence of hepatomas in males and females		Clayson et al. (1967)
Mice	Subcutaneous injection of 200 µg for up to 52 weeks	Hepatomas in 19/20 males and 6/23 females after 48–52 weeks		Gorrod et al. (1968)
Mice (BALB/ cStCrlfC3Hf/ Nctr)	0–220 mg/l in drinking-water (males), 0–300 mg/l (females), for up to 96 weeks	Significant increases in urinary bladder carcino- mas (males only), hepatocellular carcino- mas (females only), and angiosarcomas (males and females)	Hyperplasia of the bladder in most mice of both sexes receiving 75 mg/l (females) and 55 mg/l (males) or greater, but none in controls	Schieferstein et al. (1985)
Mice (newborn B6C3F1)	Different regi- mens; injected prior to weaning	Liver tumours		Dooley et al. (1988, 1992); Von Tungeln et al. (1996); Parsons et al (2005)
Rats	Subcutaneous injection in arachis oil of total dose of 3.6–5.8 g/kg bw	Mammary and intestinal tumours		Walpole et al. (1952)

## 動物における 4-Aminobiphenyl の発がん性

実験的研究では、標的組織と感受性には有意な差が認められているものの、4-Aminobiphenylは マウス、ラット、ウサギ、イヌにおいて発がん性があることが示されている(IARC、1972年)。4-Aminobiphenylは、ほとんどのばく露経路において、マウスでは主に肝臓と、低頻度で膀胱に対し て発がん性が認められるが、イヌ(及びヒト)では膀胱が標的臓器であると考えられる。これら の研究の多くが数年前に行われたものであり、詳細な情報は限られたものしか公表されていない。 さらに、試験途中における潜在的な前駆病変はほとんど記録されておらず、どの研究にも MOA で 参考となる中止/回復などの研究計画書は含まれていなかった。それにもかかわらず、研究結果に は競合する代謝的活性化及び不活性化プロセスが競合する MOA に特徴的な反応における影響の 種及び個体差(例えば、ブロックら、1978年)が示されている(表1)。

## 表 1. 各種種における 4-Aminobiphenyl の発がん性試験

動物種	投与経路/用量	発生率	コメント	参考文献
マウス	経口投与;1 mg/週を38 週間投与	90 週まで生存した 2/12 匹に膀胱がん		Clayson et al. (1965)
マウス	経口投与;0または 1.5 mg/週を52週間投与	対照群 0/19 匹に対し、 雄 1/21 匹に膀胱がん; 雌雄とも肝臓がんの発 生率が増加		Clayson et al. (1967)
マウス	200 μg を最大 52 週間皮 下注射	48~52 週後の雄 19/20 匹、雌 6/23 匹に肝臓が ん		Gorrod et al. (1968)
マウス (BALB /cStCrl fC3Hf/ Nctr)	飲料水中 0-220 mg/L (雄)、0-300 mg/L (雌)を最大 96 週間投 与	膀胱がん(雄のみ)、肝 細胞がん(雌のみ)、血 管肉腫(雄雌)の有意 な増加	75 mgL (雌) 及び 55 mg/L (雄) 以上の投与 では、雌雄ともほとん どのマウスで膀胱の過 形成が認められたが、 対照マウスでは認めら れなかった。	Schieferste in et al. (1985)
マウス (新生 児 B6C3F 1)	異なる投与用法;離乳 前に注射された	肝臟腫瘍		Dooley et al.(1988, 1992); Von Tungeln et al. (1996); Parsons et
ラット	総量 3.6~5.8 g/kg bw の ピーナッツオイルを皮 下注射	乳腺腫瘍・腸管腫瘍		al. (2005) Walpole et al. (1952)

#### Table 1 (Contd)

Species	Route/dose	Incidence	Comment	Reference
Rabbits	Oral administra- tion of unspecified dose	Bladder papillomas in 1 animal and carcinomas in 3 animals	Earliest carcinoma observed 4 years after start of treat- ment	Bonser (1962)
Dogs (2)	Gelatin capsules 6 times weekly for life for a total dose of 30 or 34 g	Carcinoma of the bladders appeared in 33 months		Walpole et al. (1954)
Dogs	Gelatin capsules 0.3 g 3 times weekly (total dose: 94.5–144 g per dog)	Bladder carcinomas after 21–34 months		Deichmann et al. (1958)
Dogs (6)	1.0 mg/kg bw 5 times weekly for 34 or 37 months (total dose 5.5–7.0 g per dog)	3 bladder papillomas and 3 bladder carcinomas (transitional cell type)		Deichmann et al. (1965)
Dogs	Single dose	Ineffective in inducing bladder tumours over a 5-year period		Deichmann & MacDonald (1968)
Dogs (24 beagles)	Oral administra- tion 5 days/week for 3 years	Negative or minimal disease in 4 dogs, with no neoplasia in 2; neoplasia developed slowly in 11 dogs, while a rapidly progressive pattern was observed in the remaining 9 dogs		Block et al. (1978)

bw, body weight

Following its oral administration by gavage (1 mg per mouse per week for 38 weeks), 2/12 mice surviving to 90 weeks developed bladder carcinoma (Clayson et al., 1965). In a separate but similar experiment, dosing mice with 1.5 mg of 4-aminobiphenyl for 52 weeks resulted in bladder carcinoma in 1/21 male mice as compared with 0/19 in controls. In this experiment, the frequency of hepatomas in both male and female mice was significantly higher than that in the controls (Clayson et al., 1967). Three subcutaneous injections of mice with 200 µg of 4-aminobiphenyl produced hepatomas in 19/20 males and 6/23 females after 48-52 weeks (Gorrod et al., 1968). Oral administration of 4-aminobiphenyl in drinking-water at concentrations of up to 220 and 300 mg/l to male and female BALB/cStCrlfC3Hf/Nctr mice, respectively, for up to 96 weeks induced dose-related, significant increases in angiosarcomas (males and females), urinary bladder carcinomas (males only), and hepatocellular carcinomas (females only). Hyperplasia of the bladder was observed in most of the mice of both sexes in groups of about 118 receiving concentrations of 75 mg/l (females) and 55 mg/l (males) or greater, whereas none was reported in the control groups of similar size (Schieferstein et al., 1985). In a number of experiments, newborn B6C3F1 mice were primarily susceptible to

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## 表 1.(続き)

動物種	投与経路/用量	発生率	コメント	参考文献
ウサギ	不定量の経口投与	1匹に膀胱乳頭腫、3匹 に膀胱がん	投与開始から4年後に 観察された最も早いが ん	Bonser (1962)
イヌ(2)	ゼラチンカプセルを週6 回終身投与、合計30ま たは34g	膀胱がんが 33 ヶ月で出 現		Walpole et al. (1954)
イヌ	ゼラチンカプセル 0.3 g を週 3 回(総投与量: イヌ 1 匹あたり 94.5~ 144 g)	21~34ヶ月後に膀胱が ん		Deichman et al. (1958)
イヌ(6)	1.0 mg/kg bw を週 5 回、 34 ヵ月または 37 ヵ月間 (イヌ 1 頭あたりの総 投与量 5.5~7.0 g)。	膀胱乳頭腫 3 匹、膀胱 がん(移行上皮型)3 匹		Deichman et al. (1965)
イヌ	単回投与	5年間にわたり膀胱腫 瘍は誘発されなかった		Deichman & MacDonal (1968)
イヌ (24、ビ ーグル)	経口投与週5回、3年間	4 匹のイヌでは陰性ま たは軽症(2 匹で腫瘍 なし) 11 匹では腫瘍はゆっく りと進行したが、残り の9 匹では急速に進行		Block et al (1978)

経口投与(マウス1週あたり1mg、38週間)の後、90週まで生存した 2/12 のマウスに膀胱が んが発生した(Clayson 5、1965年)。別の実験では、マウスに 1.5 mg の 4-Aminobiphenyl を 52 週 間投与したところ、対照群では 0/19 匹であったのに対し、1/21 匹の雄マウスで膀胱がんが発生し た。この実験では、雄マウスと雌マウスの両方で肝臓がんの頻度が対照群よりも有意に高かった (Clayson 5、1967年)。4-Aminobiphenyl 200 µg をマウスに 3 回皮下注射したところ、48~52 週 後に 19/20 匹の雄及び 6/23 匹の雌で肝臓がんが生じた(Gorrod 5、1968年)。4-Aminobiphenyl を、 BALB/cStCrlfC3Hf/Netr マウスの雄と雌に、それぞれ 220 mg/L と 300 mg/L までの濃度で飲水投与 すると、投与量に相関して、血管肉腫(雄と雌)膀胱がん(雄のみ)、肝細胞がん(雌のみ)の有 意な増加が認められた。膀胱の過形成は、75 mg/L(雌)及び 55 mg/L(雄)以上の濃度の投与を 受けた約 118 匹の群では、両性のマウスのほとんどで観察されたが、対照群では報告されていな い (Schieferstein 5、1985年)。多くの実験において、新生児 B6C3F1 マウスは、4-Aminobiphenyl 投与後に、主に肝発がんに感受性があった(Dooley 5、1988年、1992年; Von Tungeln 5、1996 年; Parsons 6、2005年)。

liver carcinogenesis following 4-aminobiphenyl administration (Dooley et al., 1988, 1992; Von Tungeln et al., 1996; Parsons et al., 2005).

Daily subcutaneous injection of rats with 4-aminobiphenyl in arachis oil to a total dose of 3.6–5.8 g/kg body weight (bw) resulted in significant increases in the incidence of mammary gland and intestinal tumours (Walpole et al., 1952).

Among seven rabbits given commercial 4-aminobiphenyl orally (dose unstated), bladder papillomas were found in one and carcinomas in three animals. The earliest carcinoma was observed 4 years after the start of treatment (Bonser, 1962).

Two dogs fed 4-aminobiphenyl in gelatin capsules 6 times weekly for life (total dose per dog: 30, 34 g) developed carcinoma of the bladder in 33 months (Walpole et al., 1954). This was confirmed by similarly feeding capsules containing 4-aminobiphenyl (0.3 g per dog) 3 times weekly. Bladder carcinomas were observed after 21–34 months (total dose: 94.5–144.0 g per dog) (Deichmann et al., 1958). When the dose of 4-aminobiphenyl was reduced to 1.0 mg/kg bw and given to six dogs 5 times weekly for 34 months or 37 months (total dose: 5.5–7.0 g per dog), three bladder papillomas and three bladder carcinomas (transitional cell type) were observed (Deichmann et al., 1965). A single dose was not effective in inducing bladder tumours over a period of 5 years (Deichmann & MacDonald, 1968). Among 24 beagles that received 4-aminobiphenyl orally 5 days per week for 3 years, three basic patterns of bladder carcinogen responses were seen. Negative or minimal disease was seen in four dogs, of which two remained completely free of neoplasia. Neoplasia developed slowly in 11 dogs, while a rapidly progressive pattern was observed in the remaining 9 dogs (Block et al., 1978).

# IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH A MODE OF ACTION (MOA) IN ANIMALS?

The first question of the IPCS HRF is an evaluation of the animal MOA itself. This is based on the process delineated by the MOA Framework developed by IPCS and published in 2001 (Sonich-Mullin et al., 2001), which evolved from the Bradford Hill criteria for causality in epidemiology studies (Hill, 1965).

## A. Postulated mode of action

4-Aminobiphenyl is metabolized by hepatic enzymes to *N*-hydroxy-4-aminobiphenyl, which can be *N*-esterified (*N*-acetylated, *N*-glucuronidated, or *N*-sulfated) in hepatic and other tissues (Miller et al., 1961; Kadlubar et al., 1977, 1991; Miller & Miller, 1977; Delclos et al., 1987; Chou et al., 1995) (Figure 1). *O*-Esterification and ring hydroxylation are competing enzymatic reactions leading to detoxification. Tissue and species differences in the activity of these reactions dictate, at least in part, variations in susceptibility to the carcinogenic effects of 4-aminobiphenyl and differences in organ specificity in the development of tumours. Ultimately, a reactive electrophilic nitrenium ion is formed in the target tissue following *N*-esterification, and this is capable of forming DNA adducts. The principal DNA adduct is *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (Talaska et al., 1990; Kadlubar et al., 1991; Flammang et al., 1992; Hatcher & Swaminathan, 1995, 2002). As a consequence of the

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

ピーナッツオイル中に 4-Aminobiphenyl を混入して合計 3.6~5.8 g/kg 体重(bw)の用量をラットに毎日皮下注射すると、乳腺及び腸の腫瘍の発生率が有意に増加した(Walpole ら、1952年)。

市販の4-Aminobiphenylを経口投与(用量は記載なし)した7匹のウサギのうち、1匹に膀胱乳 頭腫が、3匹に膀胱がんが認められた。最も早い発がんは投与開始から4年後に観察された(Bonser、 1962年)。

4-Aminobiphenyl をゼラチンカプセルで週6回投与し続けた2匹のイヌ(1匹あたりの総投与量: 30、34g)は、33ヶ月で膀胱がんを発症した(Walpole ら、1954年)。同様に4-Aminobiphenylを含むカプセル(イヌ1匹あたり0.3g)を週3回与えても、21~34ヶ月後に膀胱がんが観察された(総投与量:イヌ1匹あたり94.5~144.0g)(Deichmann ら、1958年)。4-Aminobiphenylを1.0 mg/kg bwに減量し、6匹のイヌに週5回、34ヶ月または37ヶ月間投与したところ(総投与量:1匹当た り5.5~7.0g)、それぞれ3例で膀胱乳頭腫及び膀胱がん(移行上皮型)が観察された(Deichmann ら、1965年)。単回投与では、5年間で膀胱腫瘍は誘発されなかった(Deichmann & MacDonald、 1968年)。4-Aminobiphenylを週5日、3年間経口投与した24匹のビーグル犬では、膀胱発がん物 質の反応の基本的な3つのパターンが認められた。陰性または最小限の病変は4匹にみられ、そ のうちの2匹には腫瘍が発生しなかった。腫瘍は11匹のイヌでゆっくりと進行したが、残りの9 匹では急速に進行するパターンが観察された(Block ら、1978年)。

## 動物 MOA を立証するのにエビデンスの重み付けは十分か

IPCS HRF の最初の問いかけは、動物 MOA そのものの評価である。これは、疫学研究における 因果関係を説明するための Bradford Hill 基準 (Hill、1965 年)から発展し、IPCS が開発した、2001 年発表の MOA フレームワーク (Sonich-Mullin ら、2001 年)に示されている手順に基づいている。

#### A. 推定される MOA

4-Aminobiphenyl は、肝酵素によって N-hydroxy-4-aminobiphenyl に代謝され、肝及び他の組織に おいて N-エステル化 (N-アセチル化、N-グルクロン化、または N-硫酸化) される (Miller ら、1961; Kadlubar ら、1977 年、1991 年; Miller & Miller、1977 年; Delclos ら、1987 年; Chou ら、1995 年) (図 1)。O-エステル化及び環状水酸化は、無毒化につながる競合する酵素反応である。これらの 反応の活性における組織差や種差により、少なくとも部分的には、4-Aminobiphenyl の発がん作用 に対する感受性の違いや、腫瘍の発生における臓器特異性に違いを生じさせる。最終的には、N-エステル化の後、反応性親電子ニトレニウムイオンが標的組織に形成され、これが DNA 付加体を 形成しうる。主な DNA 付加体は、N-(deoxyguanosin-8-yl)-4-aminobiphenyl である (Talaska ら、1990 年; Kadlubar ら、1991 年; Flammmang ら、1992 年; Hatcher & Swaminather、1995 年、2002 年)。

mutations that can result from these reactions at critical sites of critical genes, neoplastic cells eventually develop.

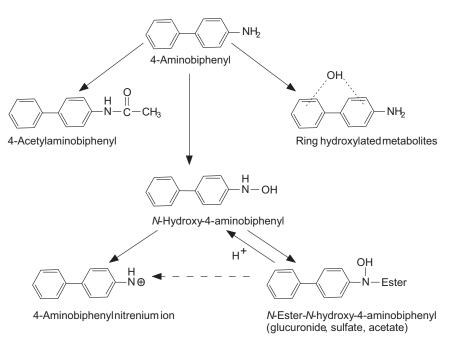


Figure 1. Metabolism of 4-aminobiphenyl

#### **B. Key events**

The major route of hepatic activation of 4-aminobiphenyl begins with its *N*-hydroxylation, catalysed, the balance of evidence indicates, by CYP1A2, at least in rats and humans (Butler et al., 1989b). In mice, there is evidence that CYP1A2 is not the only, or even the primary, form of cytochrome P-450 involved (Kimura et al., 1999). The *N*-hydroxylamine can also be produced by reaction with a variety of oxidases and peroxidases, such as by the prostaglandin synthase component of cyclo-oxygenase (Kadlubar et al., 1982). Whether any of these non-cytochrome P-450 reactions occur in vivo and are of toxicological significance remains unclear. The *N*-hydroxylamine undergoes *N*-acetylation by *N*-acetyltransferase-1 (NAT1) (Flammang & Kadlubar, 1986; Oda, 2004), resulting in an *N*-acetoxy ester that is unstable in acidic conditions, forming an arylnitrenium ion that can react directly with DNA, forming a DNA adduct at the C-8 position of guanine (Hammons et al., 1985; Flammang & Kadlubar, 1986; Hatcher & Swaminathan, 2002). Additionally, the *N*-hydroxylamine generated in liver can serve as a substrate for uridine diphosphate (UDP) glucuronosyltransferase (UGT),

これらの反応が重要な遺伝子の重要な部位で起こることにより突然変異がおこり、その結果、最 終的に腫瘍細胞が発生する。

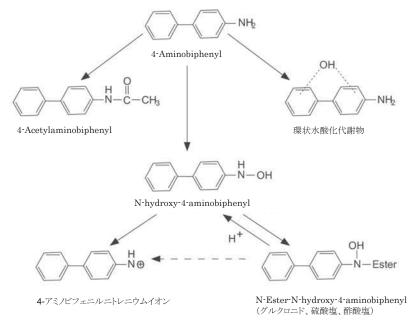


図 1.4-Aminobiphenyl の代謝

#### B. Key events

4-Aminobiphenylの肝活性化の主要経路は、少なくともラットとヒトでは CYP1A2 によって触媒 される 4-Aminobiphenylの N-水酸化から始まる (Butler ら、1989 年 b)。マウスでは、CYP1A2 や チトクローム P-450 が唯一もしくは主要に関与しているわけではないという報告がある (Kimura ら、1999 年)。また、N-hydroxylamine はシクロオキシゲナーゼのプロスタグランジン合成酵素 (Kadlubar ら、1982 年)のような様々なオキシダーゼ及びペルオキシダーゼとの反応によって生 成される。これらの非チトクローム P-450 反応のいずれかが生体内で起こるが、毒性学的に意義 があるかは不明なままである。N-hydroxylamine は、N-アセチルトランスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトランスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトシンスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトシンスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトシンスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトランスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトシンスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチルトシンスフェラーゼ 1 (NAT1) (Flammang & Kadlubar、1986 年;Oda、2004 年)による N-アセチル化を受け、酸性条件下では不 安定な N-アセトキシエステルを生じ、DNA と直接反応し得るアリルニトレニウムイオンを形成 し、グアニンの C-8 位に DNA 付加体を形成する (Hammons ら、1985 年;Flammang & Kadlubar、 1986 年;Hatcher & Swaminathan、2002 年)。さらに、肝臓で生成された N-hydroxylamine は、ウリ ジンニリン酸 (UDP) グルクロン酸転移酵素 (UGT)の基質として機能し、N-グルクロニド抱合 体を生じ、これが膀胱に運ばれる (Kadlubar ら、1977 年)。グルクロニドは尿中に排泄されるか、 酸性条件下において、加水分解後に膀胱内の N-hydroxylamine の追加供給源として機能する。N-ア セチルトランスフェラーゼ-2 (NAT2)による 4-Aminobiphenyl の N-アセチル化など、この反応と

yielding an *N*-glucuronide conjugate that is transported to the urinary bladder (Kadlubar et al., 1977). The glucuronide can either be excreted in urine or, under acidic conditions, serve as an additional source of the *N*-hydroxylamine in the urinary bladder, following hydrolysis. There are a number of reactions that can compete with this reaction scheme, including *N*-acetylation of 4-aminobiphenyl by *N*-acetyltransferase-2 (NAT2), but the resulting arylacetamide is a poor substrate for CYP1A2, and it is considered to be primarily a detoxification reaction. As a consequence, *N*-acetylation of the parent amine is considered a deactivating process. Rates of acetylation can thus affect the balance between activation and deactivation. Humans phenotypically are either rapid or slow acetylators (Lower et al., 1979). Mouse strains exist that are analogous to human slow and rapid acetylators. Thus, C57BL/6 is a rapid acetylator strain, while A/J is a slow acetylator (Hein, 1988). Interest in these differences includes a possible explanation for interspecies, interstrain, and interindividual differences in response. As a consequence of the DNA adducts formed, mutations can be produced. The key events are summarized in Table 2.

#### Table 2. Key events in the carcinogenicity of 4-aminobiphenyl in animals.

1. Metabolic activation

a) N-Hydroxylation

b) N-Esterification (glucuronide, acetyl, sulfate)

c) Hydrolysis to nitrenium ion

- 2. DNA adduct formation (dG-C8, dA-C8, dG-N2) in pluripotential cell of target organ
- 3. DNA mutation in critical gene(s) leading to cancer
- 4. Cancer

dA, deoxyadenosine; dG, deoxyguanosine

## **C.** Dose-response relationship

In view of the fact that many of the relevant studies were conducted a number of years ago, data on concordance of dose-response for precursor lesions for tumours are restricted to hyperplasia in the mouse urinary bladder. Dogs do not develop bladder tumours after a single dose of 4-aminobiphenyl (Deichmann & MacDonald, 1968), and there do not appear to have been studies of dose-response relationships in this species following multiple exposures. In the only study in which information on the incidence of precursor lesions was reported, male BALB/c mice were treated with drinking-water containing 4-aminobiphenyl at concentrations of 0, 7, 14, 28, 55, 110, or 220 mg/l for up to 96 weeks (Schieferstein et al., 1985). These treatments were associated with bladder carcinoma incidences of 0/116, 1/117, 1/118, 0/118, 6/115, 5/118, and 23/118, respectively. The incidences in the 55 mg/l group and higher were statistically significantly higher than in controls. Female mice were exposed to drinkingwater concentrations of 4-aminobiphenyl of 0, 7, 19, 38, 75, 150, and 300 mg/l. The corresponding incidences of bladder carcinomas were 0/118, 0/118, 0/118, 0/118, 0/118, 5/117, and 1/117. Incidences of hyperplasia were much higher, although severity was not indicated. In males, the incidences of hyperplasia were 0/116, 4/117, 9/118, 71/118, 108/115, 107/118, and 102/118 for doses of 0, 7, 14, 28, 55, 110, and 220 mg/l, respectively, and for females, 0/118, 0/118, 3/119, 53/119, 106/118, 97/117, and 83/117 for doses of 0, 7, 19, 38, 75, 150, and 300 mg/l, respectively. Thus, the dose-response curves for tumours and

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競合する反応が多数存在するが、得られるアリルアセトアミドは、CYP1A2 にとって貧弱な基質 であり、主に解毒反応であると考えられている。その結果、親アミンの N-アセチル化は不活性化 反応と考えられている。アセチル化の速度は、活性化と不活性化のバランスに影響を与える。ヒ トの表現型には、rapid acetylators と slow acetylators が存在する(Lower ら、1979 年)。ヒトのそれ ぞれのアセチレーターに類似したマウスの系統が存在し、C57BL/6 はrapid acetylators 系統であり、 A/J は slow acetylators 系統である(Hein、1988)。これらの違いに対する研究は、種間、系統間、 個体間での反応の違いを説明しうる。形成された DNA 付加体により、突然変異が生じる可能性が ある。key events を表 2 にまとめた。

#### 表 2. 動物における 4-Aminobiphenyl の発がん性における key events

1. 代謝的活性化

a) N-ヒドロキシル化 b) N-エステル化(グルクロニド、アセチル、硫酸塩) c) 加水分解してニトレニウムイオンにする

- 2. 標的臟器の多能性細胞における DNA 付加体形成(dG-C8, dA-C8, dG-N2)
- 3. がんにつながる重要な遺伝子の DNA 突然変異
- 4. 発がん

dA、デオキシアデノシン;dG、デオキシグアノシン

## C. 用量反応関係

関連する研究の多くが何年も前に実施されたという事実を考慮すると、腫瘍の前駆病変に対す る用量反応の一致に関するデータは、マウスの膀胱過形成に限定されている。イヌでは、4-Aminobiphenylの単回投与後に膀胱腫瘍が発生することはなく(Deichmann & MacDonald、1968 年)、 複数回投与後の用量反応関係の研究は行われていない。前駆病変の発生率に関する情報が報告さ れている唯一の研究では、雄の BALB/c マウスに 0、7、14、28、55、110、220 mg/L の濃度の 4-Aminobiphenylを含む飲料水を介して96週間まで処置したものがある(Schiefersteinら、1985年)。 これらの処置後の膀胱がん発生率は、それぞれ 0/116、1/117、1/118、0/118、6/115、5/118 及び 23/118 であった。55 mg/L 以上の群の発生率は、対照群に比べて統計的に有意に高かった。雌マウスに 4-Aminobiphenyl を濃度 0、7、19、38、75、150 及び 300 mg/Lの飲料水を介してばく露させると、 膀胱がんの発生率は、0/118、0/118、0/119、1/118、0/118、5/117及び1/117であった。重症度は示 されていなかったが、過形成の発生率ははるかに高かった。雄では、0、7、14、28、55、110及び 220 mg/L の投与量で、過形成の発生率は 0/116、4/117、9/118、71/118、108/115、107/118 及び 102/118 であった。雌では、それぞれ0、7、19、38、75、150及び300mg/Lの用量で0/118、0/118、3/119、 53/119、106/118、97/117 及び 83/117 であった。このように、腫瘍及び過形成の用量反応曲線は、 シグモイド型またはホッケースティック型であった。対照的に、尿路上皮細胞のC-8グアニンDNA 付加体の平衡レベルは直線的な用量反応関係を示した(Poirier ら、1995年)。

同研究(Schieferstein ら、1985年)では、雄では肝腫瘍の発生率の増加は認められなかったが、 雌では、それぞれ0、7、19、38、75、150及び300mg/Lの投与量で、肝腫瘍(腺腫及びがんを合 わせたもの)の発生率は0/117、0/120、2/120、4/119、11/119、17/118及び10/117であった。様々 な組織を合わせた血管肉腫の発生率は、雄と雌の高い3用量で増加したが、発生率は雄よりも雌 の方がやや高かった。

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hyperplasia were sigmoidal or hockey stick-shaped. In contrast, steady-state levels of urothelial C-8 guanine DNA adducts showed a linear dose–response (Poirier et al., 1995).

In this same study (Schieferstein et al., 1985), there was no increase in the incidence of liver tumours in the males, whereas in the females, the incidences of liver tumours (adenomas and carcinomas combined) were 0/117, 0/120, 2/120, 4/119, 11/119, 17/118, and 10/117 at doses of 0, 7, 19, 38, 75, 150, and 300 mg/l, respectively. The incidence of angiosarcomas of various tissues combined was also increased at the three highest doses in males and females, although the incidences were somewhat higher in females than in males.

#### **D.** Temporal relationship

Establishing time sequences for events in a carcinogenic process is partially, but to an important extent, dependent upon the sensitivity of the available methods for their measurement. Thus, tumours must attain a size allowing their histological detection, while the measurement of mutations and DNA adducts requires not only time but sufficient tissue. Consequently, the latter are more usually studied in liver than in urinary bladder, where the paucity of tissue available in the urothelium, particularly in rodents, causes technical difficulties that have no connection with the frequency of the biochemical and biological events. The metabolism and formation of DNA adducts are early events, which can be observed within a few minutes or hours in vitro and within a day following in vivo treatment with 4aminobiphenyl (e.g. Kadlubar et al., 1991; Swaminathan & Reznikoff, 1992; al-Atrash et al., 1995; Hatcher & Swaminathan, 1995; Doerge et al., 1999; Tsuneoka et al., 2003). Many in vivo experiments, however, continue exposure for 3-4 weeks to allow an accumulation of adducts, achieve steady-state levels, and facilitate their detection (e.g. Talaska et al., 1990; Flammang et al., 1992; Poirier & Beland, 1992; Poirier et al., 1995; Underwood et al., 1997). Mutations can also be detected within a short time in vitro, but have generally not been detected in vivo in target tissues until after several weeks or months of exposure (e.g. H-ras in mouse liver: Parsons et al., 2002), although this comparatively long period may not be a true reflection of when mutations first arise. In one study, mutations were detected in a Muta<sup>TM</sup>Mouse urinary bladder assay 14 days after a single dose of 4-aminobiphenyl (Fletcher et al., 1998). Carcinomas and hyperplasia of the urinary bladder are apparently late-occurring lesions in mice and dogs; however, time course changes have not been systematically evaluated. Although mice were killed at intervals beginning at 13 weeks in one 2-year study, and hyperplastic lesions were induced in the urinary bladder, their incidences at different times were not presented (Schieferstein et al., 1985). Tumours in the urinary bladder are commonly not discovered until after about 2 years in mice (Schieferstein et al., 1985) and longer in dogs (Walpole et al., 1954; Deichmann et al., 1958, 1965). However, neoplastic transformation of human urothelial cells (infected with SV40) treated in vitro with 4aminobiphenyl followed by in vitro culture for 6 weeks was demonstrated upon their inoculation into nude mice (Bookland et al., 1992b).

## E. Strength, consistency, and specificity of association of the tumour

## response with key events

Evidence in support of the association of the tumour response with key events comes only in part from studies on bladder; considerable evidence is provided by studies on liver. DNA adduct formation has been demonstrated in both tissues.

#### D. 時間的関連性

発がん過程における事象の順序を確立することは、その測定方法の感度に部分的ではあるが大 いに依存する。したがって、腫瘍は組織学的に検出できる大きさに達していなければならず、一 方で突然変異や DNA 付加体の測定には時間だけでなく十分な量の組織が必要である。その結果、 後者は膀胱よりも肝臓で研究されるのが一般的だが、特にげっ歯類では尿道の組織が少ないため、 生化学的・生物学的事象の頻度とは関係のない技術的な困難が生じている。DNA 付加体の代謝及 び形成は初期の事象であり、4-Aminobiphenyl による in vitro 処理後、数分または数時間以内及び1 日以内に観察される(Kadlubar ら、1991年; Swaminathan & Reznikoff、1992年; al-Atrash ら、1995 年: Hatcher & Swaminathan、1995年: Doerge ら、1999年: Tsuneoka ら、2003年)。しかしながら、 多くの in vivo 実験では、付加体を蓄積させ、平衡レベルに達し、検出を容易にするために、3~4 週間ばく露を継続する(例えば、Talaska ら、1990年; Flammang ら、1992年; Poirier & Beland、 1992 年; Poirier ら、1995 年; Underwood ら、1997 年)。また、突然変異は in vitro では短時間で検 出され得るが、生体内では数週間または数ヶ月のばく露後でないと検出されない(例えば、マウ ス肝臓における H-ras; Parsons ら、2002 年)。この期間は、突然変異が最初に生じた時期を正確に 反映しているとは限らない。ある研究では、4-Aminobiphenyl の単回投与から 14 日後に Muta™Mouse 膀胱アッセイで突然変異が検出された(Fletcher ら、1998年)。膀胱のがんや過形成 は、マウスやイヌでは明らかに遅発性の病変であるが、時間経過の変化は体系的に評価されてい ない。ある2年間の研究では、マウスを13週から一定間隔で安楽殺し、膀胱に渦形成病変を誘導 したが、異なる時期の発生率は示されていない(Schieferstein ら、1985)。膀胱の腫瘍は、一般的 に、マウスでは約2年後(Schieferstein ら、1985年)、イヌではそれより長い時間が経過するまで 発見されない(Walpole ら、1954 年; Deichmann ら、1958 年、1965 年)。しかし、ヒトの(SV40 に感染した)尿道粘膜細胞を 4-Aminobiphenyl で in vitro 処理し、6 週間 in vitro 培養した後、ヌー ドマウスに接種した場合、腫瘍性の形質転換が認められた(Bookland ら、1992 年 b)。

#### E. 腫瘍反応と key events との関連性の強さ、一貫性、特異性

腫瘍反応と key events との関連性を支持するエビデンスは、膀胱に関する研究から得られたものがあるが、肝臓に関する研究からも有力なエビデンスが得られている。DNA 付加体の形成は両 組織で実証されている。

There is an abundance of studies that demonstrate that 4-aminobiphenvl is a mutagen. including positive mutagenicity with certain frameshift mutation and base pair substitutionsensitive strains (TA1538, TA98, and TA100) of Salmonella typhimurium, but only in the presence of rodent liver S9 metabolic activating preparations. The requirement for S9 metabolic activation clearly demonstrates the lack of DNA reactivity and mutagenicity of the parent amine. In addition, 4-aminobiphenyl induces unscheduled DNA synthesis in rat liver cells in vitro (United States Environmental Protection Agency Genetic Activity Profiles). These in vitro studies provide evidence that 4-aminobiphenyl can cause genetic damage following metabolic activation. Bacterial mutation studies have also been conducted comparing metabolic activation systems based on liver homogenates from Aroclor 1254-induced male Sprague-Dawley rats and C57BL/6 mice, using S. typhimurium TA100 tester strains that expressed different levels of N- and O-acetyltransferase (OAT) activity (Dang & McQueen, 1999). TA100 has a single copy of the NAT/OAT gene; YG1029 has multiple copies of the NAT/OAT gene, and TA100/1,8DNP<sub>6</sub> is NAT/OAT-deficient. Effects with mouse and rat S9 were similar (but the effects of Aroclor 1254 treatment were not examined). Using either 4aminobiphenyl or 4-acetylaminobiphenyl as substrates, considerably more mutations were induced in YG1029 than in TA100 or TA100/1.8DNP<sub>6</sub>, in which mutation induction was similar. This supports a role for high acetylation activity in mutation induction by the Nhydroxylamine in these bacteria.

The non-enzymatic step to an arylnitrenium ion in the mechanism of mutagenesis in vivo is supported by the observation that *N*-hydroxy-4-aminobiphenyl mutagenesis in the high OAT-expressing *S. typhimurium* TG1024 strain is dependent on the pH of the medium, with an inverse relationship between mutant numbers and pH over the range 4.0–8.0 (Sarkar et al., 2002).

Administration of 4-aminobiphenyl in the drinking-water of BALB/c mice for 28 days resulted in higher levels of DNA adducts in liver than in urinary bladder of females, while the reverse occurred in males. Thus, in each sex, the DNA adduct level correlated with the susceptibility of the tissue to tumour induction by 4-aminobiphenyl (Poirier et al., 1995). However, the shape of the dose–response curve was linear for DNA adducts in both tissues (although it appears to saturate and is relatively flat in female mice), whereas the tumour dose–response curve was sigmoidal (Poirier et al., 1995).

Adduct levels were also highest in the urinary bladder of female Hsd:ICR(Br) mice that were dosed topically (the more usual exposure route in occupational settings) with 50 nmol 4-aminobiphenyl for 21 weeks. The principal adduct in all tissues examined (bladder, liver, lung, and skin) co-chromatographed with *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (Underwood et al., 1997).

One study of mutagenesis in male Muta<sup>TM</sup>Mouse transgenic mice (i.e. transgenic CD2F, [BALB/c × DBA/2]) treated orally with 4-aminobiphenyl at 10 mg/kg bw per day for 10 days reported that the mutation frequencies in urinary bladder, liver, and bone marrow were increased by 13.7-, 4.8-, and 2.4-fold, respectively (Fletcher et al., 1998).

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

4-Aminobiphenyl が変異原性物質であることを示す研究は豊富にあり、S. typhimurium 菌の特定 のフレームシフト型突然変異及び塩基対置換感受性株(TA1538、TA98及びTA100)に対する陽性 反応が、げっ歯類肝 S9 代謝的活性化製剤の存在下でのみ認められている。S9 による代謝的活性 化が必要であるということは、親アミンの DNA 反応性及び変異原性の欠如を示している。さら に、4-Aminobiphenylは、in vitro でラット肝細胞において不定期 DNA 合成を誘導する(米国環境 保護庁遺伝的活性プロファイル)。これらの in vitro 研究は、4-Aminobiphenyl が代謝的活性化後に 遺伝的損傷を引き起こす可能性があるというエビデンスを示している。また、細菌を用いた突然 変異試験でも代謝的活性化システムの解析が行われている (Dang & McQueen、1999)。Aroclor 1254 誘発雄 Sprague-Dawley ラット及び C57BL/6 マウスの肝臓ホモジネート及び、異なるレベルの N-及び O-アセチルトランスフェラーゼ (OAT) 活性を発現する S. typhimurium TA100 試験菌株が使 用された。TA100 は NAT/OAT 遺伝子のシングルコピーを有し、YG1029 は NAT/OAT 遺伝子のマ ルチコピーを有し、TA100/1.8DNP6 は NAT/OAT 欠損株である。マウス及びラット S9 を用いた効 果は類似していた(ただし、Aroclor 1254 処理の効果は調べなかった)。4-Aminobiphenyl または 4-Acetylaminobiphenyl を基質とした場合、YG1029 では TA100 または TA100/1,8DNP6 よりも多く突 然変異が誘発された。このことは、これらの細菌における N-hydroxylamine による突然変異誘発に は、高いアセチル化活性が関与していることを示唆している。

生体内での突然変異誘発機構におけるアリルニトレニウムイオンへの非酵素的ステップは、高 OAT-発現 S. typhimurium TG1024 株における N-hydroxy-4-aminobiphenyl の突然変異誘発が培地の pH に依存し、4.0~8.0 の範囲で突然変異体数と pH との間に逆相関の関係が観察されたことによ って支持される (Sarkar ら、2002 年)。

BALB/c マウスに 4-Aminobiphenyl を 28 日間飲水投与したところ、雌では膀胱よりも肝臓の方 が DNA 付加体の量が多かったのに対し、雄では逆の結果が得られた。このように、各性におい て、DNA 付加体のレベルは、4-Aminobiphenyl による腫瘍誘導に対する組織の感受性と相関して いた (Poirier ら、1995 年)。しかしながら、用量反応曲線の形状は、両組織の DNA 付加体では直 線的であった (雌マウスでは飽和して比較的平坦)のに対し、腫瘍の用量反応曲線はシグモイド 型であった (Poirier ら、1995 年)。

付加体の量は、50 nmol の 4-Aminobiphenyl を 21 週間にわたって局所投与した Hsd:ICR(Br)マウ スの雌マウスの膀胱で最も高かった (一般的な職業的ばく露経路)。検査した全組織 (膀胱、肝臓、 肺、皮膚)の主な付加体は、N-(deoxyguanosin-8-yl)-4-aminobiphenyl とのコクロマトグラフィーを 用いて検査した (Under-wood ら、1997 年)。

雄の Muta<sup>™</sup>Mouse トランスジェニックマウス(すなわち、トランスジェニック CD2F、 [BALB/c×DBA/2]) に 10 mg/kg bw/日の 4-Aminobiphenyl を 10 日間経口投与した突然変異誘発の研 究では、膀胱、肝臓及び骨髄における突然変異頻度がそれぞれ 13.7 倍、4.8 倍及び 2.4 倍に増加し たことが報告されている(Fletcher ら、1998 年)。

Newborn B6C3F1 (C57BL/6 × C3H) mice responded to treatment with 4-aminobiphenyl by developing a high frequency of liver tumours, many of which carried H-*ras* codon 61 CAA  $\rightarrow$  AAA mutations (Parsons et al., 2005). In vivo, the level of one major DNA adduct [*N*-(deoxyguanosin-8-yl)-4-aminobiphenyl] was present at 5 adducts/10<sup>6</sup> nucleotides in newborn mice treated with 0.3 µmol 4-aminobiphenyl 24 h earlier. After 8 months, the CAA  $\rightarrow$  AAA mutation was detected in 67% of the treated mice and 50% of the vehicle (dimethyl sulfoxide, or DMSO) controls, but the average mutant fraction in treated mice was 45 × 10<sup>-5</sup> compared with only 2 × 10<sup>-5</sup> in controls. After 12 months, liver tumours had developed in 79% of the treated mice and in 8% of the controls. These tumours are not those of the human target organ, but the results of this study support the general MOA proposed for bladder carcinogenesis (i.e. DNA adduct formation, followed by mutation in a key gene and the subsequent emergence of tumours).

Dogs (sex not stated) killed 24 h after a single oral dose of 4-aminobiphenyl (5 mg/kg bw) had 5.4 fmol DNA adducts/ug liver DNA and 4.8 fmol DNA adducts/ug urinary bladder DNA, whereas no DNA adducts were detected in either the liver or bladder of a dog whose bladder had been instilled with 4-aminobiphenyl. In contrast, a dog bladder instilled with the reactive intermediate N-hydroxy-4-aminobiphenyl had 3.9 fmol DNA adducts/µg bladder DNA and no detectable adducts in liver DNA. Quantification was by an immunochemical method (Roberts et al., 1988). Examination of bitches treated with tritium-labelled 4aminobiphenyl (per os, intravenously, or intraurethrally), N-hydroxy-4-aminobiphenyl (intravenously or intraurethrally), or N-hydroxy-4-aminobiphenyl N-glucuronide (intravenously) demonstrated (1) the presence of 4-aminobiphenyl-haemoglobin adducts in blood erythrocytes; (2) that after per os dosing with 4-aminobiphenyl, the major portion of total Nhydroxy-4-aminobiphenyl entering the bladder lumen was free N-hydroxy-4-aminobiphenyl (0.7%), with lower concentrations of the acid-labile *N*-glucuronide (0.3%); (3) that urothelial DNA adducts following intraurethral instillation of N-hydroxy-4-aminobiphenyl were 60 times higher than after intraurethral instillation of 4-aminobiphenyl; and (4) that exposure to N-hydroxy-4-aminobiphenyl and subsequent 4-aminobiphenyl-DNA adduct formation are directly dependent on the frequency of urination and, to a lesser extent, on urinary pH (Kadlubar et al., 1991). The urinary pH of dogs may vary from about 4.5 to 7.5, depending upon the diet (Merck, 1998), time after eating, time of day, and amount of water consumed; these are factors that might influence the carcinogenic response (Cohen, 1995). Studies in vitro with microsomal preparations from dog liver and bladder have shown the presence of transacetylation activities in both organs, so that N-hydroxy-4-aminobiphenyl binding to RNA and DNA occurs in the presence of 4-acetylaminobiphenyl, N-hydroxy-4-acetylaminobiphenyl, or acetyl coenzyme A (CoA) as acetyl donors, although the levels of binding were less with bladder than with hepatic microsomes (Hatcher & Swaminathan, 1992).

Examination of urothelial cells exfoliated into urine of dogs treated with 4-aminobiphenyl showed that DNA adducts were identical to those from DNA modified in vitro with *N*-hydroxy-4-aminobiphenyl and from dog bladder urothelial DNA isolated from 4-aminobiphenyl-dosed dogs at autopsy. A dose-related increase in 4-aminobiphenyl–DNA adduct formation was demonstrated (Talaska et al., 1990).

新生児 B6C3F1(C57BL/6×C3H)マウスは、4-Aminobiphenyl の投与に反応して高頻度の肝腫瘍を 発症し、その多くは *H-ras* 遺伝子のコドン 61 における CAA→AAA 突然変異であった (Parsons 6, 2005 年)。in vivo では、0.3 µmol の 4-Aminobiphenyl を 24 時間前に投与した新生児マウスに おいて、主要な DNA 付加 *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl 量は、5 adducts /10<sup>6</sup> ヌクレオチ ドであった。8 ヶ月後、CAA→AAA 突然変異は、投与マウスの 67%及び対照(ジメチルスルホキ シド、または DMSO)の 50%で検出されたが、投与マウスの平均突然変異率は 45×10<sup>-5</sup> であったの に対し、対照ではわずか 2×10<sup>-5</sup> であった。12 ヵ月後には、投与マウスの 79%、対照マウスの 8% に肝腫瘍が発生していた。これらの腫瘍はヒトの標的臓器の腫瘍ではないが、本研究の結果は、 膀胱がんの発生において提示されている一般的な MOA(すなわち、DNA 付加体の形成、重要な 遺伝子の突然変異、その後の腫瘍の出現)を支持するものである。

4-Aminobiphenyl (5 mg/kg bw) を単回経口投与してから24 時間後に死亡したイヌ (性別は明記 されていない)の DNA 付加体は、肝臓で5.4 fmolDNA adducts/µg、膀胱で4.8 fmolDNA adducts/µg であった。しかし、4-Aminobiphenyl を膀胱に投与したイヌの肝臓と膀胱では DNA 付加体は検出 されなかった。一方、反応性中間体である N-hydroxy-4-aminobiphenyl を膀胱に投与したイヌの膀 胱では、3.9 fmol DNA adducts/µg の DNA 付加体が検出され、肝臓では付加体は検出されなかった。 免疫化学的方法で定量を行った (Roberts ら、1988 年)。トリチウム標識された 4-Aminobiphenyl (経 口、静脈内、または尿道内)、N-hydroxy-4-aminobiphenyl (静脈内、または尿道内)、N-hydroxy-4aminobiphenyl N-glucuronide (静脈内)を投与された雌イヌの試験では以下のことが実証された。 (1)血中赤血球中の 4-aminobiphenyl -ヘモグロビン付加体の存在 (2) 4-aminobiphenyl 投与後、膀 胱内腔に入る N-hydroxy-4-aminobiphenyl の大部分は遊離 N-hydroxy-4-aminobiphenyl を尿道内 に注入した後の DNA 付加体量は、4-Aminobiphenyl を尿道内に注入した後と比較して 60 倍多かっ たこと、(4) N-hydroxy-4-aminobiphenyl へのばく露及びそれに続く 4-Aminobiphenyl-DNA 付加体 の形成は、排尿の頻度及び尿の pH に依存すること (Kadlubar ら、1991 年)。

イヌの尿中 pH は、食事内容(Merck、1998 年)、食後からの時間、時間帯及び消費水量に依存 して、約4.5 から 7.5 まで変化することがあり、これらは発がん性に影響を及ぼす可能性のある因 子である(Cohen、1995)。イヌの肝臓及び膀胱からのミクロソーム調製物を用いた in vitro の研究 では、両臓器においてトランスアセチル化活性が存在することが示されており、そのため、4-Acetylaminobiphenyl、N-hydroxy-4-acetyl-aminobiphenyl、またはアセチルコエンザイム A(CoA)が アセチル供与体として存在する場合には、N-hydroxy-4-aminobiphenylの RNA 及び DNA への結合 が起こるが、結合量は肝臓ミクロソームよりも膀胱ミクロソームの方が少なかった(Hatcher & Swaminathan、1992 年)。

4. Aminobiphenyl を投与したイヌの尿に含まれる尿膜細胞を調べたところ、N-hydroxy-4aminobiphenyl で in vitro で修飾した DNA や、4-aminobiphenyl を投与したイヌの剖検時に単離した イヌの膀胱尿路上皮細胞の DNA と同一の DNA 付加体が検出された。4- aminobiphenyl-DNA 付加 体形成の用量依存的増加が示された(Talaska ら、1990 年)。

#### F. Biological plausibility and coherence

The observations that 4-aminobiphenyl can form adducts with DNA and that it is mutagenic in organs in which tumours develop indicate, in general terms, that the proposed MOA is plausible (Fletcher et al., 1998). In addition, *N*-hydroxy-4-aminobiphenyl is able to cause neoplastic transformation of non-tumorigenic SV40-immortalized human urothelial cells (Bookland et al., 1992b). The findings with 4-aminobiphenyl are also consistent with the vast literature regarding the metabolic activation, DNA adduct formation, mutagenesis, and urinary bladder carcinogenesis in several species (including humans) of several related aromatic amine chemicals (Kadlubar et al., 1977; Miller & Miller, 1977; Delclos et al., 1987). The lack of DNA adduct formation and mutagenicity of the parent amine in various in vitro systems without metabolic activation clearly demonstrates the requirement for metabolic activation. The same DNA adducts are identified in tissues after administration of the amine or following exposure to the *N*-hydroxyl metabolite, with the structure of the adducts having been chemically confirmed. The mutagenic potential of the specific C-8 guanine DNA adduct has also been demonstrated, although the specific biophysical aspects have been better demonstrated for structurally related aromatic amines such as 2-aminofluorene (Kriek, 1992).

#### G. Other modes of action

Alternatives of components of the already described MOA have been suggested. However, they do not detract from the overall described MOA but suggest either alternative specific aspects (such as other activating enzymes) or associative processes that could affect quantitative aspects. 4-Aminobiphenyl is oxidized by hepatic enzymes other than CYP1A2 (Kimura et al., 1999) to the *N*-hydroxylated metabolite that causes liver and urinary bladder toxicity and carcinogenesis, possibly including oxidases and peroxidases (Kadlubar et al., 1982, 1991). Although the specific enzymes involved in metabolic activation may vary, the ultimate sequence of generation of a reactive electrophile, DNA adduct formation, mutagenesis, and carcinogenesis is consistent. Furthermore, it is reasonable to believe that from this point in the MOA, the same sequence occurs as that involving CYP1A2-mediated activation, regardless of the activating enzyme.

In addition to bulky adducts, there is evidence to suggest that *N*-hydroxy-4-aminobiphenyl causes oxidative damage in urothelial DNA, possibly involving endogenous peroxidases (Burger et al., 2001). The relevance of this for the carcinogenic activity of 4-aminobiphenyl is unknown.

*N*-Hydroxy-4-aminobiphenyl and its further activated forms are cytotoxic to urothelial and other cells in vitro (Reznikoff et al., 1986), but the role that this plays in its carcinogenic effects is unclear (see below for discussion of a potentiating role in urothelial carcinogenesis, rather than causative role). It is likely that this process alters the dose–response relationship, but does not alter the fundamental MOA described above.

#### H. Assessment of the postulated mode of action

The early steps in the proposed MOA are well supported by the available evidence, and it has been judged that there is good and sufficient evidence that 4-aminobiphenyl is a urinary bladder carcinogen in dogs and mice, and in other tissues (primarily the liver) in rodents. Thus, it is metabolized to products that can form DNA adducts in the liver and in other target

### F. 生物学的妥当性及び整合性

4-Aminobiphenyl が DNA と付加体を形成し、腫瘍が発生する器官で変異原性を示すという結果 は、一般的に提案されている MOA の妥当性を示している (Fletcher ら、1998 年)。さらに、*N*hydroxy-4-aminobiphenyl は、腫瘍化していない SV40 不死化ヒト尿細管細胞の腫瘍化を引き起こす ことができる (Bookland ら、1992 年 b)。また、4-Aminobiphenyl に関する知見はいくつかの関連 芳香族アミンの代謝的活性化、DNA 付加体形成、突然変異誘発及びいくつかの種 (ヒトを含む) における膀胱発がんに関する膨大な文献と一致している (Kadlubar ら、1977 年; Miller & Miller、 1977 年; Delclos ら、1987 年)。代謝的活性化を伴わない様々な in vitro 系での親アミンの DNA 付 加体形成及び変異原性の欠如は、代謝的活性化の必要性を明確に示している。アミンの投与後、 または *N*-ヒドロキシル代謝物へのばく露後に組織内で同じ DNA 付加体が確認され、付加体の構 造が化学的に確認されている。特定の生物物理学的側面は 2-aminofluorene のような構造的に関連 する芳香族アミンの方がより明らかにされているが、C-8 グアニン DNA 付加体の変異原性につい ても明らかにされている、(Kriek、1992 年)。

#### G. その他の MOA

既に記載されている MOA の構成要素の代替的なものが提案されている。しかし、それらは MOA の全体的な説明を損なうものではなく、限局的側面(他の活性化酵素など)、または定量的側面に 影響を与える可能性のある過程のいずれかを示唆している。4-Aminobiphenyl は、CYP1A2 以外の 肝酵素(Kimura ら、1999年)によって N-水酸化代謝物に酸化され、肝臓及び膀胱の毒性及び発が んを引き起こすが、これには酸化酵素及びペルオキシダーゼが関与している可能性がある (Kadlubar ら、1982年、1991年)。代謝的活性化に関与する酵素は異なるかもしれないが、反応 性親電子物質の生成、DNA 付加体の形成、変異原性、発がんという順序は一貫している。さらに、 MOA のこの時点から、活性化酵素にかかわらず、CYP1A2 を介した活性化と同じ順序で起こると 考えるのが妥当である。

巨大な付加体に加えて、N-hydroxy-4-aminobiphenylは、内因性ペルオキシダーゼが関与している 可能性があり、尿道粘膜DNAに酸化的損傷を引き起こすことを示唆するエビデンスがある(Burger ら、2001年)。このことと 4-Aminobiphenyl の発がん活性との関連性は不明である。

N-hydroxy-4-aminobiphenyl 及びその活性型は、in vitro で尿道粘膜及び他の細胞に対して細胞毒性を示す(Reznikoff ら、1986年)が、これが発がん作用に果たす役割は不明である(原因ではなく、尿道粘膜発がんにおける増強的な役割については以下を参照のこと)。この過程で用量反応関係は変化するが、上述の基本的な MOA は変化しないと考えられる。

#### H. 推定される MOA の評価

推定される MOA の初期段階は、利用可能なエビデンスによって十分に裏付けられており、4-Aminobiphenyl はイヌやマウスでは膀胱発がん物質であり、げっ歯類では他の組織(主に肝臓)で の発がん物質である十分なエビデンスがあると判断されている。このように、肝臓や他の標的臓 器では DNA 付加体を形成しうる生成物に代謝され、突然変異が生じることが実証されている。

organs, and mutations have been demonstrated to arise. Although other organs can also be targets for 4-aminobiphenyl-induced neoplasia, the urinary bladder is the main target in dogs and in some strains of mice. Evidence for the intervening steps between general genotoxicity and the emergence of neoplasia is lacking. There is a notable lack of study of the effects of 4-aminobiphenyl on cell proliferation in the urinary bladder, but information on related aromatic amines and amides is available, particularly the analysis of the interaction between DNA reactivity (and mutagenesis) and cell proliferation induced by 2-acetylaminofluorene in mouse urinary bladder utilizing data from a megamouse, ED-01 study (Cairns, 1979; Gaylor, 1979; Littlefield et al., 1979). The reliance for mutagenicity on cell proliferation can provide an explanation for the sigmoidal shape of the tumour dose–response despite a linear dose–response for DNA adducts (Cohen & Ellwein, 1990). This link has significant implications for assessing potency and dose–response for 4-aminobiphenyl-induced urinary bladder cancer (see discussion below).

#### I. Uncertainties, inconsistencies, and data gaps

Bacterial mutation studies of 4-aminobiphenyl with metabolic activation have shown that most mutations are frameshifts, whereas a single study of sequence analysis of 4-aminobiphenyl-induced mutations in the *lacZ* gene in single-stranded DNA from a bacteriophage M13 cloning vector revealed exclusively base pair substitutions, with over 80% occurring at G sites:  $G \rightarrow T$  transversions predominated, followed by  $G \rightarrow C$  transversions and  $G \rightarrow A$  transitions. The major DNA adduct, *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl, was then inserted within the M13 genome, and the mutational frequency and specificity were measured after in vivo replication. The targeted mutational efficiency was approximately 0.01%, and the primary mutation was  $G \rightarrow C$  transversion. Thus, the observations are consistent with in vivo observations, but the mutagenic activity was weak (Verghis et al., 1997).

Most in vivo investigations have been in mice. Dogs, for understandable reasons, have received less attention, although this is the species that is more sensitive to bladder carcinogenesis. Mouse strain differences in response are evident: B6C3F1 and female BALB/cStCrlfC3Hf/Nctr are more susceptible to liver carcinogenesis, whereas male BALB/cStCrlfC3Hf/Nctr mice develop bladder tumours after exposure to 4-aminobiphenyl (Schieferstein et al., 1985; Dooley et al., 1988, 1992). Nevertheless, mouse strain effects have received relatively little attention in the available studies.

The enzyme considered as fundamental for the metabolism of 4-aminobiphenyl to a product that forms adducts with DNA in liver and bladder is CYP1A2 (Butler et al., 1989a, 1989b). However, comparison of responses in CYP1A2(+/+) wild-type mice with CYP1A2(-/-) knockout mice showed that, contrary to expectations, CYP1A2 expression was not associated with 4-aminobiphenyl-induced oxidative stress or with 4-aminobiphenyl–DNA adduct formation. Furthermore, prior treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which increased hepatic CYP1A2 protein expression 5-fold along with expression of other phase I and phase II enzymes, either did not change or actually decreased the level of adducts in liver. The specific quantitative effects of such induction will depend on the balance of the enzymes induced. These results suggest either that CYP1A2 is not the major metabolic activator of 4-aminobiphenyl or that other enzymes in mice activate the compound in the absence of CYP1A2 (Tsuneoka et al., 2003). Based on studies with other aromatic amines,

他の臓器も4-Aminobiphenyl 誘発性腫瘍の標的となりうるが、イヌやマウスの一部の系統では勝 胱が主な標的となっている。一般的な遺伝毒性と腫瘍発生との間の段階についてのエビデンスは 十分ではない。4-Aminobiphenyl の膀胱の細胞増殖への影響についての研究は著しく不足している が、関連する芳香族アミンやアミドについての情報は入手可能であり、特にメガマウス ED-01 実 験(Cairns、1979年;Gaylor、1979年;Littlefieldら、1979年)のデータを用いたマウスの膀胱に おける 2-acetylaminofluorene によって誘導される DNA 反応性(及び変異原性)と細胞増殖との間 の相互作用の解析が行われている。変異原性が細胞増殖に依存していることは、DNA 付加体の用 量反応が直線的であるにもかかわらず、腫瘍の用量反応がシグモイド型であることの説明を提供 することができる(Cohen & Ellwein、1990年)。この関連性は、4-Aminobiphenyl 誘発性膀胱がん の発生と用量反応性を評価する上で重要な意味を持つ(以下の議論を参照)。

#### I. 不確実性、矛盾、データギャップ

4-Aminobiphenyl の代謝的活性化を伴う細菌を用いた突然変異試験では、ほとんどの変異がフレームシフトであることが示されているが、バクテリオファージ M13 クローニングベクターから得た一本鎖 DNA 中の lacZ 遺伝子の 4-Aminobiphenyl 誘発突然変異の配列解析を行ったところ、80% 以上が G 部位で生じる塩基対置換が明らかになった。G→T トランスバージョンが優勢で、次に G→C トランスバージョン、G→A トランジションが続いた。次に、主要な DNA 付加体である *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl を M13 ゲノム内に挿入し、生体内複製後に変異頻度と特異性を測定した。目標とする突然変異率は約 0.01%であり、一次突然変異は G→C トランスバージョンであった。このように、in vitro での観察結果は in vivo での観察結果と矛盾しないが、変異原性 は弱いものであった (Verghis 6、1997 年)。

in vivo での研究のほとんどはマウスで行われている。無理からぬ理由ではあるが、イヌは膀胱 発がんに対して感受性が高い種であるにもかかわらず、あまり注目されていない。反応における マウスの系統の違いは明らかである。B6C3F1及び雌のBALB/cStCrlfC3Hf/Netrマウスは、肝臓発 がんに対してより感受性が高く、一方、雄のBALB/cStCrlfC3Hf/Netrマウスは、4-Aminobiphenylへ のばく露後に膀胱腫瘍を発症する(Schieferstein ら、1985年;Dooley ら、1988年、1992年)。そ れにもかかわらず、利用可能な研究では、マウスの系統差による影響はあまり注目されていない。

4-Aminobiphenyl が代謝され、肝臓や膀胱で DNA と付加体を形成する基本的な酵素と考えられ ているのは CYP1A2 である (Butler ら、1989 年 a、1989 年 b)。しかし、CYP1A2(+/+)野生型マウ スと CYP1A2(-/-) ックアウトマウスの影響を比較すると、予想に反して、CYP1A2 の発現は、4-Aminobiphenyl 誘発性酸化ストレスまたは 4-Aminobiphenyl-DNA 付加体形成とは関連していない ことが示された。さらに、他の第 1 相及び第 2 相反応の酵素の発現とともに肝 CYP1A2 タンパク 質の発現を 5 倍に増加させた 2,3,7,8-テトラクロロジベンゾ-*p*-ジオキシン (TCDD) は、肝臓にお ける付加体量を変化させないか、または実際に減少させた。このような誘導の定量的効果は、誘 導された酵素のパランスに依存するであろう。これらの結果は、CYP1A2 が 4-Aminobiphenyl の主 要な代謝的活性化因子ではないか、CYP1A2 の非存在下でもマウスの他の酵素がこの化合物を活 性化させていることを示唆している (Tsuneoka ら, 2003 年) 他の芳香族アミンを用いた研究に基 づくと、活性化酵素は他にも、P-450 酵素、酸化酵素、またはペルオキシダーゼを含むかもしれな い (Lakshmi ら、1990 年; Smith ら、1991 年; Hughes ら、1992 年)。 additional activating enzymes might include other P-450 enzymes, oxidases, or peroxidases (Lakshmi et al., 1990; Smith et al., 1991; Hughes et al., 1992).

Another reaction considered to be important for carcinogenesis induced by 4-aminobiphenyl is acetylation. Acetylation plays several roles in 4-aminobiphenyl carcinogenesis. *O*-Acetylation and *N*,*O*-acetyltransfer of *N*-hydroxy-4-aminobiphenyl are expected to increase risk in humans, whereas *N*-acetylation of 4-aminobiphenyl should reduce risk (Lower et al., 1979). Acetylation can be catalysed by NAT1 or NAT2, with the latter exhibiting a marked polymorphism within the population (Hein et al., 2000; Cascorbi et al., 2001). It is predicted that a slow acetylation phenotype will increase the risk of bladder cancer, since acetylation of the parent amine, 4-aminobiphenyl, is considered to be a detoxification process in humans, whereas a rapid acetylation phenotype should be associated with a decreased risk.

However, studies of acetylator phenotype in mice have produced conflicting results. In one study, male and female homozygous rapid acetylator or homozygous slow acetylator mice that were apparently identical in every other respect were administered 4-aminobiphenyl·HCl (55–300 mg/l) in drinking-water for 28 days. The levels of hepatic DNA adducts increased with dose in both sexes, with the levels being higher in females, but were independent of the mouse acetylator phenotype. In the urinary bladder, DNA adducts increased to a plateau at 100 mg/kg in male mice and were again independent of acetylator phenotype. In female mice, the DNA adduct levels were lower than in males and decreased at the highest dose; the DNA adduct levels were higher in the rapid acetylator phenotype, contrary to expectations (Flammang et al., 1992). These results were interpreted as suggesting that acetyltransferase activities are not rate determining for DNA adduct formation in mice. A similar conclusion that there was no correlation between murine NAT2 alleles and 4-aminobiphenyl-DNA adduct levels was reached by McQueen et al. (2003), using C57BL/6, B6.A, and A/J mouse strains and the transgenic strains hNAT1:A/J and hNAT1:C57, which carry the human NAT1 transgene. However, the differences in murine NAT2 activity were modest and probably not sufficient to affect 4-aminobiphenyl genotoxicity. Recent studies suggest that in humans, NAT1, not NAT2, is responsible for the O-acetylation of N-hydroxy-4-aminobiphenyl (Oda, 2004).

There are also mouse strain-specific mutations that require explanation. Thus, in B6C3F1, 4aminobiphenyl induces predominantly  $C \rightarrow A$  mutations (reflecting  $G \rightarrow T$  transversions in the non-coding strand) in H-*ras* codon 61, whereas in CD-1 mice, the predominant mutation in H-*ras* codon 61 was  $A \rightarrow T$  transversion (Manjanatha et al., 1996).

Cell proliferation is also required for neoplasia, but there have been few studies that have investigated cell proliferation at an early stage of the carcinogenic process of 4-aminobiphenyl. It is also notable that in the carcinogenicity experiment described previously (Schieferstein et al., 1985), although urinary bladder carcinomas developed only in males, a high prevalence of hyperplasia was reported in both males and females. Apparently this observation has not been investigated further (discussed below).

In summary, the evidence is strong for the sequence of key events including metabolic activation, DNA adduct formation, and gene mutation as the MOA for 4-aminobiphenyl-induced 4-Aminobiphenyl によって誘導される発がんにおいて重要であると考えられている別の反応は、 アセチル化である。アセチル化は 4-Aminobiphenyl の発がんにいくつかの役割を果たしている。*N*hydroxy-4-aminobiphenyl の *O*-アセチル化及び *N*,*O*-アセチル化は、ヒトではリスクを増加させると 予想されるが、4-Aminobiphenyl の *N*-アセチル化はリスクを減少させるはずである(Lower ら、 1979 年)。アセチル化は、NAT1 または NAT2 によって触媒され、後者は集団内で顕著な多型が存 在する(Hein ら、2000 年; Cascorbi ら、2001 年)。親アミンである 4-Aminobiphenyl のアセチル化 はヒトでは解毒過程と考えられているため、アセチル化が遅い表現型は膀胱がんのリスクを増加 させると予測されている。それゆえ、アセチル化が速い表現型はリスクの減少と関連しているは ずである。

しかし、マウスの acetylator の表現型の研究では、相反する結果が得られている。ある研究では、 他のすべての点で明らかに同一であると思われるホモ接合性の rapid acetylator マウスまたはホモ 接合性の slow acetylator マウスの雄雌に、4-Aminobiphenyl 塩酸 (55-300 mg/L) を 28 日間飲水投与 した。肝 DNA 付加体のレベルは雌雄ともに投与量の増加とともに増加したが、acetylator の表現 型とは無関係であった。膀胱では、DNA 付加体は雄マウスでは 100 mg/kg でプラトーに到達し、 acetylator の表現型とは無関係であった。雌マウスでは、DNA 付加体量は雄マウスよりも低く、最 高用量で減少したが、予想に反して rapid acetylator 表現型では DNA 付加体量が高かった (Flammang ら、1992 年)。これらの結果は、アセチルトランスフェラーゼ活性がマウスの DNA 付加体形成の 速度を決定するものではないことを示唆していると解釈された。McQueen ら (2003 年)の研究で は、C57BL/6、B6.A 及び A/J マウス株と、ヒト NAT1 トランス遺伝子を持つトランスジェニック マウス hNAT1:A/J 及び hNAT1:C57 を用いて、マウス NAT2 対立遺伝子と 4-Aminobiphenyl-DNA 付 加体量との間には相関関係がないという同様の結論に達した。しかし、マウスの NAT2 活性の違 いは軽微であり、おそらく 4-Aminobiphenyl の遺伝毒性に影響を与えるには十分ではなかった。最 近の研究では、ヒトでは N-hydroxy-4-aminobiphenyl の 0-アセチル化には NAT2 ではなく NAT1 が 関与していることが示唆されている (Oda、2004 年)。

また、説明が必要なマウスの系統特異的突然変異もある。例えば、B6C3F1 では、4-Aminobiphenyl は、H-ras 遺伝子のコドン 61 において主に C→A 突然変異(非コード鎖の G→T トランスバージョンを反映)を誘発するのに対し、CD-1 マウスでは、H-ras 遺伝子コドン 61 における主な突然変異は A→T トランスバージョンであった(Manjanatha ら、1996 年)。

腫瘍の発生には細胞増殖も必要であるが、4-Aminobiphenylの発がん過程の初期段階での細胞増 殖を調べた研究は少ない。また、先に述べた発がん性試験(Schieferstein ら、1985年)では、膀胱 がんの発生は雄のみであったが、過形成の発生率は雌雄ともに高いことが報告されている。しか しこのことについてはそれ以上調査されていない(後述)。

要約すると、4-Aminobiphenyl 誘発性膀胱発がんの MOA は、代謝的活性化、DNA 付加体形成、 遺伝子突然変異を含む一連の key events が関与していることが明らかになった。

urinary bladder carcinogenesis. It is further strengthened by data from studies with structurally related aromatic amines. However, data gaps remain concerning details of the specific enzymes involved, the basis for differing organ specificity between species and details regarding potency, and the shape of the dose–response curve in humans. This is, perhaps, not unexpected in view of the complexity of the relevant competing metabolic pathways. While available data are considered sufficient to support the hypothesized MOA, the impact of these uncertainties needs to be considered quantitatively in the overall assessment (Table 3).

#### Table 3. Modulating factors affecting 4-aminobiphenyl urinary bladder carcinogenesis.

- 1. Competing activities of esterification enzymes
- 2. Genetic polymorphisms affecting enzymatic activation or inactivation (e.g. slow and fast acetylators)
- 3. Urinary pH (mainly affected by diet) and possibly other urinary constituents
- 4. Urothelial cell proliferation (induced by high doses of 4-aminobiphenyl or by co-administration with some other agent affecting urothelial proliferation)

## CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

There is considerable evidence in humans and human cell systems supporting each of the key events for 4-aminobiphenyl-induced urinary bladder cancer. Metabolic activation to the *N*-hydroxylamine has been demonstrated, with several different enzymes being suggested for activation and several others that might potentiate or reduce the effects of *N*-hydroxylation, such as *N*-acetylation. Genetic polymorphisms significantly affect activities of these enzymes, producing variations in the population that can affect susceptibility to the urinary bladder carcinogenesis response to 4-aminobiphenyl exposures. DNA adducts identical to those detected in DNA from mice and dogs have been identified in human urothelial cells, and consequently they have a similar mutagenic potential. Furthermore, extensive epidemiological evidence demonstrates the urinary bladder carcinogenicity of 4-aminobiphenyl in humans.

Bladder cancer is associated with smoking and occupational exposures to 4-aminobiphenyl. 4-Aminobiphenyl was manufactured in the United States of America from 1935 to 1955 (Melick et al., 1955) and was used as a highly efficient rubber antioxidant, but it is apparently no longer commercially produced. In epidemiological studies, which were confined to one series of workers occupationally exposed to commercial 4-aminobiphenyl, a high incidence of bladder carcinomas was reported (Melick et al., 1955, 1971; Melamed et al., 1960; Koss et al., 1965, 1969). Among 503 workers, 59 cases with positive cytology were identified, among which 35 cases of carcinoma of the urinary bladder were histologically verified; 7 remained cytologically positive at the time of publication, while 7 died from other causes and 10 were lost to follow-up (Koss et al., 1969). In addition to cigarette smoke, there also appear to be other, ill-defined environmental sources of exposure, possibly from other sources of combustion of substances containing carbon and nitrogen (Skipper et al., 2003). Cigarette

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このことは、構造的に関連のある芳香族アミンを用いた研究によってさらに強化されている。 しかし、関与する特定の酵素の詳細、種間での臓器特異性の違いの根拠や効力の詳細、ヒトにお ける用量反応曲線の形状については、データギャップが残っている。これは、競合する代謝経路 の複雑さを考えれば、想定内である。利用可能なデータは仮説 MOA を支持するのに十分である と考えられるが、これらの不確実性の影響については総合的な定量的評価を考慮する必要がある (表 3)。

#### 表 3. 4-Aminobiphenyl 膀胱発がん性に影響を与える調節因子

- 1 エステル化酵素の競合的活性
- 2 酵素の活性化または不活性化に影響を与える遺伝的多型(例: slow acetylator 及び rapid acetylator)
- 3 尿のpH(主に食事の影響を受ける)と他の尿成分
- 4 尿路粘膜細胞増殖(4-アミノビフェニルの高用量投与、または尿路粘膜増殖に影響を与える 他の薬剤との併用投与により誘導される)

## 実験動物とヒトとの間の key evants の根本的、質的な違いに基づいて、MOA のヒト との関連性を合理的に排除することができるか

4-Aminobiphenyl 誘発性膀胱がんの key events それぞれを支持するヒト及びヒトの細胞系におけ る有力なエビデンスがある。N-hydroxylamine への代謝的活性化が実証されており、活性化のため にいくつかの酵素が示されているほか、N-アセチル化などN-ヒドロキシル化の効果を増強または 減少させる可能性のある他の酵素も示唆されている。遺伝的多型はこれらの酵素活性に大きく影 響し、4-Aminobiphenyl ばく露に対する膀胱発がんの感受性に影響する変異を生じさせる。マウス やイヌの DNA から検出されたものと同一の DNA 付加体がヒトの尿路上皮細胞で確認されてお り、同様の変異原性を有することが明らかになった。さらに、広範な疫学的研究から、4-Aminobiphenyl のヒトにおける膀胱発がん性が証明されている。

膀胱がんは、喫煙や職業的ばく露といった 4-Aminobiphenyl の摂取と関連している。4-Aminobiphenyl は、1935 年から 1955 年まで米国で製造され(Melick ら、1955 年)、効率のいいゴ ム酸化防止剤として使用されていたが、現在では商業的には生産されていない。市販の 4-Aminobiphenyl に職業的にばく露された労働者の1系列に限定した疫学研究では、膀胱がんの発生 率が高いことが報告されている(Melick ら、1955 年、1971 年; Melamed ら、1960 年; Koss ら、 1965 年、1969 年)。503 人の労働者のうち、細胞診において 59 例が陽性、そのうち 35 例は組織学 的に膀胱がんが確認された; 公表時、7 例は細胞診において陽性のまま、7 例は他の原因で死亡し、 10 例は追跡調査できていない(Koss ら、1969 年)。たばこの煙に加えて、炭素及び窒素を含む物 質の燃焼で生じるものへのばく露や不明確な環境中の物質もあるようである(Skipper ら、2003 年)。

smoking accounts for between 40% and 70% of the bladder cancer cases in the United States and Europe (IARC, 1986; Castelao et al., 2001). Black (air-cured) tobacco is a greater source of 4-aminobiphenyl than is blonde (flue-cured) tobacco (Bryant et al., 1988).

The key events demonstrated for 4-aminobiphenyl bladder carcinogenesis in mice and dogs have also been specifically evaluated for 4-aminobiphenyl in humans, primarily in individuals exposed to 4-aminobiphenyl in cigarette smoke, but also utilizing in vitro methods with human urothelial cells (see Table 4).

## Table 4. Concordance evaluation of key events of 4-aminobiphenyl-induced urinary bladder carcinogenesis between species.

Key event	Mouse	Dog	Human
Reyevent	wouse	Dog	numan
1. Metabolic activation to reactive electrophile	+	+	+
2. DNA adduct formation	+	+	+
3. Mutagenesis	+	+	+
4. Carcinoma	+	+	+

Absorbed 4-aminobiphenyl is *N*-oxidized in the liver by CYP1A2, which, in spite of its rather high homology with CYP1A1, has an essentially different substrate specificity and is found only in liver (Lang & Pelkonen, 1999). Other enzymes have been suggested to be capable of supporting metabolic activation to the *N*-hydroxylamine.

NAT1 and NAT2 each catalyse three types of acetylation: the N-acetylation of arylamines, the O-acetylation of N-hydroxylamines, and the N,O-acetyltransfer of arylhydroxamic acids (Flammang & Kadlubar, 1986; Mattano et al., 1989; Fretland et al., 1997; Hein et al., 2000). It is believed that N-acetylation by N-acetyltransferases has a protective effect regarding bladder carcinogenicity, primarily because the acetamide of 4-aminobiphenyl formed is significantly less potent as a substrate for N-hydroxylation compared with the amine. Two genes, NAT1 and NAT2, code for the NAT isoforms, and allelic variation has been associated with susceptibility to urinary bladder cancer in humans (Hein et al., 2000). Most studies suggest that NAT2 slow acetylators are at increased risk of developing bladder cancer, whereas the contribution of the NATI genotype to aromatic amine bladder carcinogenesis is less clear (Cartwright et al., 1982; Hein et al., 2000). Among smokers, there is a higher level of 4-aminobiphenyl-haemoglobin adducts associated with the slow acetylator phenotype (Vineis et al., 1990). Interactions of *NAT1* and *NAT2* have been suggested (Cascorbi et al., 2001). In a study of 425 German bladder cancer patients, Cascorbi et al. (2001) found that there is (1) a partial linkage of the NATI\*10 genotype to the NAT2\*4 genotype, (2) a clear underrepresentation of NAT1\*10 genotypes among rapid NAT2 genotypes in the cases studied, and (3) a gene-gene-environment interaction in that NAT2\*slow/NAT1\*4 genotype combinations with a history of occupational exposure were 5.96 (2.96-12.0) times more frequent in cancer cases than in controls without a risk from occupation (P < 0.0001). Hence, the data suggest that individuals with NAT2\*4 and NAT1\*10 are at a significantly lower risk for bladder cancer, particularly when exposed to environmental risk factors.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

喫煙が原因とされているものは、米国とヨーロッパにおける膀胱がんの症例の 40%から 70%を占めている(IARC、1986年; Castelaoら、2001年)。黒タバコ(自然乾燥)は、ブロンドタバコ(熱風乾燥)に比べて 4-Aminobiphenylをより多く含んでいる(Bryant ら、1988年)。

4-Aminobiphenylの膀胱発がんにおけるマウスやイヌで示された key events は、主にタバコの煙中の4-Aminobiphenyl にばく露されたヒトを対象に評価されているが、ヒトの尿路上皮細胞を用いた in vitro 評価法も利用されている(表4を参照)。

表 4.4-Aminobiphenyl による尿路膀胱発がんの key events の種間一致性評価

key events	マウス	イヌ	ድኑ
1. 反応性親電子物質への代謝的活性化	+	+	+
2. DNA 付加体の形成	+	+	+
3. 変異原性	+	+	+
4. 発がん	+	+	+

吸収された 4-Aminobiphenyl は、CYP1A2 によって肝臓で N-酸化される。CYP1A2 は CYP1A1 との相同性がかなり高いにもかかわらず、異なる基質特異性を有しており、肝臓でのみ見出される (Lang & Pelkonen、1999)。他の酵素は、N-hydroxylamine への代謝的活性化をサポートする。

NAT1 及び NAT2 はそれぞれ、3 種類のアセチル化を触媒する。すなわち、アリルアミンの N-ア セチル化、N-hydroxylamineのO-アセチル化及びアリルヒドロキサム酸のN, O-アセチル化である (Flammang & Kadlubar、1986 年; Mattano ら、1989 年; Fretland ら、1997 年; Hein ら、2000 年)。 N-アセチルトランスフェラーゼによる N-アセチル化は、主に、形成された 4-Aminobiphenyl のア セトアミドがアミンに比べて N-ヒドロキシル化の基質として小さいために、膀胱発がん性に対し て保護効果を有すると考えられている。NAT アイソフォームをコードする 2 つの遺伝子には、 NAT1とNAT2があり、対立遺伝子の変異はヒトにおける膀胱がんの感受性と関連している(Hein ら、2000年)。ほとんどの研究は、NAT2の slow acetylator が膀胱発がんのリスク増加を示唆して いるが、芳香族アミン膀胱発がんに対する NATI 遺伝子型の寄与は明らかではない(Cartwright ら、 1982年; Hein ら、2000年)。喫煙者の間では、slow acetylator の発現に関連する 4-Aminobiphenyl-ヘモグロビン付加体がより多く存在する (Vineis ら、1990年)。また、NAT1 と NAT2 の相互作用 が示唆されている(Cascorbiら、2001年)。ドイツの膀胱がん患者 425 人を対象とした研究で、 Cascorbi ら (2001 年) は、以下のことを明らかにした。(1) NAT1\*10 遺伝子型の NAT2\*4 遺伝子 型への部分的な連鎖(2)調査した症例中の rapid NAT2 遺伝子型を有するヒトにおける、NAT1\*10 遺伝子型の発現量の低さ(3)職業ばく露歴とNAT2\* slow/NAT1\*4 遺伝子型の組み合わせは、職業 ばく露によるリスクのない対照群に比べて、がん症例は 5.96 倍(2.96-12.0)の頻度であった (P<0.0001)

Polymorphisms in *CYP1A2* (Oscarson et al., n.d.) and *NAT2* (Hein et al., 2000) genes are associated with variations in the activities of these enzymes in human populations, although the extent to which variation in CYP1A2 activity is due to genetic factors has yet to be determined (Sachse et al., 2003). Moreover, expression of the *CYP1A2* gene is induced in cigarette smokers, leading to even higher CYP1A2 enzyme activities (Sesardic et al., 1988; Eaton et al., 1995). An individual exposed to 4-aminobiphenyl and expressing high levels of CYP1A2 and slow NAT2 activity would be expected to have increased levels of *N*-hydroxy-4-aminobiphenyl and, therefore, higher levels of 4-aminobiphenyl–haemoglobin adducts and 4-aminobiphenyl–DNA adducts in liver and urinary bladder than an individual expressing low levels of CYP1A2 and rapid NAT2 activity.

The tumour suppressor genes *RB1* and *TP53* appear to be involved in bladder cancer, especially high-grade urothelial carcinomas rather than low-grade papillary tumours. Both genes are involved in the regulation of the cell cycle. In addition, TP53 plays a role in response to DNA damage, cell death, and neovascularization (Hickman et al., 2002), and its gene product regulates the expression of multiple genes (Vousden & Lu, 2002). A strong association has been found between *RB1* inactivation and muscle invasion (Cairns et al., 1991; Ishikawa et al., 1991; Presti et al., 1991; Primdahl et al., 2000). In one study of 45 bladder cancers, seven of nine TP53 mutations occurred in grade 3 tumours (i.e. invasion includes perivesicular tissue) (Martone et al., 1998). Inactivation of RB1 occurs in 30-80% of muscle-invasive bladder cancers (Cairns et al., 1991; Logothetis et al., 1992; Wright et al., 1995; Ioachim et al., 2000), most frequently as a consequence of heterozygous 13g deletions in combination with mutation of the remaining allele (Cordon-Cardo & Reuter, 1997). In studies investigating at least 30 tumours, TP53 mutations occurred in 40-60% of invasive bladder cancers (Tiguert et al., 2001; Lu et al., 2002). Although no specific mutational hotspots were identified, more than 90% of the mutations occurred in exons 4–9. In a study of the binding spectrum of N-hydroxy-4-aminobiphenyl in DNA fragments containing exons 5, 7, and 8 of TP53, preferential binding was identified at codon 285, a non-CpG site, and at codons 175 and 248, which are CpG sites, but only after C5 cytosine methylation had occurred (Feng et al., 2002). The authors concluded that the mutational spectrum in TP53 in bladder cancer strongly suggests a role of 4-aminobiphenyl in the etiology of this neoplasm.

Exposure to tobacco smoke, an environmental source of 4-aminobiphenyl, is associated with increased levels of 4-aminobiphenyl–haemoglobin adducts, in both adults and fetuses. In a study of smoking (n = 14) and non-smoking (n = 38) women, 4-aminobiphenyl–haemoglobin levels were  $183 \pm 108$  pg/g haemoglobin in smokers and  $22 \pm 8$  pg/g haemoglobin in non-smokers, whereas the levels in their respective fetuses were  $92 \pm 54$  pg/g haemoglobin adduct levels in adults in studies of tumour tissue DNA (Curigliano et al., 1996). Haemoglobin adduct levels (used as a surrogate for exposure levels and indicator for DNA adduct potential) have been associated with levels of exposure to tobacco as a source of 4-aminobiphenyl (black tobacco > blonde tobacco > non-smokers) in a male study population from Turin, Italy; the risk of bladder cancer followed the same pattern (Bryant et al., 1988). There is a substantial gap in information linking the presence of adducts, primarily an indication of exposure, and the emergence of cancer.

したがって、特に環境リスク因子にばく露される場合、NAT2\*4 及び NAT1\*10 を有する個体は、 膀胱がんのリスクが有意に低いことが示唆される。

CYP1A2 (Oscarson ら、n.d.) 及び NAT2 (Hein ら、2000 年) 遺伝子の多型は、ヒト集団における これらの酵素活性の変動と関連しているが、CYP1A2 活性の変動が遺伝的要因によるものである かはまだ明らかにされていない (Sachse ら、2003 年)。さらに、喫煙者に誘導される CYP1A2 遺伝 子の発現は、CYP1A2 酵素活性を高める (Sesardic ら、1988; Eaton ら、1995 年)。4-Aminobiphenyl にばく露され、CYP1A2 と高い slow NAT2 活性を発現する個体は、CYP1A2 と低い rapid NAT2 活 性を発現する個体よりも、N-hydroxy-4-aminobiphenyl のレベルが高い。したがって、肝臓と膀胱に おける 4-Aminobiphenyl -ヘモグロビン付加体と 4-Aminobiphenyl-DNA 付加体の高いレベルを有す ることが予測される。

腫瘍抑制遺伝子 RBI 及び TP53 は、膀胱がん、特に低悪性度の乳頭腫瘍よりも高悪性度の尿路 上皮がんに関与しているようである。両遺伝子は細胞周期の調節に関与している。さらに、TP53 は、DNA 損傷、細胞死及び血管新生の役割を果たし(Hickman ら、2002 年)、その遺伝子産物は、 複数の遺伝子の発現を調節する(Vousden & Lu、2002 年)。RB1 の不活性化と筋肉内への浸潤に強 い関連が見出されている(Cairns ら、1991年; Ishikawa ら、1991年; Presti ら、1991年; Primdahl ら、2000年)。45 例の膀胱がんを対象とした研究では、TP53 変異を有する9人のうち7人がグレ ード3の腫瘍(周囲組織への浸潤を含む)であった(Martone ら、1998年)。RB1の不活性化は、 筋肉内浸潤性膀胱がんの 30~80%で発生し (Cairns ら、1991 年; Logothetis ら、1992 年; Wright ら、1995年; Ioachim ら、2000年)、多くの場合、ヘテロ接合性 13g 欠失と残りの対立遺伝子の突 然変異の結果発生する(Cordon-Cardo & Reuter、1997年)。少なくとも 30の腫瘍を調査した研究 では、浸潤性膀胱がんの 40~60%で TP53 突然変異が認められた(Tiguert ら、2001 年: Lu ら、 2002年)。特定変異のホットスポットは確認されなかったが、変異の90%以上はエクソン4-9に発 生していた。TP53のエクソン5、7及び8を含む DNA 断片における N-hydroxy-4-aminobiphenylの 結合スペクトルの研究では、非 CpG 部位である 285 コドン及び CpG 部位である 175 及び 248 コ ドンに優先的に結合することが確認されたが、C5シトシンメチル化が起こった後にのみ確認され た(Fengら、2002年)。著者らは、膀胱がんにおける TP53 の変異スペクトルが、この腫瘍の原因 である 4-Aminobiphenyl の役割を強く示唆していると結論づけた。

4-Aminobiphenyl の環境中ばく露の源であるタバコの煙へのばく露は、成人と胎児の両方で、4-Aminobiphenyl-ヘモグロビン付加体量の増加と関連している。喫煙者(n=14)と非喫煙者(n=38)の女性の研究では、4-Aminobiphenyl-ヘモグロビンレベルは、喫煙者では183±108 pg/g ヘモグロビン、非喫煙者では22±8 pg/g ヘモグロビンであったのに対し、それぞれの胎児のレベルは92±54 pg/g ヘモグロビンと 17±13 pg/g ヘモグロビン (Coghlin ら、1991年)であった。この差は、腫瘍組織 DNA の研究において成人でも観察されている(Curigliano ら、1996年)。イタリア、トリノの男性における研究では、ヘモグロビン付加体量(ばく露量の代用として、また DNA 付加体の可能性の指標として使用される)は、4-Aminobiphenyl の供給源であるタバコへのばく露量と関連しており(黒タバコ>膀胱がんのリスクでも同様の傾向がみられた(Bryant ら、1988年)。主にばく露の指標である付加体の存在とがん発生とを結びつける情報には大きなギャップがある。

In humans, 4-aminobiphenvl has been associated only with urinary bladder cancer, whereas in mice, liver and urinary bladder tumours are induced. Although the specific reasons for these species differences in organ specificity are not known, they appear to be due to variations in competing N-esterification enzymatic activations. Sulfation appears to be primarily associated with liver carcinogenesis by aromatic amines, whereas N-glucuronidation appears to be more associated with bladder carcinogenesis. Acetylation has mixed effects, but in humans appears to be principally a detoxification process that can be influenced significantly by N-acetyltransferase polymorphisms that result in fast versus slow acetylation. Human tissues have been studied for their possible involvement in the metabolism of 4aminobiphenyl and its metabolites. CYP1A2 is responsible for the metabolism of 4-aminobiphenyl to N-hydroxy-4-aminobiphenyl by human hepatic microsomal fraction (Butler et al., 1989b). N-Hydroxy-4-aminobiphenyl can be metabolized to a product that binds covalently to calf thymus DNA by cytosolic sulfotransferases from human liver and, to a lesser extent, colon, but not from pancreas or urinary bladder. In view of this lack of sulfotransferase activity in bladder, it has been suggested that hepatic sulformasferase may actually decrease the bioavailability of N-hydroxy-4-aminobiphenyl in extrahepatic tissues and serve as a detoxification mechanism for the urinary bladder (Chou et al., 1995). On the other hand, Nacetyltransferases that are present in human urothelial cells (Frederickson et al., 1992; Swaminathan & Reznikoff, 1992) can metabolize N-hydroxy-4-aminobiphenyl, as well as the acetylated compounds N-hydroxy-4-acetylaminobiphenyl and N-acetoxy-4-acetylaminobiphenyl, to a DNA-reactive material. The major adduct co-chromatographs with N-(deoxyguanosin-8-yl)-4-aminobiphenyl. <sup>32</sup>P-postlabelling analysis of the DNA from cytosolmediated binding of N-hydroxy-4-aminobiphenyl revealed four radioactive spots. Five adducts were found when intact human urothelial cells were used, two of which were the same as two found using cytosol. This suggests the possibility of an activation pathway or pathways in addition to acetylation.

Experiments similar to those performed with dog tissues have shown that human urothelial cell microsomes possess transacetylation activity, so that *N*-hydroxy-4-aminobiphenyl binding to RNA and DNA occurs in the presence of 4-acetylaminobiphenyl, *N*-hydroxy-4-acetylaminobiphenyl, or acetylCoA as acetyl donors (Hatcher et al., 1993). These authors also found that <sup>32</sup>P-postlabelling of DNA adducts formed after reaction with *N*-hydroxy-4-aminobiphenyl, *N*-hydroxy-4-acetylaminobiphenyl, and *N*-acetoxy-4-aminobiphenyl showed similar profiles, suggesting that the arylnitrenium ion, arising from *N*-acetoxy-4-aminobiphenyl, might be the common reactive species. The structures of the adducts have been identified as the 3',5'-bisphospho derivatives of *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-aminobiphenyl), *N*-(deoxyadenosin-8-yl)-4-aminobiphenyl (dA-C8-aminobiphenyl) (Frederickson et al., 1992; Hatcher & Swaminathan, 1995), and *N*-(deoxyguanosin-*N*(2)-yl)-4-azobiphenyl (Hatcher & Swaminathan, 2002).

The results available comparing tobacco smokers with non-smokers support the relevance to humans of the hypothesized MOA. In a study of 46 T1 bladder cancer cases, mean relative staining intensity for 4-aminobiphenyl–DNA adducts was significantly higher in current smokers ( $275 \pm 81$ , n = 24) than in non-smokers ( $113 \pm 71$ , n = 22) (Curigliano et al., 1996). Similar results have been reported for laryngeal tissue (Flamini et al., 1998) and for mammary tissue (Faraglia et al., 2003). Using 4-aminobiphenyl–haemoglobin adducts as an

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ヒトでは、4-Aminobiphenylは膀胱がんとのみ関連しているが、マウスでは肝臓と膀胱の腫瘍が 誘発されている。臓器特異性におけるこれらの種差の具体的な理由は明らかにされていないが、 競合する N-エステル化酵素の活性化の違いによるものと考えられる。硫酸化は芳香族アミンによ る肝発がんと密接に関連しているようだが、N-グルクロン酸化は膀胱発がんと関連しているよう である。アセチル化には様々な効果があるが、ヒトでは主に解毒プロセスであり、アセチル化が 速いか遅いかを決定する N-アセチルトランスフェラーゼの型に大きく影響される。ヒトの組織が、 4-Aminobiphenyl とその代謝物の代謝に関与しているかについて研究されてきた。CYP1A2 は、ヒ ト肝ミクロソーム分画による 4-aminobiphenyl から N-hydroxy-4-aminobiphenyl への代謝に関与して いる (Butler ら、1989 年 b)。*N*-hydroxy-4-aminobiphenyl は、ヒト肝臓及びそれより程度は低いが、 結腸由来の細胞質性スルホトランスフェラーゼによって、牛胸腺 DNA と共有結合する生成物に 代謝されるが、膵臓や膀胱由来のものによっては代謝されない。膀胱でのスルホトランスフェラ ーゼ活性の欠如を考慮すると、肝スルホトランスフェラーゼは肝外組織での N-hydroxy-4aminobiphenyl の生物学的利用能を低下させ、膀胱の解毒機構として機能する可能性が示唆されて いる (Chou ら、1995 年)。一方、ヒト尿路上皮細胞に存在する N-アセチルトランスフェラーゼ (Frederickson ら、1992: Swaminathan & Reznikoff、1992年)は、N-hydroxy-4-aminobiphenyl 及び そのアセチル化化合物 N-hydroxy-4-acetylaminobiphenyl 及び N-アセトキシ-4-アセチルアミノビフ エニルを DNA 反応性物質に代謝することができる。N-(deoxyguanosin-8-yl)-4-aminobiphenyl と主 要な付加体はコクロマトグラフされ、N-hydroxy-4-aminobiphenyl のサイトゾル媒介結合で得た DNA を用いた <sup>32</sup>P-ポストラベリング法により、4 つの放射性スポットが明らかになった。健康な ヒト尿路上皮細胞を用いた場合には5つの付加体が認められたが、そのうち2つはサイトゾルを 用いた場合に認められた2つの付加体と同じであった。このことから、アセチル化以外の活性化 経路の存在が示唆された。

イヌ組織を用いた実験と同様の実験により、ヒトの尿路上皮細胞ミクロソームがアセチル基転移活性を有していることが示されており、N-hydroxy-4-aminobiphenylのRNAやDNAへの結合は、 4-Acetylaminobiphenyl、N-hydroxy-4-acetylaminobiphenyl、またはアセチル CoA をアセチル基供与体とした場合に起こることが示されている(Hatcher ら、1993年)。また、N-hydroxy-4-aminobiphenyl、 N-acetoxy-4-acetylaminobiphenyl、N-acetoxy-4-aminobiphenyl と反応させて生成した DNA 付加体の <sup>32</sup>P-ポストラベリングは、類似のプロファイルを示し、N-acetoxy-4-aminobiphenyl から生じるアリ ルニトレニウムイオンが共通の反応種であることを発見した。この付加体の構造は、N-(deoxyguanosin-8-yl)-4-aminobiphenyl)の 3',5'-ビスホスホ誘導体(Frederickson ら、1992年; Hatcher & Swaminathanosin、1995年)及び N-(deoxyguanosin-N(2)-yl)-4-azobiphenyl (Hatcher & Swaminathan、 2002年)として同定されている。

喫煙者と非喫煙者を比較した結果は、仮説 MOA のヒトへの関連性を支持するものである。T1 膀胱がん46例の研究では、4-Aminobiphenyl-DNA 付加体の平均相対染色強度は、非喫煙者(113±71、 n=22)よりも現在の喫煙者(275±81、n=24)の方が有意に高かった(Curiglianoら、1996年)。同 様の結果が喉頭組織(Flaminiら、1998年)及び乳腺組織(Faragliaら、2003年)についても報告 されている。4-Aminobiphenyl-ヘモグロビン付加体をばく露の指標として使用すると、膀胱がん患 者は対照者よりも高いレベルであったのに対し(Del Santoら、1991年)、肺がん患者はそうでは なかった(Westonら、1991年)。この違いの根拠は不明である。 indicator of exposure, it was found that bladder carcinoma patients had higher levels than controls (Del Santo et al., 1991), whereas lung cancer patients did not (Weston et al., 1991). The basis for this difference is unknown.

In addition to the evidence of genotoxicity generated with non-human test systems, 4-aminobiphenyl can be metabolized by human urothelial cell microsomal preparations to a mutagen in *S. typhimurium* YG1024 (a derivative of TA98 with elevated *O*-acetyltransferase activity) but not in strain TA98 itself (Hatcher et al., 1993). No other species or other human tissues were examined in this study.

6-Thioguanine-resistant mutants can be induced in a non-tumorigenic, SV40-immortalized human urothelial cell line by exposure to 4-aminobiphenyl itself or exposure to N-hydroxy-4aminobiphenyl, N-hydroxy-4-acetylaminobiphenyl, or N-acetoxy-4-acetylaminobiphenyl (Bookland et al., 1992a). No exogenous metabolic activation system was required for the observed activity. The lowest effective concentrations to produce a statistically significant increase in the mutant fraction were as follows: N-acetoxy-4-acetylaminobiphenyl, 2 µmol/l; N-hydroxy-4-acetylaminobiphenyl, 5 umol/l; N-hydroxy-4-aminobiphenyl, 20 umol/l; and 4aminobiphenyl, 100 µmol/l. Three of these substances were also tested for tumorigenic transformation using the same human immortalized urothelial cells in an in vitro-in vivo assay in which the end-point was carcinoma development when treated cells were injected subcutaneously into nude mice (Bookland et al., 1992b). Transformation was demonstrated after all treatments, the lowest concentrations being as follows: N-hydroxy-4-acetylaminobiphenyl, 0.5 µmol/l; N-hydroxy-4-aminobiphenyl, 0.5 µmol/l; and 4-aminobiphenyl, 20 µmol/l. The lower concentrations required for transformation in comparison with those for mutation are noted, but how this should be interpreted is not clear. It is consistent with the transformation being independent of mutation and with the transformation assay having a higher sensitivity, or it could merely reflect a difference in sensitivity of the methods.

In summary, on a qualitative basis, the key events in the MOA are the same in mice, dogs, and humans: metabolic activation to the *N*-hydroxylamine with subsequent formation of a reactive electrophile (presumably the nitrenium ion), formation of guanine adducts, gene mutation, and the ultimate formation of cancer. The intervening events between gene mutation and cancer, such as which genes are mutated and how cancer is induced, are not known. The MOA, nevertheless, has been clearly demonstrated and is the same in the animal models and in humans.

## CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETIC OR DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

As described in detail above, the metabolic activation, DNA adducts, and mutagenicity of 4aminobiphenyl are qualitatively the same in mice, dogs, and humans, leading to the induction of urothelial tumours of the urinary bladder in these three species and other tumours in mice, rats, and rabbits. Although detailed aspects of absorption, distribution, and excretion have not been reported, similarity in the levels of DNA adduct formation in the urothelium occurring in mice, dogs, and humans suggests that kinetic differences are not significant between these ヒト以外の試験系で得られた遺伝毒性のエビデンスに加えて、4-Aminobiphenyl は、ヒト尿路上 皮細胞のミクロソーム調製物によって、5. typhimurium YG1024 (O-アセチルトランスフェラーゼ 活性の高い TA98 の派生株)の変異原性物質に代謝されることがあるが、TA98 株自体では代謝さ れない (Hatcher ら、1993 年)。この研究では、他の種または他のヒト組織は調べられていない。

6- f + f / r = v 耐性変異体は、4-Aminobiphenyl 自体へのばく露、または N-hydroxy-4aminobiphenyl、N-hydroxy-4-aminobiphenyl、N-acetoxy-4-acetylaminobiphenyl へのばく露によって、 非腫瘍性 SV40 不死化ヒト尿路上皮細胞株で誘導することができる(Bookland ら、1992 年 a)。観 察された活性には、外因性代謝的活性化経路は必要とされなかった。変異体分画の統計的に有意 な増加をもたらすための最小濃度は以下の通りであった。N-acetoxy-4-acetylaminobiphenyl、2 µmol/L;N-hydroxy-4-acetylaminobiphenyl、5µmol/L;N-hydroxy-4-aminobiphenyl、20µmol/L;及び 4-Aminobiphenyl、100 µmol/L。また、これらの物質のうちの3つは処理した細胞をヌードマウス に皮下注射したときの発がんをエンドポイントとした in vitro-in vivo アッセイにおいて、同じヒト 不死化尿路上皮細胞を使用した腫瘍性形質転換についても検討が実施された(Bookland ら、1992 年 b)。形質転換はすべての試験で示されたが、最小濃度は以下の通りであった。N-hydroxy-4acetylaminobiphenyl、0.5 µmol/L;N-hydroxy-4-aminobiphenyl、0.5 µmol/L;及び 4-Aminobiphenyl、0.5 µmol/L;N-hydroxy-4-aminobiphenyl、0.5 µmol/L;及び 4-Aminobiphenyl、0.5 µmol/L;N-hydroxy-4-aminobiphenyl、0.5 µmol/L;N-hydroxy-4acetylaminobiphenyl、0.5 µmol/L;N-hydroxy-4-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-hydroxy-6-aminobiphenyl、0.5 µmol/L;N-

要約すると、マウス、イヌ、ヒトにおいて、MOA の key events は定性的には同じである: *N*-hydroxylamine への代謝的活性化とそれに続く反応性親電子物質(おそらくニトレニウムイオン)の形成、グアニン付加体の形成、遺伝子突然変異及びがんの形成である。どの遺伝子が変異し、どのようにしてがんが誘発されるのかなど、遺伝子変異とがんとの間に介在する事象は明らかにされていない。それにもかかわらず、MOA は明確に示されており、動物モデルでもヒトでも同じである。

# 実験動物とヒトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、MOAのヒトへの関連性を合理的に排除することができるか

以上詳述したように、4-Aminobiphenylの代謝的活性化、DNA 付加体、変異原性は、マウス、イ ヌ、ヒトでは定性的に同じであり、これら3種では膀胱腫瘍を誘発し、マウス、ラット、ウサギ ではその他の腫瘍を誘発する。吸収、分布、排泄に関する詳細は報告されていないが、マウス、 イヌ、ヒトで発生する尿路上皮における DNA 付加体形成量が類似していることから、3種の間で は速度論的な差異は有意ではないことが示唆されている。

three species. Although similar enzymatic processes occur in the three species, quantitative differences are evident. These differences may explain some of the variations seen in target organ specificity among the species and might suggest possible quantitative differences in generation of the DNA adducts. Nevertheless, these differences do not negate the overall MOA for any of the species or the different target organs and are consistent with the complexity of the competing pathways for metabolic activation and deactivation.

Presumably there is a potential for repair of the different adducts, and quantitative differences might exist among species and even among tissues. However, the detection of relatively high numbers of adducts in all three species indicates that significant numbers of stable adducts are produced.

The target tissue common among mice, dogs, and humans, the urinary bladder urothelium, is similar morphologically (Pauli et al., 1983). The urothelium has a characteristic asymmetric unit membrane at the luminal surface that provides a major part of the barrier function to urine. It is composed of urothelium-specific proteins, the uroplakins, the sequence of which is highly conserved among species (Wu et al., 1994). In addition, the urothelium is metabolically active in all three species.

Modulating urinary factors have also been identified that can quantitatively affect the ultimate formation of urothelial DNA adducts, such as pH and frequency of urination (Cohen, 1995; Sarkar et al., 2002). Although the range of pH varies among species, the pH in mice, dogs, and humans readily reaches acidic and alkaline levels as well as neutral. Again, although quantitative differences occur, these do not preclude the existence of this MOA in humans.

There is no evidence implicating another MOA besides DNA reactivity. However, significant quantitative differences exist between species with regard to apparent potency of 4aminobiphenyl with respect to urinary bladder carcinogenesis. It is clear, however, that metabolites of 4-aminobiphenyl interact with proteins (e.g. haemoglobin) as well as with DNA and that metabolites of 4-aminobiphenyl are cytotoxic (Schieferstein et al., 1985; Reznikoff et al., 1986; Kadlubar et al., 1991). Interaction with urothelial cellular proteins might be responsible for the cytotoxicity and regenerative proliferation seen in the mouse bladder at higher doses of 4-aminobiphenyl. The interaction of DNA reactivity and consequent mutagenicity and cell proliferation provide an explanation for the sigmoidal shape of the dose-response curve for tumours despite a linear dose-response for DNA adducts (Cohen & Ellwein, 1990). The high concentrations of 4-aminobiphenyl found in the urine of mice that can produce urothelial cytotoxicity are generally not attained in humans exposed to cigarette smoke. However, other (unknown) substances appear to produce urothelial hyperplasia in cigarette smokers (Auerbach & Garfinkel, 1989). This increased cell proliferation significantly potentiates the effects of 4-aminobiphenyl on the bladder, providing a significantly greater number of DNA-replicating cell targets on which to act in comparison with the small number present in the normal, slowly replicating urothelium. Thus, the apparent greater potency of 4-aminobiphenyl in humans compared with mice is unlikely, but represents the synergy of DNA reactivity and cell proliferation produced by a single substance, 4-aminobiphenyl, in mice, but by different substances in the complex mixture of cigarette smoke.

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3 種の酵素の過程は類似しているが、量的な違いは明らかである。これらの違いは、種間で見られ る標的臓器の特異性の違いの一部を説明し、DNA 付加体の生成における定量的な違いを示唆して いる可能性がある。それにもかかわらず、これらの違いは、どの種でも、あるいは異なる標的臓 器でも、全体的な MOA を否定するものではなく、代謝的活性化と不活性化のための競合する経 路の複雑さと一致している。

おそらく、異なる付加体の修復能や種間、組織間における量的な違いが存在するだろう。しか し、3種すべてで比較的多くの付加体が検出されたことから、かなり多くの安定的な付加体が産 生されていることがわかる。

マウス、イヌ、ヒトに共通する標的組織である膀胱の尿路上皮は、形態学的に類似している(Pauli ら、1983年)。尿路上皮は、腔内表面の尿に対するパリア機能の大部分を担う特徴的な非対称性単 位膜を有している。尿路上皮は、尿路上皮特異的タンパク質であるウロプラキンから構成されて おり、その配列は種間で高度に保存されている(Wu ら、1994年)。さらに、尿路上皮は、3種す べての種において代謝的に活性化している。

また、尿 pH や排尿頻度など、尿路上皮 DNA 付加体の最終的な産生量に影響を与える因子も同 定されている(Cohen ら、1995年; Sarkar ら、2002年)。pH の範囲は種によって異なるが、マウ ス、イヌ及びヒトの尿 pH は、容易に酸性及びアルカリ性、また中性に変動する。繰り返しになる が、量的な違いは生じるものの、これらはヒトにおける MOA の存在を排除するものではない。

DNA 反応性以外の MOA を示唆するエビデンスはない。しかし、4-Aminobiphenyl の膀胱発がん に対する見かけの影響に関しては、種間で量的な差が存在する。4-Aminobiphenylの代謝物はDNA と同様にタンパク質(ヘモグロビンなど)とも相互作用し、4-Aminobiphenylの代謝物は細胞毒性 を有することが明らかになっている (Schieferstein ら、1985 年; Reznikoff ら、1986 年; Kadlubar ら、1991年)。4-Aminobiphenylの高用量投与によるマウス膀胱の細胞毒性と再生性増殖には、尿 路上皮細胞タンパク質との相互作用が関与している可能性がある。DNA の反応性と、相反する変 異原性と細胞増殖の相互作用は、DNA 付加体の用量反応が直線的であるにもかかわらず、腫瘍の 用量反応曲線がシグモイド型であることを説明している(Cohen & Ellwein、1990年)。尿路上皮細 胞毒性を生じる可能性があるマウスの尿中に見られる高濃度の4-Aminobiphenylは、一般的に受動 喫煙者では達成されていない。しかし、他の(未知の)物質は、喫煙者(Auerbach & Garfinkel、 1989年)で尿路上皮の過形成を生成するように見える。この細胞増殖の増加は、標的となる DNA 複製細胞の数を大幅に増やし、正常にゆっくりと複製する少数の尿路上皮と比較して、膀胱に対 する 4-Aminobiphenyl の影響を大幅に増強する。このように、マウスと比較してヒトの方が 4-Aminobiphenyl の明らかに大きな効力は考えにくい。しかし、マウスでは 4-Aminobiphenyl 単一の 物質による DNA 反応性と細胞増殖の相乗効果がタバコの煙の複雑な混合物中の様々な物質によ って引き起こることが示されている。

Occupational exposure to 4-aminobiphenyl presumably resulted in greater doses of 4-aminobiphenyl than did exposure to cigarette smoke, since the incidence of bladder cancer in such populations was considerably higher than in smokers. However, quantitative measurements of metabolite concentrations or DNA adduct levels in urothelial cells could not be determined at the time these occupational exposures occurred, and cigarette smoking history in those individuals was not assessed (Koss et al., 1965, 1969).

In summary, although quantitative differences among species exist, they do not exclude the same MOA in mice and dogs occurring in humans.

# CONCLUSION: STATEMENT OF CONFIDENCE, ANALYSIS, AND IMPLICATIONS

The early steps in the proposed MOA are well supported by the available evidence, indicating that the key events of metabolic activation, DNA adduct formation, and mutation are the same qualitatively in mice, dogs, and humans. There is strong and sufficient evidence that 4-aminobiphenyl is a human urinary bladder carcinogen. Evidence for the intervening steps between mutation and cancer development is lacking. The associations described for adduct levels and *TP53* mutations are not compelling because these particular genetic alterations appear late in tumour progression and are often the result of endogenous causes (e.g. spontaneous depurination at methylated CpG sites). This aspect of *TP53* mutations in bladder cancer has been studied in a case–control study (Schroeder et al., 2003). In addition, most urothelial tumours in humans are low-grade papillary lesions, which generally do not have *TP53* mutations.

The mutational spectrum of N-hydroxy-4-acetylaminobiphenyl has been studied in embryonic fibroblasts of the Big Blue mouse (Besaratinia et al., 2002). Treatment of these cells for 24 h resulted in a dose-dependent increase in mutation frequency of the *cII* transgene of up to 12.8-fold over background. Single-base substitutions comprised 86% of the mutations in the treated cells and 74% of the mutations in the controls. Of these mutations, 63% and 36%. respectively, occurred at guarantee residues along the *cII* gene. Whereas  $G \rightarrow T$  transversions accounted for 47% of the mutations in the treated *cII* gene, the most common mutations in untreated cells were insertions, which accounted for 19% of the mutations. Mapping of the induced adducts established five preferred DNA adduction sites, of which four were major mutation sites for N-hydroxy-4-acetoxyaminobiphenyl, especially  $G \rightarrow T$  transversions. In the TP53 gene in human bladder cancer, however,  $G \rightarrow A$  transitions predominate (53%) and are prevalent at all of its five mutational hotspots (codons 175, 248, 273, 280, and 285), three of which are at methylated CpG hotspots (175, 248, and 273). In cII, neither the preferred adduction sites nor the induced mutational hotspots are biased towards methylated CpG dinucleotides. It is concluded from this study that there is a serious discordance between the mutation pattern induced by N-hydroxy-4-acetoxyaminobiphenyl in the cII gene and the mutational pattern observed in TP53 in human bladder cancer. However, the role of methylation status and transcriptional activity on the mutation spectrum induced by 4aminobiphenvl has vet to be determined. It is also to be noted that the TP53 mutation spectrum is a reflection of a selection process during tumour development.

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4-Aminobiphenyl への職業的ばく露は、たばこの煙よりも 4-Aminobiphenyl の用量が多いと考えら れる。なぜなら、膀胱がんの発生率が喫煙者に比べて職業的にばく露される集団の方がかなり高 かったためである。しかし、これらの職業ばく露が発生した時点では代謝物濃度や尿路上皮細胞 内の DNA 付加体レベルを定量的に測定することができず、また、個人の喫煙歴を評価することは なかった(Koss ら、1965 年、1969 年)。

要約すると、種間での量的な違いは存在するが、マウスやイヌと同じ MOA がヒトにも当ては まることを排除するものではない。

## 結論:信頼性、解析及び帰結の記述

推定される MOA の初期段階は、代謝的活性化、DNA 付加体形成及び突然変異の key events が、 マウス、イヌ及びヒトにおいて定性的に同じであることを示す。これは利用可能なエビデンスに よって十分に支持されている。4-Aminobiphenyl がヒトの膀胱発がん物質であるという強力かつ十 分なエビデンスがある。しかし、突然変異とがんの発生との間に介在するステップについてのエ ビデンスは不足している。付加体量と TP53 突然変異との関連について説明したが、これらの特定 の遺伝的変化は腫瘍の進行から遅れて現れ、しばしば内因性の原因 (例えば、メチル化された CpG 部位での脱プリン) であるため、説得力のあるものではない。膀胱がんにおける TP53 突然変異に ついては、症例対照研究で研究されている (Schroeder ら、2003 年)。さらに、ヒトの尿路上皮腫 瘍のほとんどは低悪性度の乳頭病変であり、一般に TP53 変異を有していない。

N-hydroxy-4-acetylaminobiphenylの変異スペクトルは、Big Blue mouseの胚性線維芽細胞におい て研究されている(Bessaratinia ら、2002年)。これらの細胞を24時間処理すると、cII遺伝子の突 然変異頻度がバックグラウンドと比較して最大12.8倍の用量依存的な増加をもたらした。一塩基 置換は、処理細胞では突然変異の86%、対照細胞では突然変異の74%を占めていた。これらの変 異のうち、63%と36%はそれぞれ cll 遺伝子に沿ったグアニン残基で発生した。処置細胞では cll 遺伝子の変異の 47%が G→T のトランスバージョンであったのに対し、対照細胞では最も一般的 な変異は挿入であり、これは変異の19%を占めていた。誘導された付加体のマッピングにより、 5 つの DNA 付加体部位が確立された。そのうちの 4 つは N-hydroxy-4-acetylaminobiphenyl による 主な変異部位であり、特に G→T トランスバージョンを生じていた。しかしながら、ヒト膀胱が んのTP53遺伝子では、G→Aトランジションが優勢(53%)であり、その5つの変異ホットスポ ット (コドン 175、248、273、280、285)のすべてで優勢で、そのうちの 3 つはメチル化 CpG ホ ットスポット(175、248、273)にあった。cH 遺伝子では、誘導される突然変異ホットスポット も、メチル化 CpG ジヌクレオチドに偏っていない。この研究から、cII 遺伝子における N-hydroxy-4-acetylaminobiphenyl によって誘導される突然変異パターンと、ヒト膀胱がんにおける TP53 遺伝 子で観察される突然変異パターンとの間には大きなギャップがあると結論づけられる。しかし、 4-Aminobiphenyl によって誘導される突然変異スペクトルにおけるメチル化状態や転写活性の役 割はまだ明らかにされていない。また、TP53 遺伝子の変異スペクトルは、腫瘍発生過程の選択を 反映したものであることにも留意しなければならない。

Based on the preceding analysis, it is clear that the MOA for 4-aminobiphenyl carcinogenesis is known in the animal model, and the MOA is relevant to humans both qualitatively and quantitatively. The conclusion based on this evaluation, even without epidemiological evidence, is that 4-aminobiphenyl poses a cancer hazard to humans.

To perform a full risk assessment requires additional information regarding the doseresponse and human exposures. Based on the information described above, it is clear that the data predict a cancer hazard for humans at expected exposures, at least for occupational (historical) and cigarette smoking exposures. Further analysis is required regarding the potential risk at ambient exposures in those who are not cigarette smokers. The MOA analysis provides the basis and foundation for such an assessment. The epidemiological evidence on 4-aminobiphenyl supports the conclusions suggested by the MOA HRF.

### **4-AMINOBIPHENYL AND THE HUMAN RELEVANCE FRAMEWORK**

4-Aminobiphenyl was evaluated using the proposed IPCS HRF based on an MOA analysis. The defined key events for this DNA reactivity MOA—metabolic activation, DNA adduct formation, mutagenicity, and cancer induction—clearly are the same in humans as in the animal (mice, dogs) models, indicating that 4-aminobiphenyl presents a cancer hazard for humans. The information for this MOA analysis provides a substantive foundation on which to build a complete cancer risk assessment for humans. For this chemical, there is also substantial epidemiological evidence to verify the conclusions derived from the HRF analysis.

The additional key events for this MOA—which genes are mutated and how do these genetic alterations lead to cancer—are not known for 4-aminobiphenyl. However, this does not detract from the conclusions, given the strength of evidence for the proposed MOA, based on the framework analysis presented here.

What data are necessary to conclude that a chemical produces cancer by a DNA-reactive MOA? Our suggestion is that at the very least there be a demonstration that DNA adducts are produced, preferably in the target tissue, and that the chemical is mutagenic (either with or without metabolic activation). Mutagenicity is used here in a more specific, restricted sense than the broader term genotoxicity. Demonstration of DNA adducts and mutagenicity in the target tissue after in vivo exposure increases confidence in the proposed MOA. Identification of the specific metabolic pathway and specific DNA adducts induced provides a significantly better basis for extrapolating between the animal model and humans.

This case demonstrates the potential utility of data on surrogate compounds in MOA analysis. However, the relevance of data on related compounds, whether in vivo or in vitro, needs to be adequately justified. Weight-of-evidence analysis of structure–activity relationships, which have been well developed for DNA reactivity and mutagenicity, should also contribute to framework analysis.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

以上の解析から、4-Aminobiphenyl 発がん MOA は動物モデルで知られており、その MOA は定 性的にも定量的にもヒトに関連していることが明らかになった。この評価に基づく結論は、疫学 的なエビデンスがなくても、4-Aminobiphenyl はヒトに対して発がんの危険性があるということで ある。

完全なリスク評価を行うためには、用量反応及びヒトへのばく露に関する追加情報が必要であ る。上記の情報に基づいて、職業的(過去の)ばく露と喫煙ばく露など予想されるばく露による 発がんリスクを予測している。非喫煙者の環境ばく露における潜在的リスクについては、さらな る解析が必要である。MOA 解析は、そのような評価のための基礎と基盤を提供する。4-Aminobiphenyl に関する疫学的エビデンスは、MOA の HRF によって推定される結論を支持してい る。

#### 4-Aminobiphenyl とヒト関連性フレームワーク

4-Aminobiphenyl は、提唱されている IPCS HRF を用いて MOA 解析に基づいて評価した。その 結果、DNA 反応性 MOA の key events である代謝的活性化、DNA 付加体形成、変異原性、発がん は、ヒトにおいてもマウスやイヌなどの動物モデルと同様であり、4-Aminobiphenyl はヒトに対し て発がんの危険性を示すことが明らかになった。この MOA 解析の情報は、ヒトに対する完全な がんリスク評価を構築するための実質的な基礎を提供するものである。この化学物質については、 HRF 解析から導き出された結論を検証するための実質的な疫学的エビデンスも存在する。

この MOA では、どのような遺伝子が突然変異し、その遺伝子変化がどのようにして発がんに つながるのかという追加の key events は、4-Aminobiphenyl では知られていない。しかし、ここで 提示されたフレームワーク解析に基づいて MOA のエビデンスの信頼性を考えると、このことは 結論を損なうものではない。

ある化学物質が DNA 反応性 MOA によって発がんすると結論づけるには、どのようなデータが 必要なのか?我々が提案するのは、少なくとも DNA 付加体が、標的組織で生成され、その化学物 質に変異原性(代謝的活性化の有無にかかわらず)があることを実証することである。変異原性 は、ここでは遺伝毒性という広い意味よりも、より具体的で限定的な意味で用いられている。in vivo ばく露後の標的組織における DNA 付加体及び変異原性が証明されれば、推定された MOA の 信頼性が向上する。特定の代謝経路と誘導される特定の DNA 付加体を同定することにより、動物 モデルをヒトに外挿するための優れた根拠が得られる。

この事例は、MOA 解析における代替化合物に関するデータの潜在的な有用性を示している。しかし、関連化合物に関するデータの妥当性は、in vivo であれ in vitro であれ、十分に正当化される必要がある。また、DNA 反応性や変異原性については、これまでに十分に開発されてきた構造活性相関に関するエビデンスの重み付け解析も、フレームワーク解析に貢献すべきである。

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### REFERENCES

al-Atrash J, Zhang YJ, Lin D, Kadlubar FF, Santella RM (1995) Quantitative immunohistochemical analysis of 4-aminobiphenyl–DNA cultured cells and mice: Comparison to gas chromatography/mass spectroscopy analysis. *Chemical Research in Toxicology*, **8**:747–752.

Auerbach O, Garfinkel L (1989) Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer*, **64**:983–987.

Besaratinia A, Bates SE, Pfeifer GP (2002) Mutational signature of the proximate bladder carcinogen *N*-hydroxy-4-acetylaminobiphenyl: Inconsistency with the p53 mutational spectrum in bladder cancer. *Cancer Research*, **62**:4331–4338.

Block NL, Sigel MM, Lynne CM, Ng AB, Grosberg RA (1978) The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. *Investigative Urology*, **16**:50–54.

Bonser GM (1962) Precancerous changes in the urinary bladder. In: Severi L, ed. *The morphological precursor of cancer*. Perugia, University of Perugia, p. 435.

Bookland EA, Reznikoff CA, Lindstrom M, Swaminathan S (1992a) Induction of thioguanine-resistant mutations in human uroepithelial cells by 4-aminobiphenyl and its *N*-hydroxy derivatives. *Cancer Research*, **52**:1615–1621.

Bookland EA, Swaminathan S, Oyasu R, Gilchrist KW, Lindstrom M, Reznikoff CA (1992b) Tumorigenic transformation and neoplastic progression of human uroepithelial cells after exposure in vitro to 4-aminobiphenyl or its metabolites. *Cancer Research*, **52**:1606–1614.

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR (1988) Hemoglobin adducts of aromatic amines: Associations with smoking status and type of tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, **85**:9788–9791.

Burger MS, Torino JL, Swaminathan S (2001) DNA damage in human transitional cell carcinoma cells after exposure to the proximate metabolite of the bladder carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **38**:1–11.

Butler MA, Guengerich FP, Kadlubar FF (1989a) Metabolic oxidation of the carcinogens 4aminobiphenyl and 4,4'-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Research*, **49**:25–31. Harmonization Project Document No. 4

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## 参考文献

al-Atrash J, Zhang YJ, Lin D, Kadlubar FF, Santella RM (1995) Quantitative immunohistochemical analysis of 4-aminobiphenyl–DNA cultured cells and mice: Comparison to gas chromatography/mass spectroscopy analysis. *Chemical Research in Toxicology*, **8**:747–752.

Auerbach O, Garfinkel L (1989) Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer*, **64**:983–987.

Besaratinia A, Bates SE, Pfeifer GP (2002) Mutational signature of the proximate bladder carcinogen *N*-hydroxy-4-acetylaminobiphenyl: Inconsistency with the *p53* mutational spectrum in bladder cancer. *Cancer Research*, **62**:4331–4338.

Block NL, Sigel MM, Lynne CM, Ng AB, Grosberg RA (1978) The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. *Investigative Urology*, **16**:50–54.

Bonser GM (1962) Precancerous changes in the urinary bladder. In: Severi L, ed. *The morphological precursor of cancer*. Perugia, University of Perugia, p. 435.

Bookland EA, Reznikoff CA, Lindstrom M, Swaminathan S (1992a) Induction of thioguanine-resistant mutations in human uroepithelial cells by 4-aminobiphenyl and its *N*- hydroxy derivatives. *Cancer Research*, **52**:1615–1621.

Bookland EA, Swaminathan S, Oyasu R, Gilchrist KW, Lindstrom M, Reznikoff CA (1992b) Tumorigenic transformation and neoplastic progression of human uroepithelial cells after exposure in vitro to 4-aminobiphenyl or its metabolites. *Cancer Research*, **52**:1606–1614.

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR (1988) Hemoglobin adducts of aromatic amines: Associations with smoking status and type of tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, **85**:9788–9791.

Burger MS, Torino JL, Swaminathan S (2001)DNAdamage in human transitional cell carcinoma cells after exposure to the proximate metabolite of the bladder carcinogen 4- aminobiphenyl. *Environmental and Molecular Mutagenesis*, **38**:1–11.

Butler MA, Guengerich FP, Kadlubar FF (1989a) Metabolic oxidation of the carcinogens 4aminobiphenyl and 4,49-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Research*, **49**:25–31.

Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989b) Human cytochrome P-450PA (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proceedings of the National Academy of Sciences of the United States of America*, **86**:7696–7700.

Cairns P, Proctor AJ, Knowles MA (1991) Loss of heterozygosity at the *RB* locus is frequent and correlates with muscle invasion in bladder carcinoma. *Oncogene*, **6**:2305–2309.

Cairns T (1979) The ED<sub>01</sub> study: Introduction, objectives, and experimental design. *Journal* of Environmental Pathology and Toxicology, 3:1-7.

Cartwright RA, Rogers HJ, Barham-Hall D, Glashan RW, Ahmad RA, Higgins E, Kahn MA (1982) Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **16**:842–846.

Cascorbi I, Roots I, Brockmoller J (2001) Association of *NAT1* and *NAT2* polymorphisms to urinary bladder cancer: Significantly reduced risk in subjects with *NAT1\*10. Cancer Research*, **61**:5051–5056.

Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, Ross RK, Yu MC (2001) Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute*, **93**:538–545.

Chou HC, Lang NP, Kadlubar FF (1995) Metabolic activation of the *N*-hydroxy derivative of the carcinogen 4-aminobiphenyl by human tissue sulfotransferases. *Carcinogenesis*, **16**:413–417.

Clayson DB, Lawson TA, Santana S, Bonser GM (1965) Correlation between the chemical induction of hyperplasia and of malignancy in the bladder epithelium. *British Journal of Cancer*, **19**:297–310.

Clayson DB, Lawson TA, Pringle JAS (1967) The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of  $C57 \times IF$  mouse. *British Journal of Cancer*, 1:755–762.

Coghlin J, Gann PH, Hammond SK, Skipper PL, Taghizadeh K, Paul M, Tannenbaum SR (1991) 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, **83**:274–280.

Cohen SM (1995) The role of urinary physiology and chemistry in bladder carcinogenesis. *Food and Chemical Toxicology*, **33**:715–730.

Cohen SM, Ellwein LB (1990) Proliferative and genotoxic cellular effects in 2-acetylaminofluorene bladder and liver carcinogenesis: Biological modeling of the  $ED_{01}$  study. *Toxicology and Applied Pharmacology*, **104**:79–93.

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Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989b) Human cytochrome P-450PA (P-450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3- demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proceedings of the National Academy of Sciences of the United States of America*, **86**:7696–7700.

Cairns P, Proctor AJ, Knowles MA (1991) Loss of heterozygosity at the *RB* locus is frequent and correlates with muscle invasion in bladder carcinoma. *Oncogene*, **6**:2305–2309.

Cairns T (1979) The ED<sub>01</sub> study: Introduction, objectives, and experimental design. *Journal of Environmental Pathology and Toxicology*, **3**:1–7.

Cartwright RA, Rogers HJ, Barham-Hall D, Glashan RW, Ahmad RA, Higgins E, Kahn MA (1982) Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiological approach to bladder cancer. *Lancet*, **16**:842–846.

Cascorbi I, Roots I, Brockmoller J (2001) Association of NAT1 and NAT2 polymorphisms to urinary bladder cancer: Significantly reduced risk in subjects with NAT1\*10. Cancer Research, **61**:5051–5056.

Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, Ross RK, Yu MC (2001) Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute*, **93**:538–545.

Chou HC, Lang NP, Kadlubar FF (1995) Metabolic activation of the *N*-hydroxy derivative of the carcinogen 4-aminobiphenyl by human tissue sulfotransferases. *Carcinogenesis*, **16**:413–417.

Clayson DB, Lawson TA, Santana S, Bonser GM (1965) Correlation between the chemical induction of hyperplasia and of malignancy in the bladder epithelium. *British Journal of Cancer*, **19**:297–310.

Clayson DB, Lawson TA, Pringle JAS (1967) The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of C57 × IF mouse. *British Journal of Cancer*, **1**:755–762.

Coghlin J, Gann PH, Hammond SK, Skipper PL, Taghizadeh K, Paul M, Tannenbaum SR (1991) 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, **83**:274–280.

Cohen SM (1995) The role of urinary physiology and chemistry in bladder carcinogenesis. *Food and Chemical Toxicology*, **33**:715–730.

Cohen SM, Ellwein LB (1990) Proliferative and genotoxic cellular effects in 2- acetylaminofluorene bladder and liver carcinogenesis: Biological modeling of the  $ED_{01}$  study. *Toxicology and Applied Pharmacology*, **104**:79–93

#### Harmonization Project Document No. 4

Cordon-Cardo C, Reuter VE (1997) Alterations of tumor suppressor genes in bladder cancer. *Seminars in Diagnostic Pathology*, **14**:123–132.

Curigliano G, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A, Santella RM (1996) Immunohistochemical quantitation of 4-aminobiphenyl–DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis*, **17**:911–916.

Dang LN, McQueen CA (1999) Mutagenicity of 4-aminobiphenyl and 4-acetylbiphenyl in *Salmonella typhimurium* strains expressing different levels of *N*-acetyltransferase. *Toxicology* and *Applied Pharmacology*, **159**:77–82.

Deichmann WB, MacDonald WE (1968) The non-carcinogenicity of a single dose of 4-aminobiphenyl in the dog. *Food and Cosmetics Toxicology*, **6**:143–146.

Deichmann WB, Radomski JL, Anderson WAD, Coplan MM, Woods FM (1958) The carcinogenic action of *p*-aminobiphenyl in the dog; final report. *Industrial Medicine and Surgery*, **27**:25–26.

Deichmann WB, Radomski JL, Glass E, Anderson WAD, Coplan M, Woods FM (1965) Synergism among oral carcinogens. Simultaneous feeding of four bladder carcinogens to dogs. *Industrial Medicine and Surgery*, **34**:640–649.

Delclos KB, Miller DW, Lay JO Jr, Casciano DA, Walker RP, Fu PP, Kadlubar FF (1987) Identification of C8-modified deoxyinosine and N2- and C8-modified deoxyguanosine as major products of the in vitro reaction of *N*-hydroxy-6-aminochrysene with DNA and the formation of these adducts in isolated rat hepatocytes treated with 6-nitrochrysene and 6-aminochrysene. *Carcinogenesis*, **8**:1703–1709.

Del Santo P, Moneti G, Salvadori M, Saltutti C, Delle RA, Dolara P (1991) Levels of the adducts of 4-aminobiphenyl to hemoglobin in control subjects and bladder carcinoma patients. *Cancer Letters*, **60**:245–251.

Doerge DR, Churchwell MI, Marques MM, Beland FA (1999) Quantitative analyses of 4aminobiphenyl–C8-deoxyguanosyl DNA adducts produced in vitro and in vivo using HPLC-ES-MS. *Carcinogenesis*, **6**:1055–1061.

Dooley KL, Stavenuiter JF, Westra JG, Kadlubar FF (1988) Comparative carcinogenicity of the food pyrolysis product, 2-amino-5-phenylpyridine, and the known human carcinogen, 4-aminobiphenyl, in the neonatal B6C3F1 mouse. *Cancer Letters*, **41**:99–103.

Dooley KL, Von Tungeln LS, Bucci T, Fu PP, Kadlubar FF (1992) Comparative carcinogenicity of 4-aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1 male mouse. *Cancer Letters*, **62**:205–209.

#### Harmonization Project Document No. 4

Cordon-Cardo C, Reuter VE (1997) Alterations of tumor suppressor genes in bladder cancer. *Seminars in Diagnostic Pathology*, **14**:123–132.

Curigliano G, Zhang YJ, Wang LY, Flamini G, Alcini A, Ratto C, Giustacchini M, Alcini E, Cittadini A, Santella RM (1996) Immunohistochemical quantitation of 4-aminobiphenyl–DNAadducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis*, **17**:911–916.

Dang LN, McQueen CA (1999) Mutagenicity of 4-aminobiphenyl and 4-acetylbiphenyl in *Salmonella typhimurium* strains expressing different levels of *N*-acetyltransferase. *Toxicology and Applied Pharmacology*, **159**:77–82.

Deichmann WB, MacDonald WE (1968) The non-carcinogenicity of a single dose of 4-aminobiphenyl in the dog. *Food and Cosmetics Toxicology*, **6**:143–146.

Deichmann WB, Radomski JL, Anderson WAD, Coplan MM, Woods FM (1958) The carcinogenic action of *p*-aminobiphenyl in the dog; final report. *Industrial Medicine and Surgery*, **27**:25–26.

Deichmann WB, Radomski JL, Glass E, Anderson WAD, Coplan M, Woods FM (1965) Synergism among oral carcinogens. Simultaneous feeding of four bladder carcinogens to dogs. *Industrial Medicine and Surgery*, **34**:640–649.

Delclos KB, Miller DW, Lay JO Jr, Casciano DA, Walker RP, Fu PP, Kadlubar FF (1987) Identification of C8-modified deoxyinosine and N2- and C8-modified deoxyguanosine as major products of the in vitro reaction of *N*-hydroxy-6-aminochrysene withDNAand the formation of these adducts in isolated rat hepatocytes treated with 6-nitrochrysene and 6- aminochrysene. *Carcinogenesis*, **8**:1703–1709.

Del Santo P, Moneti G, Salvadori M, Saltutti C, Delle RA, Dolara P (1991) Levels of the adducts of 4aminobiphenyl to hemoglobin in control subjects and bladder carcinoma patients. *Cancer Letters*, **60**:245–251.

Doerge DR, Churchwell MI, Marques MM, Beland FA (1999) Quantitative analyses of 4aminobiphenyl–C8-deoxyguanosylDNAadducts produced in vitro and in vivo using HPLC- ES-MS. *Carcinogenesis*, 6:1055–1061.

Dooley KL, Stavenuiter JF, Westra JG, Kadlubar FF (1988) Comparative carcinogenicity of the food pyrolysis product, 2-amino-5-phenylpyridine, and the known human carcinogen, 4- aminobiphenyl, in the neonatal B6C3F1 mouse. *Cancer Letters*, **41**:99–103.

Dooley KL, Von Tungeln LS, Bucci T, Fu PP, Kadlubar FF (1992) Comparative carcinogenicity of 4aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1 male mouse. *Cancer Letters*, **62**:205–209.

66

Eaton DL, Gallagher EP, Bammler TK, Kunze KL (1995) Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. *Pharmacogenetics*, **5**:259–274.

Faraglia B, Chen SY, Gammon MD, Zhang Y, Teitelbaum SL, Neugut AI, Ahsan H, Garbowski GC, Hibshoosh H, Lin D, Kadlubar FF, Santella RM (2003) Evaluation of 4-aminobiphenyl–DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis*, **24**:719–725.

Feng Z, Hu W, Rom WN, Beland FA, Tang MS (2002) *N*-Hydroxy-4-aminobiphenyl–DNA binding in human *p53* gene: Sequence preference and the effect of C5 cytosine methylation. *Biochemistry*, **41**:6414–6421.

Flamini G, Romano G, Curigliano G, Chiominto A, Capelli G, Boninsegna A, Signorelli C, Ventura L, Santella RM, Sgambato A, Cittadini A (1998) 4-Aminobiphenyl–DNA adducts in laryngeal tissue and smoking habits: An immunohistochemical study. *Carcinogenesis*, **19**:353–357.

Flammang TJ, Kadlubar FF (1986) Acetyl coenzyme A-dependent metabolic activation of *N*-hydroxy-3,2'-dimethyl-4-aminobiphenyl and several carcinogenic *N*-hydroxy arylamines in relation to tissue and species differences, other acyl donors, and arylhydroxamic acid-dependent acyltransferases. *Carcinogenesis*, **7**:919–926.

Flammang TJ, Couch LH, Levy GN, Weber WW, Wise CK (1992) DNA adduct levels in congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. *Carcinogenesis*, **13**:1887–1891.

Fletcher K, Tinwell H, Ashby J (1998) Mutagenicity of the human bladder carcinogen 4aminobiphenyl to the bladder of Muta<sup>TM</sup>Mouse transgenic mice. *Mutation Research*, **400**:245–250.

Frederickson SM, Hatcher JF, Reznikoff CA, Swaminathan S (1992) Acetyl transferasemediated metabolic activation of *N*-hydroxy-4-aminobiphenyl by human uroepithelial cells. *Carcinogenesis*, **13**:955–961.

Fretland AJ, Doll MA, Gray K, Feng Y, Hein DW (1997) Cloning, sequencing, and recombinant expression of NAT1, NAT2, and NAT3 derived from the C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: Functional characterization of the activation and deactivation of aromatic amine carcinogens. *Toxicology and Applied Pharmacology*, **142**:360–366.

Gaylor DW (1979) The  $ED_{01}$  study: Summary and conclusions. *Journal of Environmental Pathology and Toxicology*, **3**:179–183.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Eaton DL, Gallagher EP, Bammler TK, Kunze KL (1995) Role of cytochrome P4501A2 in chemical carcinogenesis: Implications for human variability in expression and enzyme activity. *Pharmacogenetics*, **5**:259–274.

Faraglia B, Chen SY, Gammon MD, Zhang Y, Teitelbaum SL, Neugut AI, Ahsan H, Garbowski GC, Hibshoosh H, Lin D, Kadlubar FF, Santella RM (2003) Evaluation of 4- aminobiphenyl–DNA adducts in human breast cancer: The influence of tobacco smoke. *Carcinogenesis*, **24**:719–725.

Feng Z, Hu W, Rom WN, Beland FA, Tang MS (2002) *N*-Hydroxy-4-aminobiphenyl–DNA binding in human *p53* gene: Sequence preference and the effect of C5 cytosine methylation. *Biochemistry*, **41**:6414–6421.

Flamini G, Romano G, Curigliano G, Chiominto A, Capelli G, Boninsegna A, Signorelli C, Ventura L, Santella RM, Sgambato A, Cittadini A (1998) 4-Aminobiphenyl–DNA adducts in laryngeal tissue and smoking habits: An immunohistochemical study. *Carcinogenesis*, **19**:353–357.

Flammang TJ, Kadlubar FF (1986) Acetyl coenzyme A-dependent metabolic activation of *N*- hydroxy- $3,2_{\parallel}$ -dimethyl-4-aminobiphenyl and several carcinogenic *N*-hydroxy arylamines in relation to tissue and species differences, other acyl donors, and arylhydroxamic acid- dependent acyltransferases. *Carcinogenesis*, **7**:919–926.

Flammang TJ, Couch LH, Levy GN, Weber WW, Wise CK (1992)DNAadduct levels in congenic rapid and slow acetylator mouse strains following chronic administration of 4- aminobiphenyl. *Carcinogenesis*, **13**:1887–1891.

Fletcher K, Tinwell H, Ashby J (1998) Mutagenicity of the human bladder carcinogen 4- aminobiphenyl to the bladder of Muta Mouse transgenic mice. *Mutation Research*, **400**:245–250.

Frederickson SM, Hatcher JF, Reznikoff CA, Swaminathan S (1992) Acetyl transferase- mediated metabolic activation of *N*-hydroxy-4-aminobiphenyl by human uroepithelial cells. *Carcinogenesis*, **13**:955–961.

Fretland AJ, Doll MA, Gray K, Feng Y, Hein DW (1997) Cloning, sequencing, and recombinant expression of NAT1, NAT2, and NAT3 derived from the C3H/HeJ (rapid) and A/HeJ (slow) acetylator inbred mouse: Functional characterization of the activation and deactivation of aromatic amine carcinogens. *Toxicology and Applied Pharmacology*, **142**:360–366.

Gaylor DW (1979) The ED<sub>01</sub> study: Summary and conclusions. *Journal of Environmental Pathology and Toxicology*, **3**:179–183.

Gorrod JW, Carter RL, Roe FJ (1968) Induction of hepatomas by 4-aminobiphenyl and three of its hydroxylated derivatives administered to newborn mice. *Journal of the National Cancer Institute*, **41**:403–410.

Hammons GJ, Guengerich FP, Weis CC, Beland FA, Kadlubar FF (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. *Cancer Research*, **45**:3578–3585.

Hatcher JF, Swaminathan S (1992) Microsome-mediated transacetylation and binding of *N*-hydroxy-4-aminobiphenyl to nucleic acids by hepatic and bladder tissues from dog. *Carcinogenesis*, **13**:1705–1711.

Hatcher JF, Swaminathan S (1995) Detection of deoxyadenosine-4-aminobiphenyl adduct in DNA of human uroepithelial cells treated with *N*-hydroxy-4-aminobiphenyl following nuclease P1 enrichment and <sup>32</sup>P-postlabeling analysis. *Carcinogenesis*, **16**:295–301.

Hatcher JF, Swaminathan S (2002) Identification of *N*-(deoxyguanosin-8-yl)-4-azobiphenyl by <sup>32</sup>P-postlabeling analyses of DNA in human uroepithelial cells exposed to proximate metabolites of the environmental carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **39**:314–322.

Hatcher JF, Rao KP, Swaminathan S (1993) Mutagenic activation of 4-aminobiphenyl and its *N*-hydroxy derivatives by microsomes from cultured human uroepithelial cells. *Mutagenesis*, **8**:113–120.

Hein DW (1988) Acetylator genotype and arylamine-induced carcinogenesis. *Biochimica et Biophysica Acta*, **948**:37–66.

Hein DW, Grant DM, Sim E (2000) *Arylamine* N-*acetyltransferase (NAT) nomenclature* (http://louisville.edu/medschool/pharmacology/NAT.html).

Hickman ES, Moroni MC, Helin K (2002) The role of p53 and pRB in apoptosis and cancer. *Current Opinion in Genetics and Development*, **12**:60–66.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Hughes MF, Smith BJ, Eling TE (1992) The oxidation of 4-aminobiphenyl by horseradish peroxidase. *Chemical Research in Toxicology*, **5**:340–345.

IARC (1972) 4-Aminobiphenyl. In: *Some inorganic substances, chlorinated hydrocarbons, aromatic amines,* N-*nitroso compounds, and natural products.* Lyon, International Agency for Research on Cancer, pp. 74–79 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 1).

#### Harmonization Project Document No. 4

Gorrod JW, Carter RL, Roe FJ (1968) Induction of hepatomas by 4-aminobiphenyl and three of its hydroxylated derivatives administered to newborn mice. *Journal of the National Cancer Institute*, **41**:403–410.

Hammons GJ, Guengerich FP, Weis CC, Beland FA, Kadlubar FF (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog, and human hepatic microsomes and by purified flavin-containing and cytochrome P-450 monooxygenases. *Cancer Research*, **45**:3578–3585.

Hatcher JF, Swaminathan S (1992) Microsome-mediated transacetylation and binding of *N*- hydroxy-4aminobiphenyl to nucleic acids by hepatic and bladder tissues from dog. *Carcinogenesis*, **13**:1705–1711.

Hatcher JF, Swaminathan S (1995) Detection of deoxyadenosine-4-aminobiphenyl adduct inDNAof human uroepithelial cells treated with *N*-hydroxy-4-aminobiphenyl following nuclease P1 enrichment and <sup>32</sup>P-postlabeling analysis. *Carcinogenesis*, **16**:295–301.

Hatcher JF, Swaminathan S (2002) Identification of *N*-(deoxyguanosin-8-yl)-4-azobiphenyl by <sup>32</sup>P-postlabeling analyses of DNA in human uroepithelial cells exposed to proximate metabolites of the environmental carcinogen 4-aminobiphenyl. *Environmental and Molecular Mutagenesis*, **39**:314–322.

Hatcher JF, Rao KP, Swaminathan S (1993) Mutagenic activation of 4-aminobiphenyl and its *N*-hydroxy derivatives by microsomes from cultured human uroepithelial cells. *Mutagenesis*, **8**:113–120.

Hein DW (1988) Acetylator genotype and arylamine-induced carcinogenesis. *Biochimica et Biophysica Acta*, **948**:37–66.

Hein DW, Grant DM, Sim E (2000) Arylamine N-acetyltransferase (NAT) nomenclature (http://louisville.edu/medschool/pharmacology/NAT.html).

Hickman ES, Moroni MC, Helin K (2002) The role of p53 and pRB in apoptosis and cancer. *Current Opinion in Genetics and Development*, **12**:60–66.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal* Society of Medicine, **58**:295–300.

Hughes MF, Smith BJ, Eling TE (1992) The oxidation of 4-aminobiphenyl by horseradish peroxidase. *Chemical Research in Toxicology*, **5**:340–345.

IARC (1972) 4-Aminobiphenyl. In: *Some inorganic substances, chlorinated hydrocarbons, aromatic amines,* N-*nitroso compounds, and natural products*. Lyon, International Agency for Research on Cancer, pp. 74–79 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 1).

IARC (1986) *Tobacco smoking*. Lyon, International Agency for Research on Cancer, 421 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 38).

IARC (1987) 4-Aminobiphenyl (Group 1). In: *Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42.* Lyon, International Agency for Research on Cancer, pp. 91–92 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

Ioachim E, Charchanti A, Stavropoulos NE, Skopelitou A, Athanassiou ED, Agnantis NJ (2000) Immunohistochemical expression of retinoblastoma gene product (Rb), p53 protein, MDM2, c-erbB-2, HLA-DR and proliferation indices in human urinary bladder carcinoma. *Histology and Histopathology*, **15**:721–727.

Ishikawa J, Xu HJ, Hu SX, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R (1991) Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Research*, **51**:5736–5743.

Kadlubar FF, Miller JA, Miller EC (1977) Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Research*, **37**:805–814.

Kadlubar FF, Frederick CB, Weis CD, Zenser TV (1982) Prostaglandin endoperoxide synthetase-mediated metabolism of carcinogenic aromatic amines and their binding to DNA and protein. *Biochemical and Biophysical Research Communications*, **108**:253–258.

Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, Bailey JR, Young JF, Skipper PW, Tannenbaum SR (1991) Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Research*, **51**:4371–4377.

Kimura S, Kawabe M, Ward JM, Morishima H, Kadlubar FF, Hammons GJ, Fernandez-Salguero P, Gonzalez FJ (1999) CYP1A2 is not the primary enzyme responsible for 4-aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis*, **20**:1825–1830.

Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE (1965) Carcinogenesis in the human urinary bladder. Observations after exposure to *para*-aminodiphenyl. *New England Journal of Medicine*, **272**:767–770.

Koss LG, Melamed MR, Kelly RE (1969) Further cytologic and histologic studies of bladder lesions in workers exposed to *para*-aminodiphenyl: Progress report. *Journal of the National Cancer Institute*, **43**:233–243.

Kriek E (1992) Fifty years of research on *N*-acetyl-2-aminofluorene, one of the most versatile compounds in experimental research. *Journal of Cancer Research and Clinical Oncology*, **118**:481–489.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

IARC (1986) *Tobacco smoking*. Lyon, International Agency for Research on Cancer, 421 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 38).

IARC (1987) 4-Aminobiphenyl (Group 1). In: Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1 to 42. Lyon, International Agency for Research on Cancer, pp. 91–92 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

Ioachim E, Charchanti A, Stavropoulos NE, Skopelitou A, Athanassiou ED, Agnantis NJ (2000) Immunohistochemical expression of retinoblastoma gene product (Rb), p53 protein, MDM2, c-erbB-2, HLA-DR and proliferation indices in human urinary bladder carcinoma. *Histology and Histopathology*, **15**:721–727.

Ishikawa J, Xu HJ, Hu SX, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R (1991) Inactivation of the retinoblastoma gene in human bladder and renal cell carcinomas. *Cancer Research*, **51**:5736–5743.

Kadlubar FF, Miller JA, Miller EC (1977) Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Research*, **37**:805–814.

Kadlubar FF, Frederick CB, Weis CD, Zenser TV (1982) Prostaglandin endoperoxide synthetase-mediated metabolism of carcinogenic aromatic amines and their binding toDNA protein. *Biochemical and Biophysical Research Communications*, **108**:253–258.

Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, Bailey JR, Young JF, Skipper PW, Tannenbaum SR (1991) Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladderDNAadduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Research*, **51**:4371–4377.

Kimura S, Kawabe M, Ward JM, Morishima H, Kadlubar FF, Hammons GJ, Fernandez- Salguero P, Gonzalez FJ (1999) CYP1A2 is not the primary enzyme responsible for 4- aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis*, **20**:1825–1830.

Koss LG, Melamed MR, Ricci A, Melick WF, Kelly RE (1965) Carcinogenesis in the human urinary bladder. Observations after exposure to *para*-aminodiphenyl. *New England Journal of Medicine*, **272**:767–770.

Koss LG, Melamed MR, Kelly RE (1969) Further cytologic and histologic studies of bladder lesions in workers exposed to *para*-aminodiphenyl: Progress report. *Journal of the National Cancer Institute*, **43**:233–243.

Kriek E (1992) Fifty years of research on *N*-acetyl-2-aminofluorene, one of the most versatile compounds in experimental research. *Journal of Cancer Research and Clinical Oncology*, **118**:481–489.

Lakshmi VM, Mattammal MB, Zenser TV, Davis BB (1990) Mechanism of peroxidative activation of the bladder carcinogen 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT): Comparison with benzidine. *Carcinogenesis*, **11**:1965–1970.

Lang M, Pelkonen O (1999) Metabolism of xenobiotics and chemical carcinogenesis. *IARC Scientific Publications*, **148**:13–22.

Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG (1979) Effects of dose and time in a long-term, low-dose carcinogenic study. *Journal of Environmental Pathology and Toxicology*, **3**:17–34.

Logothetis CJ, Xu HJ, Ro JY, Hu SX, Sahin A, Ordonez N, Benedict WF (1992) Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. *Journal of the National Cancer Institute*, **84**:1256–1261.

Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT (1979) *N*-Acetyltransferase phenotype and risk in urinary bladder cancer: Approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environmental Health Perspectives*, **29**:71–79.

Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C (2002) Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. *Clinical Cancer Research*, **8**:171–179.

Manjanatha MG, Li EE, Fu PP, Heflich RH (1996) H- and K-*ras* mutational profiles in chemically induced liver tumours from B6C3F1 and CD-1 mice. *Journal of Toxicology and Environmental Health*, **47**:195–208.

Martone T, Airoldi L, Magagnotti C, Coda R, Randone D, Malaveille C, Avanzi G, Merletti F, Hautefeuille A, Vineis P (1998) 4-Aminobiphenyl–DNA adducts and *p53* mutations in bladder cancer. *International Journal of Cancer*, **75**:512–516.

Mattano SS, Land S, King CM, Weber WW (1989) Purification and biochemical characterization of hepatic arylamine *N*-acetyltransferase from rapid and slow acetylator mice: Identity with arylhydroxamic acid *N*,*O*-acyltransferase and *N*-hydroxyarylamine *O*-acetyltransferase. *Molecular Pharmacology*, **68**:599–609.

McQueen CA, Chau B, Erickson RP, Tjalkens RB, Philbert MA (2003) The effects of genetic variation in *N*-acetyltransferases on 4-aminobiphenyl genotoxicity in mouse liver. *Chemico-Biological Interactions*, **146**:51–60.

Melamed MR, Koss LG, Ricci A, Whitmore WF Jr (1960) Cytohistological observations on developing carcinoma of urinary bladder in man. *Cancer (Philadelphia)*, **13**:67–74.

#### Harmonization Project Document No. 4

Lakshmi VM, Mattammal MB, Zenser TV, Davis BB (1990) Mechanism of peroxidative activation of the bladder carcinogen 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT): Comparison with benzidine. *Carcinogenesis*, **11**:1965–1970.

Lang M, Pelkonen O (1999) Metabolism of xenobiotics and chemical carcinogenesis. *IARC Scientific Publications*, **148**:13–22.

Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG (1979) Effects of dose and time in a long-term, low-dose carcinogenic study. *Journal of Environmental Pathology and Toxicology*, **3**:17–34.

Logothetis CJ, Xu HJ, Ro JY, Hu SX, Sahin A, Ordonez N, Benedict WF (1992) Altered expression of retinoblastoma protein and known prognostic variables in locally advanced bladder cancer. *Journal of the National Cancer Institute*, **84**:1256–1261.

Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT (1979) *N*- Acetyltransferase phenotype and risk in urinary bladder cancer: Approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. *Environmental Health Perspectives*, **29**:71–79.

Lu ML, Wikman F, Orntoft TF, Charytonowicz E, Rabbani F, Zhang Z, Dalbagni G, Pohar KS, Yu G, Cordon-Cardo C (2002) Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome, assessed by conventional and array-based methods. *Clinical Cancer Research*, **8**:171–179.

Manjanatha MG, Li EE, Fu PP, Heflich RH (1996) H- and K-*ras* mutational profiles in chemically induced liver tumours from B6C3F1 and CD-1 mice. *Journal of Toxicology and Environmental Health*, **47**:195–208.

Martone T, Airoldi L, Magagnotti C, Coda R, Randone D, Malaveille C, Avanzi G, Merletti F, Hautefeuille A, Vineis P (1998) 4-Aminobiphenyl–DNA adducts and *p53* mutations in bladder cancer. *International Journal of Cancer*, **75**:512–516.

Mattano SS, Land S, King CM, Weber WW (1989) Purification and biochemical characterization of hepatic arylamine *N*-acetyltransferase from rapid and slow acetylator mice: Identity with arylhydroxamic acid *N*,*O*-acyltransferase and *N*-hydroxyarylamine *O*- acetyltransferase. *Molecular Pharmacology*, **68**:599–609.

McQueen CA, Chau B, Erickson RP, Tjalkens RB, Philbert MA (2003) The effects of genetic variation in *N*-acetyltransferases on 4-aminobiphenyl genotoxicity in mouse liver. *Chemico- Biological Interactions*, **146**:51–60.

Melamed MR, Koss LG, Ricci A, Whitmore WF Jr (1960) Cytohistological observations on developing carcinoma of urinary bladder in man. *Cancer (Philadelphia)*, **13**:67–74.

70

Melick WF, Escue HM, Naryka JJ, Mezera RA, Wheeler EP (1955) The first reported cases of human bladder tumors due to a new carcinogen—Xenylamine. *Journal of Urology (Baltimore)*, **74**:760–766.

Melick WF, Naryka JJ, Kelly RE (1971) Bladder cancer due to exposure to *para*-aminobiphenyl: A 17-year follow-up. *Journal of Urology (Baltimore)*, **106**:220–226.

Merck (1998) Merck veterinary manual. Whitehouse Station, NJ, Merck & Co., Inc.

Miller JA, Miller EC (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of human cancer*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 605–627.

Miller JA, Wyatt CS, Miller EC, Hartmann HA (1961) The *N*-hydroxylation of 4-acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of *N*-hydroxy-4-acetylaminobiphenyl in the rat. *Cancer Research*, **21**:1465–1473.

Oda Y (2004) Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. *Mutation Research*, **554**:399–406.

Oscarson M, Ingelman-Sundberg M, Daly AK, Nebert DW (n.d.) *Human Cytochrome P450 (CYP) Allele Nomenclature Committee* (http://www.cypalleles.ki.se/).

Parsons BL, Culp SJ, Manjanatha MG, Heflich RH (2002) Occurrence of H-*ras* codon 61 CAA to AAA mutation during mouse liver tumor progression. *Carcinogenesis*, **23**:943–948.

Parsons BL, Beland FA, Von Tungeln LS, Delongchamp RR, Fu P, Heflich RH (2005) Levels of 4-aminobiphenyl-induced somatic H-*ras* mutation in mouse liver correlate with potential for liver tumor development. *Molecular Carcinogenesis*, **42**:193–201.

Pauli BU, Alroy J, Weinstein RS (1983) The ultrastructure and pathobiology of urinary bladder cancer. In: Bryan GT, Cohen SM, eds. *The pathology of bladder cancer, Vol. II.* Boca Raton, FL, CRC Press, pp. 41–140.

Poirier MC, Beland FA (1992) DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications for DNA adduct-based human cancer risk assessment. *Chemical Research in Toxicology*, **5**:749–755.

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995) DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis*, **16**:2917–2921.

Presti JCJ, Reuter VE, Galan T, Fair WR, Cordon-Cardo C (1991) Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Research*, **51**:5405–5409.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Melick WF, Escue HM, Naryka JJ, Mezera RA, Wheeler EP (1955) The first reported cases of human bladder tumors due to a new carcinogen—Xenylamine. *Journal of Urology (Baltimore)*, **74**:760–766.

Melick WF, Naryka JJ, Kelly RE (1971) Bladder cancer due to exposure to *para-* aminobiphenyl: A 17year follow-up. *Journal of Urology (Baltimore)*, **106**:220–226.

Merck (1998) Merck veterinary manual. Whitehouse Station, NJ, Merck & Co., Inc.

Miller JA, Miller EC (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Hiatt HH, Watson JD, Winsten JA, eds. *Origins of human cancer*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, pp. 605–627.

Miller JA, Wyatt CS, Miller EC, Hartmann HA (1961) The *N*-hydroxylation of 4- acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of *N*-hydroxy-4- acetylaminobiphenyl in the rat. *Cancer Research*, **21**:1465–1473.

Oda Y (2004) Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. *Mutation Research*, **554**:399–406.

Oscarson M, Ingelman-Sundberg M, Daly AK, Nebert DW (n.d.) *Human Cytochrome P450 (CYP) Allele Nomenclature Committee* (http://www.cypalleles.ki.se/).

Parsons BL, Culp SJ, Manjanatha MG, Heflich RH (2002) Occurrence of H-*ras* codon 61 CAA to AAA mutation during mouse liver tumor progression. *Carcinogenesis*, **23**:943–948.

Parsons BL, Beland FA, Von Tungeln LS, Delongchamp RR, Fu P, Heflich RH (2005) Levels of 4aminobiphenyl-induced somatic H-*ras* mutation in mouse liver correlate with potential for liver tumor development. *Molecular Carcinogenesis*, **42**:193–201.

Pauli BU, Alroy J, Weinstein RS (1983) The ultrastructure and pathobiology of urinary bladder cancer. In: Bryan GT, Cohen SM, eds. *The pathology of bladder cancer, Vol. II.* Boca Raton, FL, CRC Press, pp. 41–140.

Poirier MC, Beland FA (1992)DNAadduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications forDNAadduct-based human cancer risk assessment. *Chemical Research in Toxicology*, **5**:749–755.

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995)DNAadduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis*, 16:2917–2921.

Presti JCJ, Reuter VE, Galan T, Fair WR, Cordon-Cardo C (1991) Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Research*, **51**:5405–5409.

Primdahl H, von der Maase H, Christensen M, Wolf H, Orntoft TF (2000) Allelic deletions of cell growth regulators during progression of bladder cancer. *Cancer Research*, **60**:6623–6629.

Reznikoff CA, Loretz LJ, Johnson MD, Swaminathan S (1986) Quantitative assessments of the cytotoxicity of bladder carcinogens towards cultured normal human uroepithelial cells. *Carcinogenesis*, **7**:1625–1632.

Roberts DW, Benson RW, Groopman JD, Flammang TJ, Nagle WA, Moss AJ, Kadlubar FF (1988) Immunochemical quantitation of DNA adducts derived from the human bladder carcinogen 4-aminobiphenyl. *Cancer Research*, **48**:6336–6342.

Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ, Colorectal Cancer Study Group (2003) Polymorphisms in the cytochrome P450 CYP1A2 gene (*CYP1A2*) in colorectal cancer patients and controls: Allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *British Journal of Clinical Pharmacology*, **55**:68–76.

Sarkar MA, Nseyo UO, Zhong B-Z (2002) Mutagenic outcome of the urinary carcinogen 4aminobiphenyl is increased in acidic pH. *Environmental Toxicology and Pharmacology*, 11:23–26.

Schieferstein GJ, Littlefield NA, Gaylor DW, Sheldon WG, Burgers GT (1985) Carcinogenesis of 4-aminobiphenyl in BALB/cStCrlfC3Hf/Nctr mice. *European Journal of Cancer and Clinical Oncology*, **21**:865–873.

Schroeder JC, Conway K, Li Y, Mistry K, Bell DA, Taylor JA (2003) *p53* mutations in bladder cancer: Evidence for exogenous versus endogenous risk factors. *Cancer Research*, **63**:7530–7538.

Sesardic D, Boobis AR, Edwards RJ, Davies DS (1988) A form of cytochrome P450 in man, orthologous to form *d* in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *British Journal of Clinical Pharmacology*, **26**:363–372.

Skipper PL, Tannenbaum SR, Ross RK, Yu MC (2003) Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **12**:503–507.

Smith BJ, Curtis JF, Eling TE (1991) Bioactivation of xenobiotics by prostaglandin H synthase. *Chemico-Biological Interactions*, **79**:245–264.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Krose D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

#### Harmonization Project Document No. 4

Primdahl H, von der Maase H, Christensen M, Wolf H, Orntoft TF (2000) Allelic deletions of cell growth regulators during progression of bladder cancer. *Cancer Research*, **60**:6623–6629.

Reznikoff CA, Loretz LJ, Johnson MD, Swaminathan S (1986) Quantitative assessments of the cytotoxicity of bladder carcinogenes towards cultured normal human uroepithelial cells. *Carcinogenesis*, 7:1625–1632.

Roberts DW, Benson RW, Groopman JD, Flammang TJ, Nagle WA, Moss AJ, Kadlubar FF (1988) Immunochemical quantitation of DNA adducts derived from the human bladder carcinogen 4aminobiphenyl. *Cancer Research*, **48**:6336–6342.

Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ, Colorectal Cancer Study Group (2003) Polymorphisms in the cytochrome P450 CYP1A2 gene (*CYP1A2*) in colorectal cancer patients and controls: Allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *British Journal of Clinical Pharmacology*, **55**:68–76.

Sarkar MA, Nseyo UO, Zhong B-Z (2002) Mutagenic outcome of the urinary carcinogen 4aminobiphenyl is increased in acidic pH. *Environmental Toxicology and Pharmacology*, **11**:23–26.

Schieferstein GJ, Littlefield NA, Gaylor DW, Sheldon WG, Burgers GT (1985) Carcinogenesis of 4aminobiphenyl in BALB/cStCrlfC3Hf/Nctr mice. *European Journal of Cancer and Clinical Oncology*, **21**:865–873.

Schroeder JC, Conway K, Li Y, Mistry K, Bell DA, Taylor JA (2003) *p53* mutations in bladder cancer: Evidence for exogenous versus endogenous risk factors. *Cancer Research*, **63**:7530–7538.

Sesardic D, Boobis AR, Edwards RJ, Davies DS (1988) A form of cytochrome P450 in man, orthologous to form *d* in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *British Journal of Clinical Pharmacology*, **26**:363–372.

Skipper PL, Tannenbaum SR, Ross RK, Yu MC (2003) Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **12**:503–507.

Smith BJ, Curtis JF, Eling TE (1991) Bioactivation of xenobiotics by prostaglandin H synthase. *Chemico-Biological Interactions*, **79**:245–264.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Krose D, Mangelsdorf I, Meek E, Rice JM, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Swaminathan S, Reznikoff CA (1992) Metabolism and nucleic acid binding of *N*-hydroxy-4-acetylaminobiphenyl and *N*-acetoxy-4-acetylaminobiphenyl by cultured human uroepithelial cells. *Cancer Research*, **52**:3286–3294.

Talaska G, Dooley KL, Kadlubar FF (1990) Detection and characterization of carcinogen– DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by <sup>32</sup>Ppostlabelling and subsequent thin-layer and high-pressure liquid chromatography. *Carcinogenesis*, **11**:639–646.

Tiguert R, Bianco FJJ, Oskanian P, Li Y, Grignon DJ, Wood DPJ, Pontes JE, Sarkar FH (2001) Structural alteration of p53 protein in patients with muscle invasive bladder transitional cell carcinoma. *Journal of Urology*, **166**:2155–2160.

Tsuneoka Y, Dalton TP, Miller ML, Clay CD, Shertzer HG, Talaska G, Medvedovic M, Nebert DW (2003) 4-Aminobiphenyl-induced liver and urinary bladder DNA adduct formation in Cyp1a2(-/-) and Cyp1a2(+/+) mice. *Journal of the National Cancer Institute*, **95**:1227–1237.

Underwood PM, Zhou Q, Jaeger M, Reilman R, Pinney S, Warshawsky D, Talaska G (1997) Chronic, topical administration of 4-aminobiphenyl induces tissue-specific DNA adducts in mice. *Toxicology and Applied Pharmacology*, **144**:325–331.

Verghis SBM, Essigmann JM, Kadlubar FF, Morningstar ML, Lasko DD (1997) Specificity of mutagenesis by 4-aminobiphenyl: Mutations at G residues in bacteriophage M13 DNA and  $G \rightarrow C$  transversions at a unique dG<sup>8-ABP</sup> lesion in single-stranded DNA. *Carcinogenesis*, **18**:2403–2414.

Vineis P, Caporaso N, Tannenbaum SR, Skipper PL, Glogowski J, Bartsch H, Coda M, Talaska G, Kadlubar FF (1990) Acetylation phenotype, carcinogen–hemoglobin adducts, and cigarette smoking. *Cancer Research*, **50**:3002–3004.

Von Tungeln LS, Bucci TJ, Hart RW, Kadlubar FF, Fu PP (1996) Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) in the CD1 newborn mouse bioassay. *Cancer Letters*, **104**:133–136.

Vousden KH, Lu X (2002) Live or let die: The cell's response to p53. *Nature Reviews. Cancer*, **2**:594–604.

Walpole AL, Williams MHC, Roberts DC (1952) The carcinogenic action of 4aminodiphenyl and 3:2'-dimethyl-4-aminodiphenyl. *British Journal of Industrial Medicine*, **9**:255–263.

Walpole AL, Williams MHC, Roberts DC (1954) Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl. *British Journal of Industrial Medicine*, **11**:105–109.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Swaminathan S, Reznikoff CA (1992) Metabolism and nucleic acid binding of *N*-hydroxy-4-acetylaminobiphenyl and *N*-acetoxy-4-acetylaminobiphenyl by cultured human uroepithelial cells. *Cancer Research*, **52**:3286–3294.

Talaska G, Dooley KL, Kadlubar FF (1990) Detection and characterization of carcinogen–DNAadducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by <sup>32</sup>P- postlabelling and subsequent thin-layer and high-pressure liquid chromatography. *Carcinogenesis*, **11**:639–646.

Tiguert R, Bianco FJJ, Oskanian P, Li Y, Grignon DJ, Wood DPJ, Pontes JE, Sarkar FH (2001) Structural alteration of p53 protein in patients with muscle invasive bladder transitional cell carcinoma. *Journal of Urology*, **166**:2155–2160.

Tsuneoka Y, Dalton TP, Miller ML, Clay CD, Shertzer HG, Talaska G, Medvedovic M, Nebert DW (2003) 4-Aminobiphenyl-induced liver and urinary bladderDNAadduct formation in Cyp1a2( $\mathbf{i}/\mathbf{i}$ ) and Cyp1a2( $\mathbf{i}/\mathbf{i}$ ) mice. *Journal of the National Cancer Institute*, **95**:1227–1237.

Underwood PM, Zhou Q, Jaeger M, Reilman R, Pinney S, Warshawsky D, Talaska G (1997) Chronic, topical administration of 4-aminobiphenyl induces tissue-specificDNAadducts in mice. *Toxicology and Applied Pharmacology*, **144**:325–331.

Verghis SBM, Essigmann JM, Kadlubar FF, Morningstar ML, Lasko DD (1997) Specificity of mutagenesis by 4-aminobiphenyl: Mutations at G residues in bacteriophage M13DNAand G  $\stackrel{\bullet}{1}$  C transversions at a unique dG<sup>8-ABP</sup> lesion in single-stranded DNA. *Carcinogenesis*, **18**:2403–2414.

Vineis P, Caporaso N, Tannenbaum SR, Skipper PL, Glogowski J, Bartsch H, Coda M, Talaska G, Kadlubar FF (1990) Acetylation phenotype, carcinogen–hemoglobin adducts, and eigarette smoking. *Cancer Research*, **50**:3002–3004.

Von Tungeln LS, Bucci TJ, Hart RW, Kadlubar FF, Fu PP (1996) Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6- phenylimidazo-[4,5-b]pyridine (PhIP) in the CD1 newborn mouse bioassay. *Cancer Letters*, **104**:133–136.

Vousden KH, Lu X (2002) Live or let die: The cell's response to p53. *Nature Reviews. Cancer*, **2**:594–604.

Walpole AL, Williams MHC, Roberts DC (1952) The carcinogenic action of 4- aminodiphenyl and 3:2<sub>1</sub>-dimethyl-4-aminodiphenyl. *British Journal of Industrial Medicine*, **9**:255–263.

Walpole AL, Williams MHC, Roberts DC (1954) Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl. *British Journal of Industrial Medicine*, **11**:105–109.

Weston A, Caporaso NE, Taghizadeh K, Hoover RN, Tannenbaum SR, Skipper PL, Resau JH, Trump BF, Harris CC (1991) Measurement of 4-aminobiphenyl–hemoglobin adducts in lung cancer cases and controls. *Cancer Research*, **51**:5219–5223.

Wright C, Thomas D, Mellon K, Neal DE, Horne CH (1995) Expression of retinoblastoma gene product and p53 protein in bladder carcinoma: Correlation with Ki67 index. *British Journal of Urology*, **75**:173–179.

Wu XR, Lin JH, Walz T, Haner M, Yu J, Aebi U, Sun TT (1994) Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. *Journal of Biological Chemistsy*, **269**:13716–13724.

#### Harmonization Project Document No. 4

Weston A, Caporaso NE, Taghizadeh K, Hoover RN, Tannenbaum SR, Skipper PL, Resau JH, Trump BF, Harris CC (1991) Measurement of 4-aminobiphenyl–hemoglobin adducts in lung cancer cases and controls. *Cancer Research*, **51**:5219–5223.

Wright C, Thomas D, Mellon K, Neal DE, Horne CH (1995) Expression of retinoblastoma gene product and p53 protein in bladder carcinoma: Correlation with Ki67 index. *British Journal of Urology*, **75**:173–179.

Wu XR, Lin JH, Walz T, Haner M, Yu J, Aebi U, Sun TT (1994) Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. *Journal of Biological Chemistsy*, **269**:13716–13724.

# FORMALDEHYDE AND GLUTARALDEHYDE AND NASAL CYTOTOXICITY: CASE-STUDY WITHIN THE CONTEXT OF THE IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Douglas McGregor, Hermann Bolt, Vincent Cogliano, & Hans-Bernhard Richter-Reichhelm

Formaldehyde and glutaraldehyde cause toxicity to the nasal epithelium of rats and mice upon inhalation. In addition, formaldehyde above certain concentrations induces dose-related increases in nasal tumours in rats and mice, but glutaraldehyde does not. Using the 2006 International Programme on Chemical Safety (IPCS) human framework for the analysis of cancer mode of action (MOA), an MOA for formaldehyde was formulated and its relevance tested against the properties of the non-carcinogenic glutaraldehyde. These compounds produce similar patterns of response in histopathology and in genotoxicity tests (although formaldehyde has been much more extensively studied). The MOA is based on the induction of sustained cytotoxicity and reparative cell proliferation induced by formaldehyde at concentrations that also induce nasal tumours upon long-term exposure. Data on dose dependency and temporal relationships of key events are consistent with this MOA. While a genotoxic MOA can never be ruled out for a compound that is clearly genotoxic, at least in vitro, the non-genotoxic properties fundamental to the proposed MOA can explain the neoplastic response in the nose and may be more informative than genotoxicity in risk assessment. It is not yet fully explained why glutaraldehyde remains non-carcinogenic upon inhalation, but its greater inherent toxicity may be a key factor. The dual aldehyde functions in glutaraldehyde are likely to produce damage resulting in fewer kinetic possibilities (particularly for proteins involved in differentiation control) and lower potential for repair (nucleic acids) than would be the case for formaldehyde. While there have been few studies of possible glutaraldehyde-associated cancer, the evidence that formaldehyde is a human carcinogen is strong for nasopharyngeal cancers, although less so for sinonasal cancers. This apparent discrepancy could be due in part to the classification of human nasal tumours with tumours of the sinuses, which would receive much less exposure to inhaled formaldehyde. Evaluation of the human relevance of the proposed MOA of formaldehyde in rodents is restricted by human data limitations, although the key events are plausible. It is clear that the human relevance of the formaldehyde MOA in rodents cannot be excluded on either kinetic or dynamic grounds.

# INTRODUCTION

Formaldehyde and glutaraldehyde are aliphatic mono- and dialdehydes, respectively, that undergo reactions typical of aldehydes to form acetals, cyanohydrins, oximes, hydrazones, and bisulfite complexes. They are highly reactive chemicals and produce covalently crosslinked complexes with DNA and proteins. Their metabolism has some commonality in that they are both oxidized by aldehyde dehydrogenases. Several studies have demonstrated that inhalation exposure to formaldehyde causes nasal tumours in rats, whereas no nasal tumours were observed in the only 2-year inhalation study of rats exposed to glutaraldehyde. IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

# ホルムアルデヒド及びグルタルアルデヒドの鼻腔内細胞毒性: 発がん MOA のヒトへの関連性を解析するための IPCS フレームワークを用いた事例研究<sup>1</sup>

#### Douglas McGregor, Hermann Bolt, Vincent Cogliano, & Hans-Bernhard Richter-Reichhelm

ホルムアルデヒド及びグルタルアルデヒドは、吸入によりラット及びマウスの鼻腔上皮に毒性を 示す。また、一定濃度以上のホルムアルデヒドはラットとマウスの鼻腔腫瘍を用量依存的に増加さ せるが、グルタルアルデヒドにそのような作用はない。発がん MOA 解析のための 2006 年国際化 学物質安全性計画(International Programme on Chemical Safety: IPCS)のヒトフレームワークを用い て、ホルムアルデヒドの MOA を策定し、非発がん性のグルタルアルデヒドの特性との関連性につ いて検討した。これらの化合物は、病理組織学的検査及び遺伝毒性試験において同様の反応パター ンを示した (ホルムアルデヒドの方が広範囲に研究されているものの)。MOA は、持続的な細胞毒 性と再生細胞増殖の誘発に基づいており、長期ばく露によりホルムアルデヒドが鼻腔内腫瘍を誘 発する濃度で生じるというものである。key events の用量依存性と時間的関係に関するデータは、 この MOA と一致している。少なくとも in vitro 評価で遺伝毒性が明らかな化合物については、遺 伝毒性 MOA を除外することはできない。しかし、提示されている MOA の基本となる非遺伝毒性 は、鼻の腫瘍性病変を説明することができ、リスク評価において遺伝毒性よりも有益な情報を提供 するかもしれない。グルタルアルデヒドを吸入しても発がん性を示さない理由はまだ完全には解 明されていないが、固有の毒性が重要な要因である可能性がある。グルタルアルデヒドの二つのア ルデヒド官能基は、ホルムアルデヒドに比べて、(特に分化制御に関与するタンパク質の場合)運 動性の低下や、(核酸の)修復機能の低下へとつながる損傷を引き起こす可能性が高い。グルタル アルデヒドに関連した発がん性に関する研究はほとんどない。一方、ホルムアルデヒドがヒトの発 がん物質であるというエビデンスは、副鼻腔がんでは少ないが、鼻咽頭がんについては多い。ホル ムアルデヒドの吸入ばく露がはるかに少ない副鼻腔の腫瘍を鼻腔腫瘍と区別せず分類しているこ とが一因となり、見かけの不一致を生み出していると考えられる。提示されているげっ歯類におけ るホルムアルデヒドの MOA はヒトへの関連性評価については、kev events は妥当ではあるが、ヒ トのデータが限られているため限定的である。げっ歯類におけるホルムアルデヒドの MOA のヒト への関連性は、動態的または薬力学的な理由から除外できないことは明らかである。

# 序文

ホルムアルデヒド、グルタルアルデヒドはそれぞれ脂肪族のモノアルデヒド、ジアルデヒドで あり、アルデヒドに典型的な反応を経てアセタール、シアノヒドリン、オキシム、ヒドラゾン、 重亜硫酸塩錯体を形成する。これらは反応性の高い化学物質であり、DNA やタンパク質と共有結 合で架橋した錯体を生成する。これらの代謝には、アルデヒド脱水素酵素によって酸化されると いう共通点がある。いくつかの研究では、ホルムアルデヒドの吸入ばく露はラットに鼻腔腫瘍を 引き起こすことが示されているが、グルタルアルデヒドをラットに2年間吸入ばく露させた試験 では鼻腔腫瘍は観察されなかった。

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## Formaldehyde

Formaldehyde has been tested for carcinogenicity by the inhalation route in mice, rats, and Syrian hamsters, by oral administration (drinking-water) in rats, by skin application in mice, and by subcutaneous injection in rats. There is conclusive evidence from the inhalation studies that formaldehyde is a carcinogen in rats.

There is considerable evidence that prolonged inhalation exposure to formaldehyde induces highly non-linear dose-related increases in the incidence of tumours of the anterior and posterior lateral meatus of rats (Morgan et al., 1986; Feron et al., 1988; Woutersen et al., 1989; Monticello et al., 1996; Kamata et al., 1997; CIIT, 1999). There are sharp increases in tumour incidence at formaldehyde concentrations equal to and greater than 7.2 mg/m<sup>3</sup>. Exposure to concentrations of 2.4 mg/m<sup>3</sup> and lower induced no malignant nasal tumours. Table 1 combines the data from two published rat studies (Kerns et al., 1983a; Monticello et al., 1996) conducted at the same laboratory and some additional information from one of these studies on a number of rats that had not been examined at the time of the publications (Schlosser et al., 2003). The majority of formaldehyde-induced neoplasms were squamous cell carcinomas.

# Table 1. Combined incidence of nasal squamous cell carcinomas in rats exposed to formaldehyde.

Formaldehyde concentration		
(mg/m <sup>3</sup> )	Number of rats at risk <sup>a</sup>	Actual number of tumours <sup>b</sup>
0	122	0
0.84	27	0
2.4	126	0
7.2	113	3
12	34	22
18	182	157

Note: Adapted from Schlosser et al. (2003).

<sup>a</sup> Rats at risk are those that survived to 2 years and were examined at that time plus those that died before 2 years in which tumours were found.

<sup>b</sup> Rats in which tumours were found at or before 2 years.

In contrast, inhalation studies in Syrian hamsters showed no carcinogenic effect at a single dose of 12.3 mg/m<sup>3</sup> (Dalbey, 1982), and one of two inhalation studies in mice showed no effect in females and squamous cell carcinomas in 2/17 males killed at 2 years at a high-dose concentration of 17.6 mg/m<sup>3</sup> (Kerns et al., 1983a, 1983b), whereas the other was inadequate for evaluation (Horton et al., 1963).

Studies on rats using other routes of exposure produced no significant results in two of four drinking-water studies (Takahashi et al., 1986; Tobe et al., 1989), forestomach papillomas in one study (Til et al., 1989), and leukaemia and gastrointestinal tract tumours in another (Soffritti et al., 1989), but the interpretation of the last study has been questioned (Feron et al., 1990). Mouse skin application and subcutaneous injection studies were not suitable for evaluation. In no study in rodents was there a significant increase in nasal tumours other than in the five inhalation exposure studies in rats—that is, at the entry portal.

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#### ホルムアルデヒド

ホルムアルデヒドの発がん性試験は、マウス、ラット、シリアンハムスターを対象とした吸入 試験、ラットを対象とした経口投与(飲料水)試験、マウスを対象とした皮膚貼付試験、ラット を対象とした皮下投与試験が行われている。ラットではホルムアルデヒドが発がん物質であると いう決定的なエビデンスは吸入試験の結果から得られている。

ホルムアルデヒドへの長期吸入ばく露は、ラットの前方及び後方の側鼻道腫瘍の発生率を、非 線形性用量相関的に増加させるというエビデンスがある(Morgan ら、1986; Feron ら、1988年; Woutersen ら、1989年; Monticello ら、1996年; Kamata ら、1997年; CIIT、1999年)。ホルムア ルデヒド濃度が 7.2 mg/m<sup>3</sup>以上になると、腫瘍発生率が急激に増加する。2.4 mg/m<sup>3</sup>以下の濃度で はばく露しても、悪性の鼻腔腫瘍は誘発されなかった。表1は、同じ研究室で実施された2つの ラットの試験データ(Kerns ら、1983年a; Monticello ら、1996年)と、これらの研究のうちの1 つで、公表時には調査されていなかった数匹のラットについての追加情報を組み合わせたもので ある(Schlosser ら、2003年)。ホルムアルデヒド誘発性腫瘍の多くは扁平上皮がんであった。

# 表1. ホルムアルデヒドにばく露されたラットにおける鼻腔扁平上皮がんの複合発生率

ホルムアルデヒド濃度	リスクを抱えるラットの数。	実際の腫瘍数り
$(mg/m^3)$		
0	122	0
0.84	27	0
2.4	126	0
7.2	113	3
12	34	22
18	182	157

注:Schlosser ら(2003)からの引用

\* リスクを抱えるラットとは、2年まで生存し、その時点で検査を受けたラットと、腫瘍が見つかった2年以内に死亡したラットのことである。

<sup>b</sup>2年以内に腫瘍が認められたラット

対照的に、シリアンハムスターを対象とした吸入試験では、12.3 mg/m<sup>3</sup>の単回投与で発がん性 を示さなかった(Dalbey、1982年)。マウスを用いた2つの吸入試験のうち1つは、17.6 mg/m<sup>3</sup>の 高用量濃度で雌には変化がなく、2年の時点でと殺した雄17匹のうち2匹には扁平上皮がんが認 められた(Kerns ら、1983年 a、1983年 b)。もう1つの試験は評価が不十分であった(Horton ら、 1963年)。

ラットを対象とした他のばく露経路を用いた試験では、4 つの飲水投与試験のうち 2 つの試験 (Takahashi ら、1986年; Tobe ら、1989年) では有意な結果は得られなかった。もう1 つの試験 (Til ら、1989年) では前胃乳頭腫、さらにもう一つの試験(Soffritti ら、1989年) では白血病及 び消化管腫瘍を認めた。しかし、最後に挙げた試験の解釈は疑問視されている(Feron ら、1990)。 マウスの皮膚貼付試験及び皮下投与試験は評価に適さなかった。げっ歯類を対象とした研究では、 ラットを対象とした 5 つの吸入ばく露試験以外に、鼻腔内腫瘍の有意な増加が認められた研究は なかった。

# Glutaraldehyde

Glutaraldehyde has been tested for carcinogenicity by the inhalation route in mice and rats and by oral administration (drinking-water) in rats. Inhalation studies showed no carcinogenic effect in either B6C3F1 mice exposed to a single concentration of 400  $\mu$ g/m<sup>3</sup> for 78 weeks (Zissu et al., 1998) or multiple concentrations up to 1000  $\mu$ g/m<sup>3</sup> for 2 years (NTP, 1999) or F344 rats exposed to concentrations of up to 3000  $\mu$ g/m<sup>3</sup> for 2 years (NTP, 1999). In a drinking-water study in which male and female F344 rats were exposed to glutaraldehyde concentrations of up to 4000 mg/m<sup>3</sup> for 2 years, increased incidences of large granular cell lymphatic leukaemia were found in the spleen of females at all exposure concentrations (Ballantyne, 1995; Van Miller et al., 1995).

# **1. IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH A MODE OF ACTION (MOA) IN ANIMALS?**

# A. Postulated mode of action

Prolonged exposure to formaldehyde above a critical concentration induces sustained cytotoxicity and cell proliferation. As a result of genetic changes within this proliferating cell population, neoplasia emerges. The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by formaldehyde. Formal-dehyde is a genotoxic substance in vitro and forms DNA–protein cross-links (DPX). DPX are a well established indicator of formaldehyde exposure, but it is not clear whether they are premutational lesions required to produce neoplasia (by initiating DNA replication errors, resulting in mutation). Apart from the abundance of DPX observations in rats, there is little evidence that formaldehyde is mutagenic to mammalian cells in vivo.

This postulated MOA is mainly based on observations of consistent, non-linear doseresponse relationships for all three key events (sustained cell proliferation, DPX, and tumours) and concordance of incidence of these effects across regions of the nasal passages.

# **B. Key events**

# Formaldehyde

Limitation of damage to the entry portal following exposure to formaldehyde is clearly important, with metabolism playing a significant role in the process. The importance of the entry portal for formaldehyde-induced nasal tumours is supported by the observation that the principal non-neoplastic effect in rats exposed orally to formaldehyde solutions is the development of histological changes within the forestomach and glandular stomach (Til et al., 1989; Tobe et al., 1989).

Formaldehyde is an endogenous metabolic product of *N*-, *O*-, and *S*-demethylation reactions within cells (Hardman et al., 2001), and circulating concentrations of about 2.0–2.6  $\mu$ g/g blood are normal in unexposed mammals (Heck et al., 1982, 1985; Casanova et al., 1988). Exogenous formaldehyde is rapidly detoxified upon absorption. It has a half-life in plasma of about 1 min in rats exposed intravenously (Rietbrock, 1965), and it readily and spontaneously combines with reduced glutathione to form *S*-hydroxymethylglutathione, the substrate for alcohol dehydrogenase 3 (ADH3, also known as glutathione-dependent formaldehyde

## グルタルアルデヒド

グルタルアルデヒドの発がん性については、マウス及びラットでは吸入試験、ラットでは経口 投与(飲水投与)試験が行われている。吸入試験では、B6C3F1マウスに400µg/m<sup>3</sup>の単濃度で78 週間ばく露させた場合(Zissu ら、1998年)、または1000µg/m<sup>3</sup>までの複数濃度で2年間ばく露さ せた場合(NTP、1999年)、またはF344 ラットに3000µg/m<sup>3</sup>までの濃度で2年間ばく露させた場 合(NTP、1999年)のいずれにおいても発がん性は認められなかった。雌雄のF344 ラットを最大 4000 mg/m<sup>3</sup>のグルタルアルデヒド濃度に2年間ばく露した飲水試験では、すべてのばく露濃度で 雌の脾臓に大顆粒リンパ球性白血病の発生率増加が認められた(Ballantyne、1995; Van Miller ら、 1995年)。

# 1. 動物における作用機序(MOA)を立証するのにエビデンスの重み付けは十分か

# A. 推定される MOA

ー定濃度以上のホルムアルデヒドを長時間ばく露させると、持続的な細胞毒性と細胞増殖が誘 発される。この増殖細胞塊内における遺伝的変化の結果、腫瘍が発生する。このような遺伝的変 化は、ホルムアルデヒドによって明らかに誘導される細胞毒性、化生、過形成の二次的なもので あると推測されている。ホルムアルデヒドは in vitro では遺伝毒性物質であり、DNA-タンパク質 架橋 (DPX)を形成する。DPX はホルムアルデヒドばく露の指標として確立されているが、それ らが腫瘍発生に必要な突然変異前病変 (DNA 複製エラーを起こし、突然変異をもたらす)である かは明らかではない。ラットで DPX が豊富であることを除けば、ホルムアルデヒドが生体内で哺 乳類の細胞に変異原性を示すというエビデンスはほとんどない。

この MOA は、主に3 つの key events (持続的な細胞増殖、DPX、腫瘍) すべてについて一貫した非線形の用量反応関係が観察されたこと及び鼻腔全体でこれらの影響の発生率が一致していることに基づいて推測される。

## **B** Key events

## ホルムアルデヒド

ホルムアルデヒドばく露後の障害が侵入口に限定されることは明らかに重要であり、その過程 では代謝が重要な役割を果たしている。ホルムアルデヒド誘発性鼻腔腫瘍における侵入口の重要 性は、ホルムアルデヒド溶液を経口ばく露させたラットにおいて、主要な非腫瘍性の影響が前胃 及び腺胃の組織学的変化の進展であったことから裏付けられる(Til ら、1989 年; Tobe ら、1989 年)。

ホルムアルデヒドは、細胞内の *N*・*O*-及び *S*・脱メチル化反応の内因性代謝産物であり(Hardman 6,2001年)、ばく露を受けていない哺乳類の正常な血中濃度は約2.0~2.6 μg/g である(Heck ら、 1982年、1985年; Casanova ら、1988年)。外因性ホルムアルデヒドは吸収されると急速に解毒さ れる。ホルムアルデヒドを静脈内投与されたラットにおける血漿中の半減期は約1分で(Rietbrock、 1965年)、還元されたグルタチオンと容易かつ自然に結合する。そして、アルコール脱水素酵素 3 (ADH3,グルタチオン依存性ホルムアルデヒド脱水素酵素としても知られている)の基質である *S*-ヒドロキシメチルグルタチオンを形成し、*S*-ホルミルグルタチオンを産生する(Uotila & Koivusalo、1974年; Koivusalo ら、1989年)。 dehydrogenase) (Uotila & Koivusalo, 1974; Koivusalo et al., 1989), to form *S*-formylglutathione, which is further metabolized to formic acid and reduced glutathione by *S*-formylglutathione hydrolase (Uotila & Koivusalo, 1997). The  $K_{\rm M}$  for initial binding of hydroxymethylglutathione with ADH3 is about 0.004 mmol/l, and the concentration of free formaldehyde is likely to be even lower (Uotila & Koivusalo, 1997; Hedberg et al., 1998). It may be toxicologically significant that formaldehyde also combines with thiols such as cysteine and cysteinylglycine (Holmquist & Vallee, 1991). In addition to this efficient metabolic detoxification mechanism, the mucociliary apparatus provides protection of the underlying epithelium from gases and vapours. Thus, in order to attain free formaldehyde concentrations that may be cytotoxic to the target tissue, relatively high concentrations of formaldehyde vapour must be delivered to the target site to overcome these protective mechanisms. Mechanistic events of clear significance for carcinogenicity occur at dose levels where formaldehyde detoxification mechanisms are saturated in rats (Casanova & Heck, 1987).

The predominant non-neoplastic and preneoplastic events that have been measured and associated with nasal cancer formation following inhalation exposure of the nasal epithelium to formaldehyde include cytotoxicity, DPX formation, nasal epithelial cell regenerative proliferation, squamous metaplasia, and inflammation, which are site-specific, highly non-linear response processes in concordance with the incidence of nasal tumours.

The relative magnitude of an increase in cell proliferation is dependent upon the size of the target cell population within specific regions of the nasal cavity and not always directly related to the length of exposure, or total cumulative exposure (Swenberg et al., 1983, 1986; Monticello et al., 1991, 1996; Monticello & Morgan, 1994). These factors have been well defined and measured in a number of studies in rat, monkey, and human epithelial cells. In a 24-month carcinogenicity assay with interim sacrifices at 3, 6, 12, and 18 months, cell proliferation was demonstrated in rats exposed to 7.2, 12, and 18 mg/m<sup>3</sup> at all times (Monticello et al., 1991, 1996).

An immunohistochemical technique was used to assess the presence of p53 protein, a marker of cell proliferation (proliferating cell nuclear antigen, or PCNA), and tumour growth factor (TGF)- $\alpha$  in the histopathological sections of the same tumours. In addition to the p53-positive immunostaining in squamous cell carcinomas, especially in cells with keratinization, p53-positive immunostaining was observed in preneoplastic hyperkeratotic plaques, while normal nasal mucosa did not stain. A correlation was found between the distribution of immunostaining of PCNA and that of p53 (Wolf et al., 1995).

The formation of DPX in rats is a non-linear function of concentration (Casanova & Heck, 1987; Casanova et al., 1989, 1994; Heck & Casanova, 1995) and correlates with the site specificity of tumours (Casanova et al., 1994). Cross-links were not detected in the olfactory mucosa or in the bone marrow of rats (Casanova-Schmitz et al., 1984; Casanova & Heck, 1987). DPX have been found in rhesus monkeys following inhalation exposure to formal-dehyde, with the highest concentrations in the middle turbinates, followed by the anterior lateral wall septum and nasopharynx (Casanova et al., 1991).

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これはS-ホルミルグルタチオンヒドロラーゼ (Uotila & Koivusalo、1997 年) によってさらにギ 酸に代謝され、グルタチオンを減少させる。ADH3 とヒドロキシ-メチルグルタチオンの初期結合 のための K<sub>M</sub> 値は約 0.004 mmol/L であり、遊離ホルムアルデヒドの濃度はさらに低い可能性が高 い (Uotila & Koivusalo、1997 年; Hedberg ら、1998 年)。また、ホルムアルデヒドがシステイン及 びシステイニルグリシンのようなチオール類と結合することは、毒性学的に重要であるかもしれ ない (Holmquist & Vallee、1991 年)。この効率的な解毒代謝メカニズムに加えて、絨毛粘膜はガス や蒸気から下層の上皮を保護する。したがって、標的組織において細胞毒性が発生する遊離ホル ムアルデヒドを高濃度で得るためには、これらの保護機構を克服することが必要であり、比較的 高濃度のホルムアルデヒド蒸気を標的部位に到達させる必要がある。発がん性にとって明確で意 義のある機序的な事象は、ラットにおけるホルムアルデヒドの解毒機構が飽和している用量で発 現することである (Casanova & Heck、1987 年)。

ホルムアルデヒド吸入ばく露後に測定された鼻のがん形成と関連している主な非腫瘍性及び前 腫瘍性徴候には、細胞毒性、DPX形成、鼻腔上皮細胞の再生性増殖、扁平上皮化生及び炎症が含 まれる。これらは部位特異的で高度に非線形な反応過程であり、鼻腔がんの発生率と一致してい る。

相対的な細胞増殖の亢進の程度は、鼻腔内の特定領域内の標的細胞集団の大きさに依存し、ば く露期間または累積ばく露の総量と常に相関するわけではない(Swenberg ら、1983年、1986年; Monticello ら、1991年、1996年; Monticello & Morgan、1994年)。これらの因子は、ラット、サル 及びヒト上皮細胞を対象とした多くの研究で明らかにされ、測定されている。3、6、12及び18ヶ 月の途中計画殺を伴う24ヶ月の発がん性試験では、7.2、12及び18 mg/m<sup>3</sup>の濃度でばく露された ラットはすべての時点で細胞増殖活性が実証された(Monticello ら、1991、1996年)。

免疫組織化学的手法を用いて、同一腫瘍の病理組織切片中の細胞増殖マーカーである p53 タン パク質(増殖細胞核抗原、または PCNA)及び腫瘍成長因子(TGF)を評価した。正常な鼻粘膜は 陰性であったが、扁平上皮がんでは p53 陽性となり、特に前腫瘍性病変である角化亢進性プラー ク内において角化した細胞での p53 陽性が観察された。PCNAの免疫染色の分布と p53 の免疫染 色の分布との間に相関関係が認められた(Wolf ら、1995 年)。

ラットにおける DPX の形成は、濃度の非線形関数であり(Casanova & Heck、1987年; Casanova
 ら、1989年、1994年; Heck & Casanova、1995年)、腫瘍の部位特異性と一致している(Casanova
 ら、1994年)。架橋はラットの嗅粘膜または骨髄では検出されなかった(Casanova-Schmitz ら、1984年; Casanova & Heck、1987年)。DPX はホルムアルデヒド吸入ばく露後のアカゲザルで認められており、その濃度が最も高い部位は中鼻甲介で、次いで前側壁中隔及び鼻咽頭であった(Casanova
 ら、1991年)。

Studies of rats, mice, Syrian hamsters, and rhesus monkeys exposed to formaldehyde for 13 (mice) or 26 weeks found that squamous metaplasia in the nasal turbinates developed in rats and rhesus monkeys at 3.7 mg/m<sup>3</sup>, but not in Syrian hamsters or, at 4.9 mg/m<sup>3</sup>, in mice (Rusch et al., 1983; Maronpot et al., 1986). Cell replication is also a feature of the more tumour-susceptible areas of the nasal epithelium of rats (Casanova et al., 1994).

# Glutaraldehyde

Inhalation exposure to glutaraldehyde at 400  $\mu$ g/m<sup>3</sup> for 78 weeks resulted in non-neoplastic lesions in the nasal vestibule of female mice, consisting of hyperplasia of the squamous epithelium lining the dorsal wall and the lateral aspect of the atrioturbinate (Zissu et al., 1998).

In the United States National Toxicology Program (NTP) studies of glutaraldehyde, the nasal changes observed in male and female rats included the following:

- 1. In the squamous epithelium in the most rostral part of the nasal passage, behind the external nares, there were increased incidences of hyperplasia and inflammation. The hyperplasia was a minimal to marked change characterized by variable thickening of the epithelium due to an increase in the number of cell layers and, in the more severe cases, varying degrees of keratin accumulation.
- 2. In the respiratory epithelium, there was hyperplasia, minimal goblet cell hyperplasia (primarily along the nasal septum and ventral meatus), inflammation, and squamous metaplasia, with accumulation of keratin on the epithelial surface in the more severe cases.
- 3. In the olfactory epithelium of the dorsal meatus, there were slightly increased incidences of hyaline degeneration.

The glutaraldehyde-associated inflammation that was observed in the squamous epithelial and respiratory epithelial regions was a minimal to marked change consisting of multifocal to locally extensive infiltrates of neutrophils, lymphocytes, and plasma cells. Occasionally, there were a few macrophages within the lamina propria and, in severe cases, within the epithelium itself. In male and female mice of this same study, the lesions were qualitatively similar to those found in rats. Females were more severely affected than male mice.

Glutaraldehyde induced DPX in a TK6 human lymphoblast cell line (St. Clair et al., 1991). In vivo, glutaraldehyde induced cell proliferation (S-phase nuclei) in nasal cells in rats and mice exposed by inhalation (Gross et al., 1994) and nasal instillation (St. Clair et al., 1990). In a parallel nasal instillation study by the same authors, formaldehyde induced the same level of cell proliferation at 20-fold higher molar concentrations.

# C. Dose-response relationship

## Formaldehyde

Available data from rats exposed to formaldehyde show a highly non-linear dose–response pattern for the key events, with no observed effects at 2.4 mg/m<sup>3</sup>, a minimal response at 7.2 mg/m<sup>3</sup>, and a sharp increase at 12 and 18 mg/m<sup>3</sup>.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

ラット、マウス、シリアンハムスター及びアカゲザルにホルムアルデヒドを 13 週間 (マウス) または 26 週間ばく露した研究において、ラット及びアカゲザルでは鼻尖部の扁平上皮化生が 3.7 mg/m<sup>3</sup>で発現したが、シリアハムスター及びマウスでは 4.9 mg/m<sup>3</sup>でも発現しなかった (Rusch ら、 1983 年; Maronpot ら、1986 年)。細胞の複製は、ラット鼻腔上皮のうち腫瘍感受性がより高い領 域の特徴でもある (Casanova ら、1994 年)。

## グルタルアルデヒド

400 μg/m<sup>3</sup>の濃度でグルタルアルデヒドを 78 週間吸入ばく露させた結果、雌マウスの鼻前庭に 非腫瘍性の病変が生じた。その病変は、背側壁を覆う扁平上皮の過形成及びアトリオタービネート (atrioturbinate)の側方面の過形成である (Zissu ら、1998 年)。

グルタルアルデヒドの米国国家毒性プログラム (NTP)の研究では、雌雄ラットにおいて以下のような鼻の変化が認められた。

- 鼻腔の最も吻側にある扁平上皮、外鼻腔の後ろにある扁平上皮では、過形成と炎症の発生率が 増加していた。過形成は、細胞層数の増加による上皮の不均一な肥厚と、より重篤な症例では、 様々な程度のケラチンの蓄積を特徴とする変化であった。
- 2. 呼吸上皮では、過形成、軽微な杯細胞過形成(主に鼻中隔及び腹側中隔に沿って)、炎症及び扁 平上皮化生が認められ、重度の症例では上皮表面にケラチンが蓄積していた。
- 3. 背側中膜の嗅覚上皮では、硝子変性がわずかに認められた。

扁平上皮及び呼吸上皮で観察されたグルタルアルデヒド関連の炎症は、好中球、リンパ球及び 形質細胞の多巣性から広範な浸潤にわたる様々な程度の変化があった。時折、固有層内に数個の マクロファージが存在し、重度の場合には上皮にも存在していた。この研究では雌雄のマウスに おいて、病変の質はラットと類似していたが、雌の方が雄よりも重症度は高かった。

グルタルアルデヒドは、TK6 ヒトリンパ芽細胞株において DPX を誘発した(St. Clair ら、1991年)。in vivo では、グルタルアルデヒドは、吸入(Gross ら、1994年)及び鼻腔内投与(St. Clair ら、1990年)によってばく露されたラット及びマウスの鼻腔内細胞において細胞増殖(S期)を誘導した。同じ著者による並行して実施された鼻腔内投与試験において、ホルムアルデヒドでは、20 倍高いモル濃度で同様の細胞増殖を誘発した。

# C. 用量反応関係

# ホルムアルデヒド

ホルムアルデヒドをばく露したラットから得られたデータによると、key eventsの用量反応パタ ーンは非線形であり、2.4 mg/m<sup>3</sup>では反応を認めず、7.2 mg/m<sup>3</sup>では最小の反応を認め、12 及び18 mg/m<sup>3</sup>では急激に反応が増加している。

In rats exposed to formaldehyde, no increases in cell turnover or DNA synthesis were found in the nasal mucosa after subchronic or chronic exposure to concentrations of  $\leq 2.4 \text{ mg/m}^3$ (Rusch et al., 1983; Zwart et al., 1988; Monticello et al., 1991; Casanova et al., 1994). Small, site-specific increases in the rate of cell turnover were noted at 3.7 mg/m<sup>3</sup> (6 h/day, 5 days/week, for 13 weeks) in Wistar rats (Zwart et al., 1988) and in the rate of DNA synthesis at 7.2 mg/m<sup>3</sup> in Fischer 344 rats exposed for a similar period (Casanova et al., 1994). At these concentrations, however, an adaptive response would seem to occur in rat nasal epithelium, since cell turnover rates after 6 weeks (Monticello et al., 1991) or 13 weeks (Zwart et al., 1988) are lower than those after 1–4 days of exposure. The unit length labelling index (ULLI) method was used to establish the proliferation in male Fischer 344 rats exposed to formaldehyde concentrations of 0, 0.84, 2.4, 7.2, 12, or 18 mg/m<sup>3</sup> for 6 h/day, 5 days/week, for 3, 6, 12, 18, or 24 months. Significant increases in ULLI were present only in the 12 and 18 mg/m<sup>3</sup> groups, with the greater increases on the anterior lateral meatus and the medial maxilloturbinate. Elevated ULLI in the anterior dorsal septum developed later in the course of the exposure. This belated elevation of ULLI may have been secondary to changes in airflow patterns and thus local formaldehyde concentrations associated with growth of lesions and distortion of the airspace in those areas of the nose more susceptible to neoplasia (Monticello et al., 1996).

The non-linear relationships for formaldehyde-induced DPX formation, epithelial cell proliferation, and subsequently nasal tumours are demonstrated in Table 2. It is arguable that the designations of high- and low-tumour areas proposed by Casanova et al. (1994) are not the most appropriate, and consequently the truly high tumour incidence region DPX response may have been diluted by that of the intermediate tumour incidence (posterior lateral meatus) region.

Other studies showed that Fischer 344 rats exposed to 1.2 mg/m<sup>3</sup> (22 h/day, 7 days/week, for 26 weeks) developed no detectable nasal lesions, whereas at 3.6 mg/m<sup>3</sup>, the only histological change was squamous metaplasia in the nasal turbinates (Rusch et al., 1983). The development of mild squamous metaplasia was similarly demonstrated in the nasal turbinates of Fischer 344 rats exposed to 2.4 mg/m<sup>3</sup> (6 h/day, 5 days/week, for 24 months) (Kerns et al., 1983b). Epithelial dysplasia and rhinitis were also observed in these rats. The occurrence of squamous metaplasia appears to be the histological feature requiring the lowest formaldehyde concentration of any of the in vivo responses reported.

A rat, anatomically accurate computational fluid dynamics model was used to test whether the distribution of formaldehyde-induced squamous metaplasia was related to the location of high-flux regions posterior to the squamous epithelium. Squamous metaplasia was considered present when  $\geq$ 50% of a subsection was lined by squamous epithelium. No squamous metaplasia was present in sections of nose from rats exposed to 2.4 mg/m<sup>3</sup> or less. Squamous metaplasia was present on the lateral meatus after exposure to 7.2 mg/m<sup>3</sup> or more and on the lateral and medial walls of the airway after exposure to 12 or 18 mg/m<sup>3</sup> (Kimbell et al., 1997).

There is evidence that glutathione-mediated detoxification of formaldehyde within nasal tissues becomes saturated in rats at inhalation exposures above 4.8 mg/m<sup>3</sup>. This saturation of

ホルムアルデヒドにばく露されたラットでは、2.4 mg/m<sup>3</sup>以下の濃度に亜慢性または慢性的にば く露した後の鼻粘膜で、細胞の再生及び DNA 合成の増加は認められなかった (Rusch 6、1983 年; Zwart 6、1988 年; Monticello 6、1991 年; Casanova 6、1994 年)。Wistar ラットでは細胞の再生 速度の部位特異的な軽度の増加が 3.7 mg/m<sup>3</sup> (6 時間/日、5 日/週、13 週間) で認められた (Zwart 6、1988 年)。同様の期間ばく露した Fischer 344 ラットでは DNA 合成速度の増加が 7.2 mg/m<sup>3</sup> で 認められた (Casanova 6、1994 年)。しかしながら、これらの濃度では、6 週間後 (Monticello 6、 1991 年) または 13 週間後 (Zwart 6、1988 年)の細胞再生率がばく露 1~4 日後よりも低いため、 ラットの鼻腔上皮では適応反応が起こると思われる。単位長ラベリング指数 (ULLI) 法を用いて、 0、0.84、2.4、7.2、12、または 18 mg/m<sup>3</sup> のホルムアルデヒド濃度に 6 時間/日、5 日/週、3、6、12、 18、または 24 ヶ月間ばく露した雄の Fischer 344 ラットにおける増殖を測定した。ULLI の有意な 上昇は 12 及び 18 mg/m<sup>3</sup> 群でのみ認められ、前側側鼻道及び内側上顎鼻甲介での上昇が大きかっ た。前背側中隔における ULLI の上昇は、ばく露後しばらくしてから発現した。この遅発性の ULLI 上昇は、鼻腔腫瘍の影響を受けやすい部分における、病変の成長と空隙の歪みに関連する局所的 なホルムアルデヒド濃度の変化でおこる気流変化による二次的なものと考えられた (Monticello 6、 1996 年)。

ホルムアルデヒド誘発性 DPX 形成、上皮細胞増殖及びその後の鼻腔腫瘍の非線形関係を表 2 に 示す。Casanova ら (1994 年) が提示した高腫瘍領域と低腫瘍領域の指定は最適なものではなく、 真に腫瘍発生率の高い領域の DPX 反応は、中間的な腫瘍発生率(後方側鼻道)領域の DPX 反応 によって希釈されている可能性がある。

他の研究では、1.2 mg/m<sup>3</sup> (1日 22 時間、7日/週、26 週間)にばく露した Fischer 344 ラットで は、検出可能な鼻腔病変は認められなかったが、3.6 mg/m<sup>3</sup>において組織学的変化は鼻甲介の扁平 上皮化生のみであった(Rusch ら、1983 年)。同様に 2.4 mg/m<sup>3</sup> (6 時間/日、5日/週、24ヶ月間) にばく露した Fischer 344 ラットの鼻甲介においても、軽度な扁平上皮化生の発生が示された (Kerns ら、1983 年 b)。これらのラットでは上皮異形成及び鼻炎も観察された。扁平上皮化生の 発生は、報告されている生体内反応の中で最も低濃度のホルムアルデヒドで発生する組織学的所 見だと思われる。

ラットにおいて正確に計算された解剖学的流体力学モデルを用いて、ホルムアルデヒドによっ て誘発される扁平上皮化生の分布が、扁平上皮の後方にある高流量領域に関係しているかどうか を検証した。50%以上が扁平上皮に置き換わっている場合、扁平上皮化生が生じているとみなさ れた。2.4 mg/m<sup>3</sup>以下でばく露されたラットの鼻には扁平上皮化生は認められなかった。7.2 mg/m<sup>3</sup> 以上のばく露では側鼻道に、12 または 18 mg/m<sup>3</sup>のばく露では気道の側壁及び内側壁に扁平上皮化 生がみられた (Kimbell ら、1997 年)。

4.8 mg/m<sup>3</sup>以上の吸入ばく露では、ラットで鼻組織内のホルムアルデヒドのグルタチオン介在性の解毒が飽和状態に達するというエビデンスがある。ホルムアルデヒド代謝の飽和は、この濃度以上のばく露における DPX、細胞増殖及び腫瘍発生率の用量反応関係の非線形性に寄与する可能性がある(Casanova & Heck、1987 年)。

formaldehyde metabolism may contribute to the non-linearity of the dose–response relationships for DPX, cell proliferation, and tumour incidence at exposures above this level (Casanova & Heck, 1987).

## Table 2. Comparative effects of formaldehyde exposure upon cell proliferation, DNAprotein cross-linking, and tumour incidence.

Formalde- hyde	Cell proliferation ([³H]thymidine-labelled cells/mm basement membrane)³		DNA-protein cross-link formation (pmol [ <sup>14</sup> C]- formaldehyde bound/mg DNA) <sup>b</sup>		Incidence of nasal carcinoma <sup>c</sup>				
concen- tration (mg/m <sup>3</sup> )	Anterior lateral meatus	Posterior lateral meatus	Anterior mid- septum	"High- tumour region"	"Low- tumour region"	All sites	Anterior lateral meatus	Posterior lateral meatus	Anterior mid- septum
0	10.11	7.69	6.58	0	0	0/90	0/90	0/90	0/90
0.84	10.53	7.82	8.04	5	5	0/90	0/90	0/90	0/90
2.4	9.83	11.24	12.74	8	8	0/96	0/96	0/96	0/96
7.2	15.68	9.96	4.15	30	10	1/90	1/90	0/90	0/90
12	76.79	15.29	30.01	-	-	20/90	12/90	2/90	0/90
18	93.22	59.52	75.71	150	60	69/147	17/147	9/147	8/147

<sup>a</sup> Cell proliferation measured in three locations of the nasal epithelium in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for 3 months (Monticello et al., 1996).

<sup>b</sup> Extent of DNA-protein cross-link formation measured in two regions of the nasal cavity (respiratory mucosa) in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for about 12 weeks; the complete lateral meatus was designated the "high-tumour region"; the "low-tumour region" comprised the medial aspects of naso- and maxilloturbinates, posterior lateral wall, posterior dorsal septum excluding olfactory region, and nasopharyngeal meatuses (Casanova et al., 1994). Data were derived from graphical representations in the reference cited.

<sup>c</sup> Incidence of nasal tumours within the entire nasal cavity or the anterior lateral meatus, posterior lateral meatus, or anterior mid-septum in male F344 rats exposed to the indicated concentrations of formaldehyde, 6 h/day, 5 days/week, for 24 months (Monticello et al., 1996).

## Glutaraldehyde

A series of repeated-dose experiments with rats and mice exposed to glutaraldehyde has been summarized by NICNAS (1994). Among these, the lowest concentration producing lesions of the nasal cavity of rats was 1000  $\mu$ g/m<sup>3</sup> (6 h/day, 5 days/week, for 13 weeks) (NTP, 1993). The most severe lesions occurred in the anterior portions of the nasal passages and involved both the respiratory and olfactory epithelium. Hyperplasia and squamous metaplasia were most commonly noted on the lateral wall of the nasal cavity and on the tips of the nasoturbinates. Lesions were most extensive in rats exposed to 4000  $\mu$ g/m<sup>3</sup>. In another study in rats, no nasal lesions were observed at concentrations up to 776  $\mu$ g/m<sup>3</sup> delivered for 14 weeks (Bushy Run, 1983).

Mice appeared to be more sensitive to glutaraldehyde inhalation in a 13-week study, with inflammation of the nasal cavity being observed in female mice even at the lowest concentration of 250  $\mu$ g/m<sup>3</sup> and in male mice at 1000  $\mu$ g/m<sup>3</sup>. The species difference in

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# 表 2. ホルムアルデヒドばく露の影響の比較:細胞増殖、DNA-タンパク質架橋及び 腫瘍発生率

				DNA-夕. 架橋形成					
		細胞増殖		[ <sup>14</sup> C]-ホル	レムアル				
	([³H] ℱ	ミジン標識	新用泡/mm	デヒ ド糸	<i>昔合/mg</i>				
ホルムア ルデヒド =		<i>基底膜)</i> 。		DNA	) ь		鼻腔がんの	の発生率。	
ルテヒド - 濃度 (mg/m <sup>3</sup> )	<i>前側</i> 鼻道	<i>後側</i> 鼻道	前中隔	<i>高腫瘍</i> <i>領域</i>	<i>低腫瘍</i> <i>領域</i>	全体	前側 鼻道	後側 鼻道	前中隔
0	10.11	7.69	6.58	0	0	0/90	0/90	0/90	0/90
0.84	10.53	7.82	8.04	5	5	0/90	0/90	0/90	0/90
2.4	9.83	11.24	12.74	8	8	0/96	0/96	0/96	0/96
7.2	15.68	9.96	4.15	30	10	1/90	1/90	0/90	0/90
12	76.79	15.29	30.01	_	_	20/90	12/90	2/90	0/90
18	93.22	59.52	75.71	150	60	69/147	17/147	9/147	8/147

a 指定された濃度のホルムアルデヒドに6時間/日、週5日/日、3ヶ月間ばく露した雄のF344 ラットの鼻腔上皮の3箇所で測定した細胞増殖 (Monticello ら、1996年)。

b 指定された濃度のホルムアルデヒドに6時間/日、5日/週、約12週間ばく露した雄のF344 ラットの鼻腔(呼吸器粘膜)の2つの領域で測定した DNA-タンパク質架橋形成の程度。腫瘍低発生領域は、鼻腔及び側鼻道の内側壁、後側壁、嗅覚領域を除く後背側鼻道及び鼻中隔からなる(Casanova ら、1994年)。データは引用文献中のグラフに基づいている。

c 指定された濃度のホルムアルデヒドに1日6時間、1日5日/週、24ヶ月間ばく露した雄性F344 ラットにおけ る鼻腔全体または鼻腔内の鼻腔腫瘍の発生率、または前側鼻道、後側鼻道、または前中隔の発生率 (Monticello ら、1996年)。

# グルタルアルデヒド

グルタルアルデヒドにばく露したラット及びマウスを用いた一連の反復投与試験が NICNAS (1994 年)にまとめられている。その中で、ラットの鼻腔に病変を生じる最も低い濃度は 1000 µg/m<sup>3</sup>(6時間/日、5日/週、13週間)であった(NTP、1993 年)。最も重篤な病変は鼻腔前部に発 生し、呼吸上皮と嗅上皮の両方を障害した。過形成及び扁平上皮化生は、鼻腔の側壁及び鼻尖部 に最もよく認められた。病変は 4000 µg/m<sup>3</sup>にばく露されたラットで最も広範囲に認められたが、 1000 及び 2000 µg/m<sup>3</sup>群でも認められ、500 µg/m<sup>3</sup>群の雄 1 匹でも認められた。ラットを用いた別 の研究では、最大 776 µg/m<sup>3</sup>の濃度で14週間投与しても鼻の病変は観察されなかった(Bushy Run、 1983 年)。

マウスはグルタルアルデヒド吸入による感受性が高いようであり、13週間の試験で雌マウスで は最低濃度の250µg/m<sup>3</sup>で、雄マウスでは1000µg/m<sup>3</sup>でも鼻腔の炎症が観察された。このような感 受性の違いは、マウスのほうが気道が狭いためゴミなどで閉塞しやすいためと考えられる(NTP、 1993年)。呼吸器の病理組織学的病変は4000µg/m<sup>3</sup>群のマウスで最も重篤であり、喉頭上皮の扁 平上皮の軽度から中程度の扁平上皮化生、鼻腔前部の膿瘍性炎症がみられた。 sensitivity is probably due to the smaller airways of mice being more prone to blockage by debris (NTP, 1993). Histopathological lesions in the respiratory tract were most severe in mice in the 4000  $\mu$ g/m<sup>3</sup> group and consisted of minimal to mild squamous metaplasia of the laryngeal epithelium, suppurative inflammation in the anterior parts of the nasal cavity, and minimal squamous metaplasia on the tips of the nasoturbinates. Necrosis and inflammation were noted at lower concentrations, primarily in the anterior portion of the nasal passage.

In the NTP (1993) 13-week studies with glutaraldehyde, there were significant, exposurerelated increases in ULLI in the squamous epithelium of the nasal vestibule and, to a lesser extent, the respiratory epithelium of the atrioturbinate of the dorsal meatus. The exposurerelated increase in cell replication was generally greater in rats than in mice. Upon examining the results in individual mice, it was found that there was an increased rate of cell replication in the squamous epithelium of the nasal vestibule only of those mice in which there was also neutrophilic infiltration of the mucosa; however, the severity of the infiltrate did not correlate with the degree of cell proliferation. These observations were clearest at 13 weeks, particularly in female mice. In rats, in addition to increased replication in the squamous epithelium of the vestibule, there was an equally prominent increase in replication in the respiratory epithelium of the dorsal atrioturbinate, whereas in mice, the response in this area was weak.

## **D.** Temporal association

## Formaldehyde

A number of short-, medium-, and long-term studies of the effect of formaldehyde exposure on cell proliferation within the respiratory epithelium of rats have indicated a sustained increase in proliferation of nasal epithelial cells following exposure to concentrations greater than 2.4 mg/m<sup>3</sup>, irrespective of the exposure period. Cell proliferation was observed in rats exposed to formaldehyde for periods from as short as 3 days. In the ULLI study already described, the magnitude of increased cell proliferation generally decreased over time but remained significantly increased by approximately 2- to 10-fold over controls, for certain nasal locations, up to and including the 18-month observation period when this effect was last examined (Monticello et al., 1996).

Data relating to temporal associations for DPX are limited, as most formaldehyde inhalation studies of DPX formation are of short duration (i.e. exposure duration up to 1 day). Formaldehyde-induced DPX in the nasal epithelium of rats and rhesus monkeys was shown consistently in these studies (Casanova et al., 1991). However, a well conducted study investigating both acute and cumulative DPX yields in rats exposed to formaldehyde for about 12 weeks (Casanova et al., 1994) found that the acute DPX yield in the lateral meatus (a high tumour yield site) of previously exposed rats was about half that in naive rats at concentrations greater than 7.2 mg/m<sup>3</sup>, while there were no differences in the medial and posterior meatuses (low tumour yield sites). No significant accumulation of DPX occurred in previously exposed rats.

Regenerative cell proliferation following formaldehyde-induced cytotoxicity increases the number of DNA replications and thus increases the probability of DPX-initiated DNA

鼻尖部の扁平上皮化生は軽度であった。壊死と炎症は低濃度で、主に鼻腔前部で認められた。

グルタルアルデヒドを用いた NTP (1993 年) の 13 週間の試験では、鼻前庭の扁平上皮と、そ れよりも軽度ではあるが、背側鼻道のアトリオタービネートの呼吸器上皮において、ばく露に関 連した ULLI の有意な増加が認められた。ばく露に関連した細胞増殖活性は、一般的にマウスよ りもラットの方が高かった。個々のマウスを調べたところ、鼻前庭の扁平上皮では、粘膜に好中 球浸潤が認められたマウスでのみ細胞増殖率の増加が認められた。ただし、細胞増殖の程度と浸 潤の重症度は相関していなかった。これらの観察は、特に雌マウスでは 13 週目に最も明らかにな った。ラットでは、前庭の扁平上皮の増殖増加に加えて、背側のアトリオタービネートの呼吸器 上皮でも同様に顕著な増殖増加がみられたが、マウスではこの領域での反応は弱かった。

## D. 時間的関連性

## ホルムアルデヒド

ラットの呼吸器上皮内の細胞増殖に対するホルムアルデヒドばく露の影響について、短期、中 期、長期の研究では、ばく露期間にかかわらず、2.4 mg/m<sup>3</sup>以上の濃度にばく露した後、鼻腔上皮 細胞の増殖が持続的に増加することが示されている。ホルムアルデヒドにばく露したラットでは、 3 日間という短い期間から細胞増殖が観察された。すでに述べた ULLI 研究では、一般的に細胞増 殖の増加率は時間の経過とともに減少したが、この影響は、特定の鼻腔部位では対照群の約 2~ 10 倍の増加を示し、最長で 18 ヶ月間の観察期間を含めて試験終了まで持続していた (Monticello ら、1996 年)。

ほとんどのホルムアルデヒド吸入による DPX 形成の研究は短期間(すなわち、ばく露期間が1 日まで)であるため、DPX の時間的関連性に関するデータは乏しい。ホルムアルデヒド誘発性 DPX は、ラットとアカゲザルの鼻腔上皮における研究で一貫して示された(Casanova ら、1991年)。 しかし、ホルムアルデヒドに約12週間ばく露したラットの急性及び累積 DPX 収量を調べる研究 (Casanova ら、1994年)において、7.2 mg/m<sup>3</sup>以上の濃度では、事前にばく露されたラットの側鼻 道(腫瘍発生率の高い部位)の急性 DPX 収量は、対照群の約半分であったが、内側及び後側の鼻 道(腫瘍発生率の低い部位)では差が見られなかった。事前にばく露されたラットでは、DPX の 有意な蓄積は認められなかった。

ホルムアルデヒド誘発細胞毒性後の再生性細胞増殖は、DNA 複製数を増加させる。その結果、 DPX が誘発する DNA 複製エラーを増加させ、突然変異を引き起こす。この仮説は、高濃度にお けるラットの鼻での DNA 複製の阻害(Heck & Casanova、1995年)及び前腫瘍病変における p53 発現の増加(Wolf ら、1995年)によって支持される。 increased cell replication (ULLI) in the nasal vestibule of rats occurred early (within a few days) and either remained elevated or decreased slightly through the course of the study. Increases in ULLI in the nasal vestibule of mice tended to develop with time. In an inhalation study with mice (Zissu et al., 1994), the earliest lesions were observed in the respiratory epithelium of the septum and the naso- and maxilloturbinates after 4 days of exposure to 1.2 mg/m<sup>3</sup>. Severe histopathological changes were still observed 2 weeks after the end of the exposure to 4.0 mg/m<sup>3</sup>. No exposure-related histological abnormalities were detected in the trachea and lungs.

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replication errors, resulting in mutations. This hypothesis is supported by the observed

inhibition of DNA replication in the rat nose at elevated concentrations (Heck & Casanova,

1995) and increased p53 expression in preneoplastic lesions (Wolf et al., 1995). In 5 of 11 squamous cell carcinomas from rats exposed to  $18 \text{ mg/m}^3$  for up to 2 years, there were point

mutations at the GC base pairs in the p53 complementary DNA (cDNA) sequence (Recio et

The study of cell replication in the 13-week rat and mouse inhalation studies with

glutaraldehyde (NTP, 1993) showed that, in contrast to the results obtained for mice, the

# E. Strength, consistency, and specificity of association of tumour response with key events

#### Formaldehyde

al., 1992).

Glutaraldehvde

There are extensive studies investigating formaldehyde-induced neoplasia. Available data revealed formaldehyde-induced DPX formation and increased epithelial cell proliferation within the upper respiratory tract in a range of species including rats and monkeys and a variety of rat and human cells in vitro. It was found that at similar levels of exposure, concentrations of DPX were approximately an order of magnitude lower in rhesus monkeys than in rats. Increased human epithelial cell proliferation following in situ exposure to formaldehyde was reported in a model system in which rat tracheae populated with human tracheobronchial epithelial cells were xenotransplanted into athymic mice.

There is good correlation between key events and regional tumour incidence and tumour sites. Cell proliferation, metaplasia, and increased DPX were seen in the regions of the nasal cavity where tumours have been observed. The highly non-linear dose–response relationships for DPX, cytotoxicity, cell proliferation, metaplasia, and tumours are consistent, with significant increases in metaplasia occurring at 2.4 mg/m<sup>3</sup> in one study and all end-points being observed at concentrations of greater than 4.8 mg/m<sup>3</sup>. This is also in good correlation with the concentration at which mucociliary clearance is inhibited and glutathione-mediated metabolism is saturated—that is, 4.8 mg/m<sup>3</sup>. The study by Morgan et al. (1986) examining effects of inhaled formaldehyde on the nasal mucociliary apparatus in male rats also included 18-h recovery groups following days 1, 9, and 14 of exposure to concentrations of 2.4 mg/m<sup>3</sup>, 7.2 mg/m<sup>3</sup>, and 18 mg/m<sup>3</sup>. Inhibition of mucociliary clearance was progressively more extensive with increasing duration of exposure, but showed little or no evidence of recovery 18 h after cessation of exposure.

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18 mg/m<sup>3</sup>に最大 2 年間ばく露して誘発されたラットの扁平上皮がんの 11 例中 5 例では、p53 相補 的 DNA (cDNA) 配列の GC 塩基対に点突然変異が認められた(Recio ら、1992 年)。

#### グルタルアルデヒド

ラット及びマウスの13週間のグルタルアルデヒド吸入試験(NTP、1993年)における細胞増殖 の研究では、マウスで得られた結果とは対照的に、ラットの鼻前庭における細胞増殖の増加(ULLI) は、早い時期(数日以内)に発生し、試験期間中は上昇したままであるか、またはわずかに減少 した。マウスの鼻前庭におけるULLIの増加は時間の経過とともに増加する傾向があった。マウ スを用いた吸入試験(Zissuら、1994年)では、1.2 mg/m<sup>3</sup>に4日間ばく露した後、鼻中隔の呼吸 上皮及び鼻及び顎甲介に最初の病変が観察された。4.0 mg/m<sup>3</sup>ばく露終了2週間後にも重篤な病理 組織学的変化が認められた。気管及び肺ではばく露に関連した組織学的異常は認められなかった。

### E. 腫瘍発生と key events との関連性の強さ、一貫性及び特異性

#### ホルムアルデヒド

ホルムアルデヒド誘発性腫瘍に関する広範な研究がある。入手可能なデータを見る限り、ラッ トやサル、ラットやヒトの様々な動物種や、ラットやヒトの様々な細胞の in vitro で明らかになっ ている。。同程度のばく露量では、DPX の濃度はラットよりもアカゲザルの方が約一桁低いことが 明らかになった。ホルムアルデヒドへの組織ばく露後のヒト上皮細胞増殖の亢進が、ヒトの気管 支上皮細胞を移植したラット気管を胸腺欠損マウスに異種移植したモデル系で報告された。

key events と局所的な腫瘍発生率及び腫瘍発生部位との間には相関関係がある。腫瘍が観察され ている鼻腔領域では、細胞増殖、化生及び DPX の増加が認められた。DPX、細胞毒性、細胞増殖、 化生、腫瘍についての非線形用量反応関係は一貫しており、ある試験では 2.4 mg/m<sup>3</sup> で化生の有意 な増加が認められ、4.8 mg/m<sup>3</sup> 以上の濃度では全てのエンドポイントが観察された。これはまた、 粘膜クリアランスが抑制され、グルタチオンを介した代謝が飽和する濃度、すなわち 4.8 mg/m<sup>3</sup> と 密接な相関関係を示している。Morgan ら (1986 年)による雄ラットの鼻粘膜に対する吸入ホルム アルデヒドの影響を検討した研究では、2.4 mg/m<sup>3</sup>、7.2 mg/m<sup>3</sup>、18 mg/m<sup>3</sup>の濃度で1日、9日及び 14 日間ばく露後、18 時間の回復期間を設けた群が含まれていた。粘膜クリアランスの阻害は、ば く露時間が長くなるにつれて徐々に範囲が広がったが、ばく露停止 18 時間後の回復はほとんど、 あるいは全く認められなかった。

Mice appear to be less susceptible than rats to the development of nasal tumours following exposure to a given concentration of formaldehyde. However, it is well known that mice decrease their minute volume in response to inhalation of noxious chemicals (Brown et al., 1986, in CIIT, 1999).

# Glutaraldehyde

In comparison with formaldehyde, the glutaraldehyde-induced lesions were located in a more anterior part of the nose, involving the squamous epithelium. Also, they were of a different character, with none of the focal hyperkeratosis and hyperplasia with cellular atypia and dysplasia found in animals receiving formaldehyde for 13 weeks (Monticello, 1990; Morgan & Monticello, 1990).

# F. Biological plausibility and coherence

# Formaldehyde

Evidence supporting the hypothesis that prolonged regenerative cell proliferation can be a causal mechanism in chemical carcinogenesis continues to accumulate (IPCS, 2002). This proposed MOA for formaldehyde-induced nasal tumours in animals exposed by inhalation is consistent with biological plausibility and the available data. Sustained increased cell proliferation has been observed in the nasal cavity in extensive short- and medium-term toxicity studies in rats and a few studies in other species. Histopathological effects in the nasal cavity (epithelial cell dysplasia and metaplasia) were consistent in a range of sub-chronic and chronic animal studies. It should be noted, however, that the respective roles of DPX, mutation, and cellular proliferation in the induction of nasal tumours in the rat have not been fully elucidated.

# Glutaraldehyde

Effects of inhaled glutaraldehyde have not been as extensively studied as those of formaldehyde. In inhalation studies, glutaraldehyde did not induce nasal tumours in rats and mice. However, the same key events that are considered key events in the nasal carcinogenicity of formaldehyde—cytotoxicity and cell proliferation—have been demonstrated in rats and mice exposed to glutaraldehyde. This might appear to reduce the plausibility of these processes being important for formaldehyde.

# G. Possible alternative modes of action

# Formaldehyde

There is the possibility that mutagenicity could play a role in the development of formaldehyde-induced tumours. Evaluation of the available data indicates that formaldehyde is genotoxic in vitro, but is generally not genotoxic in standard in vivo assays, although there are many studies demonstrating that it produces DPX.

Formaldehyde has been extensively studied for genotoxicity in vitro, with positive results in studies with bacterial and mammalian cells (Ames test, gene mutation), and produced DNA single-strand breaks and DPX (reviewed in IARC, 2005). In vivo, formaldehyde has reproducibly induced mutations in *Drosophila*, but there is no convincing evidence of its genotoxic activity in rodent bone marrow cell tests. There is limited evidence that formaldehyde expo-

ある濃度のホルムアルデヒドにばく露後発生する鼻腔腫瘍において、マウスはラットよりも感 受性が低いようである。しかしながら、マウスでは有害化学物質の吸入に反応して換気量/分が減 少することがよく知られている(Brown ら、1986年; CIIT、1999年)。

# グルタルアルデヒド

ホルムアルデヒドと比較して、グルタルアルデヒドによって誘発された病変は、扁平上皮を含む鼻の前方に位置していた。また、それらの病変は異なる特徴を有しており、ホルムアルデヒドを 13 週間投与した動物にみられる細胞異型及び異形成を伴う局所的な角化亢進及び過形成は認められなかった(Monticello、1990 年; Morgan & Monticello、1990 年)。

# F. 生物学的妥当性及び整合性

# ホルムアルデヒド

長期間にわたる再生性細胞増殖が化学物質による発がんの原因であるという仮説を支持するエ ビデンスが蓄積され続けている (PCS、2002 年)。吸入ばく露された動物におけるホルムアルデヒ ド誘発性鼻腔腫瘍に対する MOA は、生物学的に妥当性があり、利用可能なデータと一致してい る。ラットを対象とした短期・中期毒性試験や他種の研究では、鼻腔内の持続的な細胞増殖活性 が観察されている。鼻腔内の病理組織学的影響(上皮細胞の異形成と化生)は、亜慢性及び慢性 の動物実験で一貫していた。しかし、ラットの鼻腔腫瘍誘導における DPX、突然変異及び細胞増 殖のそれぞれの役割は完全には解明されていないことに留意すべきである。

# グルタルアルデヒド

吸入したグルタルアルデヒドの影響は、ホルムアルデヒドほど研究されていない。吸入試験で は、グルタルアルデヒドはラットとマウスの鼻腔腫瘍を誘発しなかった。しかし、ホルムアルデ ヒドの鼻発がん性において key events とみなされる細胞毒性と細胞増殖が、グルタルアルデヒド にばく露されたラットとマウスでも実証されている。このことは、これらの過程がホルムアルデ ヒドにとって重要であることの妥当性を低下させるように見えるかもしれない。

# G. 考えられる代替 MOA

# ホルムアルデヒド

変異原性がホルムアルデヒド誘発性腫瘍の発生に関与している可能性がある。利用可能なデー タを評価すると、ホルムアルデヒドは in vitro では遺伝毒性はあるが、標準的な in vivo アッセイ では概して遺伝毒性はないことが示されている。しかし、DPX を産生することを示す研究は数多 くある。

ホルムアルデヒドは in vitro での遺伝毒性について広く研究されており、細菌や哺乳類細胞を用 いた試験(Ames 試験、遺伝子突然変異)で陽性結果が得られ、DNAの一本鎖切断や DPXの産生 を認めた(IARC、2005 年レビュー)。生体内では、ホルムアルデヒドはショウジョウバエで再現 性のある突然変異を誘発したが、げっ歯類の骨髄細胞試験では、その遺伝毒性について有力なエ ビデンスはない。しかし、ホルムアルデヒドのばく露が、ヒトの鼻や頬の細胞や末梢血リンパ球 における染色体異常や小核発現頻度の増加と関連しているというわずかなエビデンスはある (IARC、2005 年;付録参照)。

sure is associated with increased chromosomal aberration and micronucleus frequencies in human nasal and buccal cells and peripheral blood lymphocytes (reviewed in IARC, 2005; see Appendix).

It is unclear to what extent DPX contributes to the mutagenesis and carcinogenicity of formaldehyde (Recio, 1997; Merk & Speit, 1998; Speit et al., 2000; Liteplo & Meek, 2003). The presence of DPX has been considered mainly as an indicator of exposure, although some have also seen these lesions as premutagenic in character and therefore evidence of a direct genotoxic mechanism. DPX are, however, potentially damaging to the afflicted cell, and cell death is a likely outcome should they occur at high frequency. They also indicate that protein–protein cross-linkage (PPX) may occur, with potentially less serious effects for the cell. Should key proteins be involved in the PPX formation, this could have consequences on the regulatory machinery of the cell, including the regulation of differentiation. Such a change clearly occurs in the nasal epithelium of rats exposed to formaldehyde, since areas of metaplasia emerge. Neoplasia could be viewed as simply a different kind of metaplasia, unless there is compelling evidence for a genotoxic mode of action.

A different interpretation of the data has been offered by Gaylor et al. (2004), who analysed the concentration-response relationship for formaldehyde-induced cell proliferation in rats using statistical methods designed to identify J-shaped concentration curves. Cell proliferation data were used because there were insufficient quantal data on cancer incidence to perform the analysis. Their analysis supports the hypothesis that the threshold-type dose-response for nasal tumour incidence is the result of a minor genotoxicity at low dose that is superimposed by a J-shaped dose-response for cell proliferation at high cytotoxic dose levels (Lutz, 1998). At low doses, the effect of incremental DNA damage may be cancelled out by a reduction in cell proliferation; therefore, in spite of the apparent threshold, the data remain consistent with a genotoxic mechanism.

In rats exposed to formaldehyde, point mutations at GC base pairs in the cDNA sequence of the evolutionarily conserved regions II–V of the *p53* gene were found in 5 of 11 primary nasal squamous cell carcinomas (Recio et al., 1992). This observation may be interpreted to indicate genotoxic processes induced by formaldehyde in the carcinogenic process; however, the presence of specific mutations in the emergent tumour is not evidence that they were present in the early stages of neoplasia or that they were directly induced by the chemical. While there is the possibility of a direct mutagenic event occurring, it is also possible that these mutations arose indirectly of exposure as a result of functional changes in chromatin proteins induced by the chemical. At what stage in the life history of the tumour these observed mutations occurred is also open to speculation: they are relatively common events, it is clear, but it is also clear that they are not essential events (since they do not occur in all tumours that are apparently of the same type). The occurrence of these mutations indicates that a genotoxic mechanism has not been excluded, but this evidence does not necessarily support one.

Specific changes in gene expression have also been observed in vivo. The results indicated that exposure to formaldehyde can cause alteration in the expression levels of genes involved in several functional categories, including xenobiotic metabolism, cell cycle regulation, DNA synthesis and repair, oncogenes, and apoptosis (Hester et al., 2003). It is not clear at present

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DPX がホルムアルデヒドの突然変異誘発性及び発がん性にどの程度寄与しているかは不明であ る (Recio、1997 年; Merk & Speit、1998 年; Speit ら、2000 年; Liteplo & Meek、2003 年)。DPX の存在は主にばく露の指標として考えられてきたが、これらの病変の特徴は前変異原性であり、 それゆえに直接的な遺伝毒性メカニズムのエビデンスであるという見方もある。しかし、DPX は 形成された細胞にダメージを与える可能性があり、高頻度で発生すると細胞死が起こる可能性が 高い。また、本研究では細胞への影響はそれほど深刻ではないものの、タンパク質-タンパク質架 橋 (PPX) が起こる可能性があることも示唆されている。もし主要なタンパク質が PPX の形成に 関与していれば、分化の制御を含む細胞の制御機構に影響を及ぼす可能性がある。化生領域が出 現しているため、このような変化は、ホルムアルデヒドにばく露されたラットの鼻腔上皮で生じ ている。遺伝毒性の MOA を示す有力なエビデンスがない限り、腫瘍は単に化生の一種と見なす ことができる。

Gaylor ら (2004 年) は、J 型濃度曲線を特定するように設計された統計手法を用いて、ラット におけるホルムアルデヒド誘発性細胞増殖の濃度-反応関係を解析し、異なるデータの解釈を提示 した。解析の際、がん発生率に関する定量的なデータが不十分であったため、細胞増殖のデータ を使用した。彼らの解析は、鼻腔腫瘍の発生に対する閾値を有する用量反応性が、低用量におけ る軽度な遺伝毒性の結果であり、これにより細胞毒性を有する高用量において J 字型の細胞増殖 曲線を呈するという仮説を支持するものである(Lutz、1998 年)。低用量では、漸増する DNA 損 傷は細胞増殖の減少によって打ち消される可能性があるため、明らかな閾値があるにもかかわら ず、データは遺伝毒性メカニズムと一致している。

ホルムアルデヒドにばく露されたラットにおいて、p53 遺伝子の進化的に保存された領域 II-V の cDNA 配列の GC 塩基対における点突然変異が、原発性鼻腔扁平上皮がん 11 例のうち 5 例で認 められた (Recio ら、1992 年)。この結果は、発がん過程におけるホルムアルデヒドにより誘発さ れた遺伝毒性過程を示すものと解釈される。しかし、出現した腫瘍における特定の突然変異の存 在は、それらが腫瘍の初期段階に存在していたエビデンスでも、それらが化学物質によって直接 誘導されたことを示すものではない。直接的な突然変異原性を示す可能性はあるが、これらの突 然変異は、化学物質によって誘発されたクロマチンタンパク質の機能的変化により間接的に生じ た可能性もある。これらの観察された突然変異が腫瘍の発生段階のどの段階で生じたかは推測の 域を出ない。これらの突然変異は一般的な事象であることは明らかであるが、すべて同じタイプ の腫瘍が発生するわけではないので、本質的な事象ではないこともまた明らかである。これらの 突然変異の発生は、遺伝毒性のメカニズムが排除されていないことを示しているが、必ずしも支 持するものでもない。

また、遺伝子発現の特異的な変化も生体内で観察されている。その結果、ホルムアルデヒドへ のばく露は、異物代謝、細胞周期制御、DNA 合成と修復、がん遺伝子、アポトーシスを含むいく つかの機能に関与する遺伝子の発現レベルに変化をもたらすことが示された(Hester ら、2003 年) これらの変化がホルムアルデヒドにどの程度特異的であるか、あるいは発がん性においてどのよ うな役割を果たしているのかは、現時点では明らかになっていない。

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how specific these changes are to formaldehyde or what their role is, if any, in carcinogenicity.

## Glutaraldehyde

Glutaraldehyde has been less extensively tested than formaldehyde for genotoxicity in vitro and in vivo. It produces weak and inconsistent positive findings in tests in vitro and is not active in the vast majority of in vivo studies. The genetic toxicity of glutaraldehyde has been recently reviewed (Zeiger et al., 2005).

Glutaraldehyde induced DNA repair systems in bacterial cells and was a weak mutagen in *Salmonella* and *Escherichia coli*. Unscheduled DNA synthesis (UDS), DPX, and double-strand breaks were seen in human cell lines, but not in primary rat cells. There were weak and inconsistent responses in chromosomal aberration and sister chromatid exchange (SCE) studies with mammalian cells, and glutaraldehyde did not induce transformation in cultured Syrian hamster embryo (SHE) cells.

In vivo, glutaraldehyde induced S-phase DNA synthesis in nasal cells in rats and mice following direct nasal administration. Glutaraldehyde did not produce DNA damage in rat liver or cross-links in rat testes DNA or sperm cells. Tests for induction of chromosomal aberration in bone marrow cells in rats and mice were generally negative. Glutaraldehyde did not induce micronuclei in bone marrow cells or dominant lethal mutations in mice. Thus, glutaraldehyde does possess genotoxic potential, and, although the database is not as extensive as it is for formaldehyde, it might be anticipated that site of contact genotoxicity would occur. Consequently, if genotoxicity is a major carcinogenic MOA for formaldehyde, it remains to be explained why glutaraldehyde is not active.

# H. Uncertainties, inconsistencies, and data gaps

#### Formaldehyde

In most of the cancer bioassays for formaldehyde, data on intermediate end-points such as proliferative response as a measure of cytotoxicity and DPX are limited. Consequently, direct comparison of the incidence of intermediate lesions and tumours is restricted. Additionally, information on a direct relationship between DPX and mutation induction and the probability of converting a DPX into a mutation is desirable, while the mode by which regenerative cell proliferation is involved in the production of mutations required for tumour development needs to be determined.

Studies on the *hprt* mutation spectrum in formaldehyde-exposed human cells revealed that 50% of the mutations are deletions, whereas 50% are due to point mutation at the A:T base pair (Crosby et al., 1988; Liber et al., 1989). The finding of deletions as part of the formaldehyde mutation spectrum may explain the homozygous nature of base pair mutations observed in p53 in formaldehyde-induced squamous cell carcinomas. However, there is an inconsistency with regard to the base pair that is mutated. It was found to be A:T in *hprt* in human and mammalian cell lines and G:C at p53 in formaldehyde-induced squamous cell carcinomas are induced by formaldehyde in vitro, these types of mutation may not be fundamental to its carcinogenicity.

#### グルタルアルデヒド

グルタルアルデヒドは、ホルムアルデヒドに比べて in vitro 及び in vivo での遺伝毒性に関する 試験があまり行われていない。グルタルアルデヒドは、in vitro 試験では軽微かつ不規則な陽性所 見を示し、in vivo 試験の大部分では活性がない。最近、グルタルアルデヒドの遺伝毒性に関する レビューが発表された(Zeiger ら、2005 年)。

グルタルアルデヒドは細菌では DNA 修復系を誘導し、サルモネラ菌や大腸菌では弱い変異原 性を示した。不定期 DNA 合成(UDS)、DPX、二本鎖切断はヒト細胞株で認められたが、ラット 初代細胞では認められなかった。哺乳類細胞を用いた染色体異常及び姉妹染色体交換(SCE)試験 では、軽微かつ一貫性のない反応がみられ、培養シリアンハムスター胚(SHE)細胞においてグル タルアルデヒドは形質転換を誘導しなかった。

生体内では、ラット及びマウスの鼻腔内投与後、グルタルアルデヒドは鼻腔内細胞にS期のDNA 合成を誘導した。グルタルアルデヒドはラット肝臓ではDNA 損傷を生じず、ラット精巣 DNA や 精子細胞では架橋を生じなかった。ラット及びマウスの骨髄細胞を用いた染色体異常試験では、 概ね陰性であった。グルタルアルデヒドは、マウスの骨髄細胞に小核や優性致死性突然変異を誘 発しなかった。このように、グルタルアルデヒドは遺伝毒性を有する可能性があり、データベー スはホルムアルデヒドほど豊富ではないが、接触部位における遺伝毒性の発生が予想される。し たがって、遺伝毒性がホルムアルデヒドの主要な発がん MOA であるとすれば、なぜグルタルア ルデヒドが効果を発揮しないのかを説明する必要がある。

# H. 不確実性、矛盾、データギャップ

## ホルムアルデヒド

ホルムアルデヒドを用いたがんのバイオアッセイのほとんどは、細胞毒性の指標としての増殖 反応や DPX などの中間エンドポイントに関するデータが限られている。そのため、中間病変と腫 瘍の発生率を直接比較することには制限がある。加えて DPX と突然変異誘発との直接的な関係に 関する情報及び DPX が突然変異につながる確率に関する情報が望まれる。一方で、腫瘍の発生に 必要な突然変異の生成にはどの再生性細胞増殖が関与しているかを決定する必要がある。

ホルムアルデヒドばく露ヒト細胞における hprr 遺伝子突然変異スペクトルの研究により、突然 変異の 50%は欠失であり、50%は A:T 塩基対での点突然変異によるものであることが明らかに なった (Crosby ら、1988 年; Liber ら、1989 年)。ホルムアルデヒド誘発性扁平上皮がんにおいて、 ホルムアルデヒド突然変異スペクトルの一部として欠失が認められたことで、p53 遺伝子で観察 されるホモ塩基対変異の性質を説明できるかもしれない。しかし、変異している塩基対に関して は矛盾がある。ヒト及び哺乳類細胞株では hprr 遺伝子の A:T であり、ホルムアルデヒド誘発性 扁平上皮がんでは p53 の G:C であることが明らかになっている(Recio、1997 年)。in vitro では ホルムアルデヒドによってこのような突然変異が誘導されるが、このタイプの突然変異はホルム アルデヒドの発がん性の根本ではない可能性がある。

# Glutaraldehyde

Glutaraldehyde is clearly much more cytotoxic than formaldehyde, perhaps because it is a bifunctional alkylating agent. Intranasal instillation studies have demonstrated that, on a molar basis, glutaraldehyde is 10- to 20-fold more toxic than formaldehyde when delivered to the nasal mucosa as a single treatment in aqueous solution (St. Clair et al., 1990). Comparison of results from a 13-week inhalation study of glutaraldehyde (NTP, 1993) with similar inhalation studies with formaldehyde (Heck et al., 1990; Monticello, 1990; Monticello et al., 1991) shows that glutaraldehyde is about 20-fold more toxic than formaldehyde by this route also. Pulmonary damage and mortality occur at much higher glutaraldehyde concentrations. Cytotoxicity is manifest closer to the external nares in the case of inhaled glutaraldehyde. This difference in the site of toxic action may be particularly important because, if the only difference was toxic potency, then glutaraldehyde would be expected to produce effects similar to those of formaldehyde, although only at lower doses.

# I. Assessment of postulated mode of action

# Formaldehyde

From a weight-of-evidence point of view, the hypothesized MOA for formaldehyde-induced nasal tumours satisfies several criteria, including consistency, concordance of dose–response relationships across all key events, and biological plausibility and coherence of the database. Given the extensive experimental data that address and are consistent with the proposed MOA of formaldehyde in the induction of tumours in the nasal cavity, a high degree of confidence may be ascribed to it.

## Glutaraldehyde

The key events of cytotoxicity, cell proliferation, and DPX formation (in vitro) have been demonstrated with exposure to glutaraldehyde. However, glutaraldehyde has not produced nasal tumours in rats and mice. Therefore, if the proposed MOA for formaldehyde is to be maintained, an explanation for this discrepancy is necessary. A reason for the difference has not been identified, but a hypothesis can be proposed. The dialdehyde function of glutaraldehyde is an important factor that may inhibit the macromolecules with which it reacts from further reaction within the cellular environment. Should these macromolecules be proteins involved in the maintenance of survival, then their immobility perhaps more likely leads to cell death than to a change in differentiation state. This immobilization of macromolecules by glutaraldehyde is the property that makes it a better fixative for high-resolution microscopy (e.g. electron microscopy) than formaldehyde. It almost certainly contributes to the very much higher toxicity of the dialdehyde. The monoaldehyde function of formaldehyde also causes cellular damage, but a proportion of proteins involved in cellular differentiation may be able to continue in that role, although with an altered outcome that may be the beginning of a path to neoplasia. If, on the other hand, these aldehydes react with nucleic acids (the evidence for glutaraldehyde reacting in this way is not substantial), then the repair of the alkylated nucleotides may be more difficult or even impossible in the case of glutaraldehyde, whereas repair does occur following formaldehyde interaction with DNA. Thus, irrespective of whether the mode of formaldehyde action in carcinogenicity is as proposed or is primarily due to genetic toxicity, the different response to glutaraldehyde exposure can be explained.

## グルタルアルデヒド

グルタルアルデヒドは二官能性アルキル化剤であるためか、ホルムアルデヒドよりも明らかに 細胞毒性が強い。経鼻投与試験では、水溶液を鼻粘膜に単回投与した場合、モル比でグルタルア ルデヒドはホルムアルデヒドの10~20倍の毒性があることが実証されている(St. Clair 6、1990 年)。グルタルアルデヒドの13週間吸入試験(NTP、1993年)の結果とホルムアルデヒドの同様 の吸入試験(Heck 6、1990年; Monticello 6、1991年)との比較は、こ の経路でもグルタルアルデヒドはホルムアルデヒドより約20倍の毒性があることを示している。 肺障害及び壊死は、かなり高い濃度のグルタルアルデヒドで発生する。細胞毒性は、グルタルア ルデヒドを吸入した場合には外鼻に近いところで発現するため、主に影響を受ける組織はホルム アルデヒドを吸入した場合と同じではない。唯一の違いが毒性の強さであるならば、グルタルア ルデヒドは、低用量でもホルムアルデヒドと同様の影響をもたらすと予想されるため、毒性作用 部位における違いは特に重要かもしれない。

# I. 推定される MOA の評価

# ホルムアルデヒド

重要なエビデンスの観点より、ホルムアルデヒド誘発性鼻腔腫瘍の仮説 MOA は一貫して、key events すべてにおける用量反応関係の一致、データベースの生物学的妥当性と整合性など、いく つかの基準を満たしている。鼻腔内腫瘍誘発におけるホルムアルデヒドの推定される MOA は多 くの実験データと一致しているため、この MOA は信頼性が高いと考えられる。

# グルタルアルデヒド

細胞毒性、細胞増殖及び DPX 形成の key events (in vitro) は、グルタルアルデヒドへのばく露 で実証されている。しかし、グルタルアルデヒドはラットやマウスでは鼻腔内腫瘍を発生させな い。したがって、推定されているホルムアルデヒドの MOA を維持するためには、この矛盾を説 明する必要がある。この矛盾の理由は特定されていないが、仮説を提示することは可能である。 グルタルアルデヒドの二つのアルデヒド官能基は、細胞環境内で高分子のさらなる反応を阻害す る重要な因子である。これらの高分子は、生存に関与するタンパク質であり、それらの不動化は、 おそらく分化状態の変化よりも細胞死につながる。このグルタルアルデヒドの高分子を不動化さ せる特性が、ホルムアルデヒドよりも高分解能顕微鏡(例えば電子顕微鏡)用の優れた固定剤に なる理由である。そのことはほぼ確実にジアルデヒドの非常に高い毒性につながっている。また、 ホルムアルデヒドのアルデヒド官能基は細胞障害を引き起こすが、細胞の分化に関与するタンパ ク質は、腫瘍の初期変化となりうる分化方向の変化を伴うものの、その役割を維持することがで きるかもしれない。一方、これらのアルデヒドが核酸と反応する場合(グルタルアルデヒドがこ のように反応するというエビデンスは実質的なものではない)、ホルムアルデヒドが DNA と相互 作用した後にアルキル化されたヌクレオチドの修復が起こるのに対し、グルタルアルデヒドの場 合は、困難、あるいは不可能かもしれない。このように、発がん性におけるホルムアルデヒドの 作用様式が推定されている通りなのか、それとも遺伝毒性が主な原因なのかにかかわらず、グル タルアルデヒドばく露に対する反応の違いを説明することができる。

# 2. CAN HUMAN RELEVANCE OF THE MOA BE EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

# A. Formaldehyde

In rhesus monkeys exposed to formaldehyde at 7.2 mg/m<sup>3</sup> for between 1 and 6 weeks, formaldehyde-induced lesions were associated with increases in cell proliferation rates of up to 18-fold over controls and remained significantly elevated after 6 weeks of exposure. Histological lesions and increases in cell proliferation were most extensive in the nasal passages and were minimal in the lower airways, whereas the maxillary sinuses showed no evidence of a response to formaldehyde exposure. Based on the extent of lesions and cell proliferation data, it appeared that rhesus monkeys are more sensitive than rats to the acute and subacute effects of formaldehyde at 7.2 mg/m<sup>3</sup> (Monticello et al., 1989). The absence of response in the maxillary sinuses in rhesus monkeys is an observation deserving special attention in the design of epidemiological studies (or, perhaps, in the reporting of tumour sites). Most epidemiological studies of sinonasal cancer have not distinguished tumours arising in the nose from those developing in the nasal sinuses. Thus, the risk for nasal cancer specifically would tend to be diluted if there was no corresponding risk for cancer in the sinuses and could go undetected through lack of statistical power.

Many epidemiological studies have investigated formaldehyde exposure and cancer of the respiratory tract. The strongest evidence of an association has been observed for naso-pharyngeal cancers. A statistically significant excess of deaths from nasopharyngeal cancer has been observed in the largest cohort study of industrial workers (Hauptmann et al., 2004), with statistically significant exposure–response relationships for peak and cumulative exposure. An excess of deaths from nasopharyngeal cancer was observed in a proportionate mortality analysis of the largest cohort of embalmers in the United States (Hayes et al., 1990). An excess of cases of nasopharyngeal cancer was observed in a Danish study of proportionate cancer incidence among workers at companies that manufactured or used formaldehyde (Hansen & Olsen, 1995). Other cohort studies reported fewer cases of nasopharyngeal cancer than expected (Walrath & Fraumeni, 1983; Coggon et al., 2003; Pinkerton et al., 2004). Of seven case–control studies of nasopharyngeal cancer, five found elevations of risk for exposure to formaldehyde.

Several case–control studies have investigated the association between exposure to formaldehyde and sinonasal cancer. A pooled analysis of 12 studies showed an increased risk of adenocarcinoma in men and women thought never to have been exposed to wood dust or leather dust, with an exposure–response trend for an index of cumulative exposure (Luce et al., 2002). One other case–control study (Olsen & Asnaes, 1986) and a proportionate incidence study (Hansen & Olsen, 1995) showed an increased risk of sinonasal cancer, particularly squamous cell carcinoma. However, the three most informative cohort studies of industrial workers showed no excesses of sinonasal cancer (Coggon et al., 2003; Hauptmann et al., 2004; Pinkerton et al., 2004).

In evaluating this body of evidence, the International Agency for Research on Cancer (IARC) concluded that there was sufficient epidemiological evidence that formaldehyde causes

# 2. 実験動物とととの間の key evants の根本的、質的な違いに基づいて、MOA のととの 関連性を合理的に排除することができるか

## A. ホルムアルデヒド

7.2 mg/m<sup>3</sup>のホルムアルデヒドに 1~6 週間ばく露したアカゲザルにおいて、ホルムアルデヒド に誘発された病変は、対照群の最大 18 倍を示す細胞増殖率の増加を伴っており、6 週間のばく露 後も有意に高い値が続いた。組織学的な病変と細胞増殖の増加は鼻腔で最も広範囲に見られ、下 気道では最小限であったが、上顎洞ではホルムアルデヒドばく露に対する反応のエビデンスは認 められなかった。病変の程度及び細胞増殖のデータに基づいて、7.2 mg/m<sup>3</sup>の用量でのホルムアル デヒドの急性及び亜急性影響に対する感受性はラットよりもアカゲザルの方が高いと考えられた (Monticello ら、1989 年)。アカゲザルの上顎洞における反応がないことは、疫学研究において(あ るいは、腫瘍部位の報告において)特別な注意を払うに値する所見である。副鼻腔がんのほとん どの疫学研究では、鼻に発生した腫瘍と副鼻腔に発生した腫瘍を区別していない。そのため、副 鼻腔に発生するがんに対応するリスクがなければ、鼻腔がんのリスクは希釈される傾向にあり、 統計的に検出されない可能性がある。

多くの疫学研究では、ホルムアルデヒドばく露と気道がんとの関連が調査されている。最も有 力なエビデンスは、鼻咽頭がんで観察されている。工場労働者を対象とした最大規模のコホート 研究(Hauptmann ら, 2004 年)では、鼻咽頭がんによる死亡者数の増加が観察されており、最大 ばく露量と累積ばく露量についてばく露-反応関係を示している。死体防腐処理業者を対象とした 米国最大のコホート研究では、上咽頭がんによる死亡者の増加が死亡率分析で観察されている (Hayes ら、1990 年)。ホルムアルデヒドを製造または使用している企業の労働者を対象としたデ ンマークの研究では、鼻咽頭がんの症例数の増加が観察された(Hansen & Olsen、1995 年)。他の コホート研究では、予想よりも鼻咽頭がんの症例数は少なかったと報告されている(Walrath & Fraumeni、1983 年; Coggon ら、2003 年; Pinkerton ら、2004 年)。鼻咽頭がんに関する7件の症例 対照研究のうち、5件ではホルムアルデヒドばく露によるリスク増加が認められた。

いくつかの症例対照研究では、ホルムアルデヒドへのばく露と副鼻腔がんとの関連が調査され ている。12 件のプール解析では、木粉または革粉にばく露されたことがない男女における腺がん のリスク増加が示され、累積ばく露の指標に対し、ばく露用量反応性を示す傾向が示された(Luce ら、2002 年)。他の1 件の症例対照研究(Olsen & Asnaes、1986 年)と発生率研究(Hansen & Olsen、 1995 年)では、副鼻腔がん、特に扁平上皮がんのリスクの増加が示された。しかしながら、工場 労働者を対象とした 3 件の最も有力なコホート研究では、副鼻腔がんの増加は認められなかった (Coggon ら、2003 年; Hauptmann ら、2004 年; Pinkerton ら、2004 年)。

この一連のエビデンスを評価するにあたり、国際がん研究機関(IARC)は、ホルムアルデヒドがヒトで鼻咽頭がんを引き起こすという十分な疫学的エビデンスがあるが、ホルムアルデヒドがヒトで副鼻腔がんを引き起こすという疫学的エビデンスは限られている。

nasopharyngeal cancer in humans; only limited epidemiological evidence that formaldehyde causes sinonasal cancer in humans; and strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde (Cogliano et al., 2005).

There are no publications describing DPX in nasal cells from formaldehyde-exposed personnel. Assessment of DPX in peripheral lymphocytes from formaldehyde-exposed workers demonstrated an association with overall exposure (Shaham et al., 2003). The single DPX study involved 399 workers from 14 hospital pathology departments, and formaldehyde exposure categories were low-level (mean 0.5 mg/m<sup>3</sup>, range 0.05–0.8 mg/m<sup>3</sup>) and high-level (mean 2.7 mg/m<sup>3</sup>, range 0.86–6.7 mg/m<sup>3</sup>). Adjusted mean DPX were significantly higher in the exposed groups. There appear to be some doubts regarding the sensitivity and reproducibility of the physical separation method used in this study (Heck & Casanova, 2004).

Some studies have investigated the histological changes within the nasal epithelium of workers occupationally exposed to formaldehyde; however, the extent to which nasal epithelial cell regenerative proliferation occurs is unresolved because the results are mixed and there was co-exposure to wood dust in some studies (Berke, 1987; Edling et al., 1988; Holmström et al., 1989; Boysen et al., 1990; Ballarin et al., 1992).

Mucociliary clearance in the anterior portion of the nasal cavity was reduced following exposure of volunteers to formaldehyde at  $0.30 \text{ mg/m}^3$  (Andersen & Mølhave, 1983).

The concordance of animal and human key events for formaldehyde is summarized in Table 3.

## Table 3. Formaldehyde concordance table.

Key event	Evidence in animals	Evidence in humans
Cytotoxicity	Positive in vivo (target cells)	Plausible
Proliferation	Positive in vivo (target cells)	Plausible (some evidence but confounded by co-exposure)
Genotoxicity	DPX (target cells in vivo)	DPX (non-target cells, i.e. lymphocytes)
Mutations	Positive in vitro; unconvincing in vivo	Positive (? cells)
Nasal tumours	Positive (mainly anterior lateral meatus)	Positive (nasopharyngeal) ? (sinonasal)

# **B. Glutaraldehyde**

There are few epidemiological studies for exposure to glutaraldehyde and human cancer. No increase in the number of cancer deaths was observed among 186 male glutaraldehyde production workers. The average time since first exposure to glutaraldehyde was 20.6 years, and the period of exposure was 3–7 years. During periods of monitoring exposure, glutaraldehyde concentrations in air ranged from 0.04 to 1.4 mg/m<sup>3</sup> (NICNAS, 1994). Studies of embalmers, pathologists, and members of the American Association of Anatomists for possible effects of glutaraldehyde have all shown increases in risk of cancer; however, all of

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また、白血病とホルムアルデヒドの職業ばく露との間に因果関係があるという十分なエビデンスはない、と結論づけた (Cogliano ら、2005)。

ホルムアルデヒドにばく露されたヒトの鼻の細胞における DPX について記述している論文は ない。ホルムアルデヒドにばく露された労働者の末梢リンパ球における DPX の評価では、全体的 なばく露との関連性が示された(Shaham ら、2003 年)。単一の DPX 試験では、14 の病院の各病 理部門で働く 399 人が参加し、ホルムアルデヒドばく露のカテゴリーは低レベル(平均0.5 mg/m<sup>3</sup>、 範囲 0.05~0.8 mg/m<sup>3</sup>)及び高レベル(平均 2.7 mg/m<sup>3</sup>、範囲 0.86~6.7 mg/m<sup>3</sup>)であった。調整後の 平均 DPX は被曝群で有意に高かった。この研究で用いられた物理的分離法の感度及び再現性には 疑問があるようである(Heck & Casanova、2004 年)。

いくつかの研究では、ホルムアルデヒドに職業ばく露された労働者の鼻腔上皮内の組織学的変 化を調査しているが、結果はまちまちであり、いくつかの研究では木粉への共ばく露があったた め、鼻腔上皮細胞の再生性増殖がどの程度起こるのかは未解明である(Berke, 1987年; Edling ら、 1988年; Holmström ら、1989年; Boysen ら、1990年; Ballarin ら、1992年)。

鼻腔前部の粘膜クリアランスは、ホルムアルデヒドの 0.30 mg/m<sup>3</sup> でのばく露後に減少した (Andersen & Mølhave、1983 年)。

ホルムアルデヒドに関する動物とヒトの key events の一致を表 3 にまとめた。

# 表 3. ホルムアルデヒドの用語索引表

key events	動物におけるエビデンス	ヒトにおけるエビデンス
細胞毒性	in vivo で陽性(標的細胞)	妥当性あり
細胞増殖	in vivo で陽性(標的細胞)	妥当性あり (エビデンスはあるが、共 ばく露によって交絡されている)
遺伝毒性	DPX (in vivo 標的細胞)	DPX (非標的細胞、リンパ球など)
突然変異	in vitro では陽性、in vivo では説得 力がない	陽性(?細胞)
鼻腔腫瘍	陽性(主に前外側鼻腔)	陽性(鼻咽頭) ?(副鼻腔)

# B. グルタルアルデヒド

グルタルアルデヒドへのばく露とヒトのがんに関する疫学的研究はほとんどない。グルタルア ルデヒド製造に携わる男性労働者 186 名のがん死亡者の増加は認められなかった。グルタルアル デヒドに初めてばく露されてからの平均期間は 20.6 年、ばく露期間は 3~7 年であった。モニタ リングばく露期間中、大気中のグルタルアルデヒド濃度は 0.04~1.4 mg/m<sup>3</sup> の範囲であった (NICNAS、1994 年)。グルタルアルデヒドの潜在的な影響について、遺体整復師、病理医及び米 国解剖学会のメンバーを対象とした研究では、すべての研究ががんリスクの増加を示している。 しかしこのグループはホルムアルデヒドにもばく露されていた(Walrath & Fraumeni、1983 年、遺 伝毒性変異;ホルムアルデヒドに関するコンセンサスワークショップ、1984 年; Stroup ら、1986 年)。

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these groups were also exposed to formaldehyde (Walrath & Fraumeni, 1983; Consensus Workshop on Formaldehyde, 1984; Stroup et al., 1986).

There are no studies examining glutaraldehyde exposure and DPX formation, cytotoxicity, and cell proliferation in human nasal tissues.

The concordance of animal and human key events for glutaraldehyde is summarized in Table 4.

### Table 4. Glutaraldehyde concordance table.

Key event	Evidence in animals	Evidence in humans
Cytotoxicity	Positive	Plausible
Proliferation	Positive in vivo	Plausible
Genotoxicity	DPX in vitro	Unknown
Mutations	Positive in vitro	Unknown
Nasal tumours	Negative (no evidence at any site)	Unknown

# 3. CAN HUMAN RELEVANCE OF THE MOA BE EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETIC OR DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

## A. Formaldehyde

Quantitative differences between experimental animals and humans for the postulated MOA will be a function of the concentration of formaldehyde at the target tissue. It is formaldehyde per se, and not its metabolites, that causes cytotoxicity. Exogenous inhaled formaldehyde is rapidly metabolized upon absorption, to formate, by a number of widely distributed cellular enzymes, particularly formaldehyde dehydrogenase. In addition to this efficient metabolic detoxification mechanism, the mucociliary apparatus provides protection of the underlying epithelium from gases and vapours. Thus, in order to attain free formaldehyde concentrations that may be cytotoxic to the target tissue, relatively high concentrations of formaldehyde vapour must be delivered to the target site to overcome these protective mechanisms. Mechanistic events of clear significance for carcinogenicity occur at dose levels where formaldehyde detoxification mechanisms are saturated in rats (Casanova & Heck, 1987).

It is critical to take dosimetry into consideration when considering quantitative species differences for formaldehyde-induced toxicity in the respiratory tract. Inhaled formaldehyde is predominantly deposited and readily absorbed in the regions of the upper respiratory tract with which it comes into initial contact, owing to its high reactivity with biological macromolecules (Heck et al., 1983; Swenberg et al., 1983). A complex relationship between nasal anatomy, ventilation, and breathing patterns (nasal or oronasal) determines where in the upper respiratory tract formaldehyde absorption occurs in species. In rodents, which are obligate nasal breathers, deposition and absorption occur primarily in the nasal passage. In contrast, primates are oronasal breathers; although absorption and deposition are likely to occur primarily in the oral mucosa and nasal passages, they can also occur in the trachea and ヒトの鼻組織におけるグルタルアルデヒドばく露と DPX 形成、細胞毒性及び細胞増殖を検討した研究はない。

グルタルアルデヒドに関する動物とヒトの key events の一致を表4にまとめた。

表4. グルタルアルデヒドの用語索引表

key events	動物におけるエビデンス	ヒトにおけるエビデンス
細胞毒性	陽性	妥当性あり
細胞増殖	in vivo で陽性	妥当性あり
遺伝毒性	DPX (in vitro)	不明
突然変異	in vitro で陽性	不明
鼻腔腫瘍	陰性(どの部位にもエビデンスが	不明
	ない)	

# 3. 実験動物ととトとの間の動態的または薬力学的要因のいずれかの量的差異に基づいて、MOAのとトへの関連性を合理的に排除することができるか

## A. ホルムアルデヒド

推定される MOA に関する実験動物とヒトとの間の定量的差異は、標的組織におけるホルムア ルデヒドの濃度がもたらす作用にある。細胞毒性を引き起こすのはホルムアルデヒドそのもので あり、その代謝物ではない。吸入された外因性のホルムアルデヒドは、広く分布する多数の細胞 酵素、特にホルムアルデヒド脱水素酵素によって急速に代謝されギ酸塩になる。この効率的な代 謝解毒メカニズムに加えて、粘膜絨毛機構が、その下の上皮をガスや蒸気から保護する。したが って、標的組織において細胞毒性が発生するのに十分な遊離ホルムアルデヒドの濃度を達成する ためには、これらの保護機構を克服することが必要であり、比較的高濃度のホルムアルデヒド蒸 気を標的部位に到達させる必要がある。明らかに有意な発がん性をもたらす薬力学的事象は、ラ ットのホルムアルデヒド解毒機構が飽和した用量で発生する (Casanova & Heck、1987 年)。

呼吸器系におけるホルムアルデヒド誘発性毒性の定量的な種差を考慮する際には、投与量を考 慮することが重要である。吸入されたホルムアルデヒドは、生体高分子との高い反応性のため、 最初に接触する上気道の領域に多く沈着し、容易に吸収される(Heck ら、1983 年; Swenberg ら、 1983 年)。鼻の生体機構、換気量、呼吸パターン(鼻または鼻腔)の間の複雑な関係が、種によっ て上気道のどこでホルムアルデヒドの吸収が起こるかを決定する。鼻呼吸であるげっ歯類では、 沈着と吸収は主に鼻腔内で起こる。対照的に霊長類は口鼻呼吸であり、吸収と沈着は主に口腔粘 膜と鼻腔で起こる可能性が高いが、気管と気管支でも起こる可能性がある(Monticello ら、1991 年)。

bronchus (Monticello et al., 1991). This hypothesis is supported by effects (histopathological changes, increased epithelial cell proliferation, and DPX formation) being observed farther along within the upper respiratory tract in monkeys.

Species differences in dosimetry have been taken into account in a two-stage clonal growth model that has been developed to predict the nasal carcinogenic risk of formaldehyde in humans (Conolly et al., 2004). The model also incorporates data on normal growth curves for rats and humans, cell cycle times, and cells at risk in the different regions of the respiratory tract.

Mice are better able to reduce both their respiratory rate and tidal volume upon repeated exposures; therefore, mice have less formaldehyde available for deposition than rats, resulting in less tissue damage and a lower rate of cell turnover in the nasal epithelium (Chang et al., 1981, 1983). These are characteristics that may help explain the lack of neoplastic response in the nose of mice.

Although there are likely to be quantitative differences between animal species and humans due to differences in dosimetry in the respiratory tract, there do not appear to be fundamental differences that would indicate that the proposed MOA does not occur in humans.

# **B. Glutaraldehyde**

Much less is known of the kinetics of glutaraldehyde in experimental animals compared with formaldehyde. Inhalation studies do not appear to have been conducted. The terminal half-lives for elimination are long for both intravenous injection (rat 10 h, rabbit 15–30 h) and dermal application (rat 40–110 h, rabbit 20–100 h), probably due to the binding of glutaraldehyde to protein and the slow excretion of metabolites. The metabolites have not been identified, but it has been proposed that the metabolism of glutaraldehyde dehydrogenase. The glutaric acid formed by oxidation is probably further metabolized by reaction with coenzyme A (CoA) to give glutaryl CoA, which is then oxidized by glutaryl CoA dehydrogenase to glutaconyl CoA, leading to its eventual degradation to carbon dioxide via acetate (Beauchamp et al., 1992; NTP, 1993; NICNAS, 1994; Ballantyne, 1995).

Glutaraldehyde reacts readily with proteins as a cross-linking agent, mainly between amino groups. The reaction is rapid and pH dependent (rate increases at pH >9), to give Schiff bases. Further reaction occurs to give a number of complex reaction products, with the mechanism of the cross-linking process not yet fully understood.

Little information is available on the interaction between glutaraldehyde and DNA, but it has been reported (Hopwood, 1975) that glutaraldehyde reacts with DNA only at >60 °C (summarized by NICNAS, 1994), and there are data implying that there is no reaction under physiological conditions (Sewell et al., 1984; Douglas & Rogers, 1998; Vock et al., 1999).

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この仮説は、サルの上気道内のより遠位まで病理組織学的変化、上皮細胞の増殖及び DPX 形成 が観察されることによって支持されている。

ヒトにおけるホルムアルデヒドの鼻腔発がんリスクを予測するために開発された 2 段階のクロ ーン増殖モデルでは、用量評価における種差が考慮されている (Conolly ら、2004 年) このモデル には、ラットとヒトの正常成長曲線、細胞周期の時間及び気道の異なる領域におけるリスクのあ る細胞に関するデータも組み込まれている。

マウスは反復ばく露により呼吸速度と一回換気量の両方を低下させることができるため、マウスはラットよりもホルムアルデヒドの沈着が少なく、その結果、鼻腔上皮の組織損傷と細胞の再 生率が低下する(Changら、1981年、1983年)。これらの特徴は、マウスの鼻で腫瘍発生がないこ とを説明するのに役立つかもしれない。

動物とヒトとの間には、気道の用量評価の違いによる定量的な違いがあると思われるが、推定 される MOA がヒトでは発生しないことを示すような根本的な違いはないようである。

# B. グルタルアルデヒド

実験動物におけるグルタルアルデヒドの動態は、ホルムアルデヒドに比べてあまり知られてい ない。吸入試験は実施されていないようである。半減期は、静脈内投与(ラット10時間、ウサギ 15~30時間)と経皮投与(ラット40~110時間、ウサギ20~100時間)の両方で長くなっている が、これはグルタルアルデヒドがタンパク質に結合し、代謝物の排泄が遅いためと考えられる。 代謝物は同定されていないが、おそらくグルタルアルデヒドの代謝は、アルデヒド脱水素酵素に よる対応するカルボン酸への初期酸化を伴うと推定されている。酸化によって産生されたグルタ ル酸は、コエンザイム A (CoA)と反応してグルタリル CoA を産生する。そして、グルタリル CoA デヒドロゲナーゼによってグルタコニル CoA に酸化され、最終的にはアセテートを介して二酸化 炭素に分解される(Beauchamp ら、1992年; NTP、1993年; NICNAS、1994年; Ballantyne、1995 年)。

グルタルアルデヒドは、主にアミノ基間の架橋剤としてタンパク質と容易に反応する。この反応は迅速でpHに依存しており (pH>9 で反応速度が増加)、Schiff塩基を生成する。さらに反応すると、多くの複雑な反応生成物が得られるが、架橋反応のメカニズムはまだ完全には解明されていない。

グルタルアルデヒドと DNA との相互作用についてはほとんど情報がないが、グルタルアルデ ヒドが DNA と反応するのは 60℃以上の場合のみであることが報告されており(Hopwood、1975 年)、生理的条件下では反応しないことを示唆するデータもある(Sewell ら、1984 年; Douglas & Rogers、1998 年; Vock ら、1999 年)。

# 4. STATEMENT OF CONFIDENCE, ANALYSIS, AND IMPLICATION

# A. Formaldehyde

Sustained cytotoxicity and cell proliferation are key events in the proposed MOA for the induction of several types of animal tumours. There are substantial data to support this postulated MOA for formaldehyde-induced nasal tumours in rats. Cytotoxicity, DPX formation, nasal epithelial cell regenerative proliferation, squamous metaplasia, and inflammation have been measured in rat studies and are site-specific, highly non-linear concentration–response processes in concordance with the incidence of nasal tumours.

Based on the weight of evidence, it is likely that the MOA is relevant to humans, at least qualitatively. Increased cell proliferation and DPX formation within epithelia of the upper respiratory tract have been observed in monkeys exposed to formaldehyde vapour. Increased human epithelial cell proliferation following in situ exposure to formaldehyde has also been observed in a model system in which rat tracheae populated with human tracheobronchial epithelial cells were xenotransplanted into athymic mice. Limited evidence on histopathological lesions in the nose of humans exposed primarily to formaldehyde in the occupational environment is consistent with a qualitatively similar response of the upper respiratory tract in experimental animals. In addition, several epidemiological studies have indicated an increased risk of nasal cancers with formaldehyde exposure.

Therefore, the MOA is considered relevant to humans, and animal nasal tumour and other supporting data should be taken forward to evaluate human risk. This process would include consideration of the data suggesting that formaldehyde induces tumours in a non-linear, dose-dependent manner. There may also be quantitative differences in response between species for the proposed MOA due to differences in dosimetry.

# **B. Glutaraldehyde**

The epidemiological studies for glutaraldehyde are very limited and do not show an association with nasal tumours. In animal studies, glutaraldehyde has been shown to cause cytotoxicity, cell proliferation, and DPX production, but not nasal tumours, in inhalation studies in rats and mice. The fact that glutaraldehyde is clearly more toxic than formaldehyde should not constitute a reason for the difference in carcinogenic potential. Although, dose for dose, glutaraldehyde exposure may tend to result in more cell death than formaldehyde exposure, if glutaraldehyde is a carcinogen, this should be demonstrable at doses lower than those used for formaldehyde.

The MOA postulated for formaldehyde—that is, sustained cytotoxicity and cell proliferation—would appear to be relevant to glutaraldehyde, but tumour formation has not been demonstrated. It has been tentatively suggested here that the difference in pathological responses to these aldehydes is due to formaldehyde being a monoaldehyde whereas glutaraldehyde is a dialdehyde. This difference may result in a different form of cross-linking so that glutaraldehyde cross-link products are neither likely to retain any biological function nor likely to be repairable. The case-study highlights the difficulties in applying the HRF when the animal tumour data are inadequate.

# 4. 信頼性、解析及び帰結の記述

# A. ホルムアルデヒド

持続的な細胞毒性と細胞増殖は、いくつかの動物種における腫瘍誘発に対して提唱されている MOA にとっての key events である。ラットにおけるホルムアルデヒド誘発性鼻腔腫瘍の MOA を 支持するデータは多数存在する。細胞毒性、DPX 形成、鼻腔上皮細胞の再生性増殖、扁平上皮化 生、炎症はラットの研究で観察されており、部位特異的で非線形の濃度反応過程であり、鼻腔腫 瘍の発生率と一致している。

エビデンスの重み付けに基づいて、動物 MOA は少なくとも定性的にはヒトに関連している可 能性が高い。ホルムアルデヒド蒸気にばく露されたサルでは、上気道上皮内の細胞増殖及び DPX 形成の増加が観察されている。また、ホルムアルデヒドへの組織ばく露後のヒト上皮細胞の増殖 は、ヒト気管支上皮細胞を移植したラット気管を胸腺欠損マウスに異種移植したモデル系でも観 察されている。主にホルムアルデヒドにばく露されたヒトの鼻の病理学的病変に関する数少ない エビデンスは、定性的に類似している実験動物の上気道の病変と一致している。さらに、いくつ かの疫学研究では、ホルムアルデヒドばく露に伴う鼻腔がんのリスク増加が示唆されている。

したがって、MOA はヒトに関連していると考えられ、ヒトのリスク評価のために、動物の鼻腔 腫瘍やその他の裏付けとなるデータを活用すべきである。このプロセスには、ホルムアルデヒド が非線形で用量依存的に腫瘍を誘発することを示唆するデータを考慮することが含まれる。また、 定量法の違いにより推定される MOA については種間の反応に量的な差異があるかもしれない。

# B. グルタルアルデヒド

グルタルアルデヒドの疫学研究は非常に限られており、鼻腔腫瘍との関連性は示されていない。 動物実験では、ラットとマウスの吸入試験において、グルタルアルデヒドは細胞毒性、細胞増殖、 DPX 産生を引き起こすことが示されているが、鼻腔腫瘍は引き起こさないことが示されている。 グルタルアルデヒドの方がホルムアルデヒドよりも明らかに毒性が強いという事実は、発がん性 の違いの説明にはならない。グルタルアルデヒドはホルムアルデヒドよりも低用量での細胞死が 多いため、発がん性があるとすれば、ホルムアルデヒドよりも低い用量でそのことが証明されな ければならない。

ホルムアルデヒドで推定される MOA、すなわち持続的な細胞毒性と細胞増殖は、グルタルアル デヒドに関連しているように思われるが、腫瘍形成では実証されていない。これらのアルデヒド に対する病理学的反応の違いは、ホルムアルデヒドがモノアルデヒドであるのに対し、グルタル アルデヒドはジアルデヒドであるためであることが暫定的に示唆されている。この違いが、グル タルアルデヒドの架橋生成物が生物学的機能を保持する可能性も修復する可能性もないように、 架橋の形態が異なるという結果になっているのかもしれない。この事例研究は、動物の腫瘍デー タが不十分な場合に HRF を適用することの難しさを浮き彫りにしている。

## REFERENCES

Andersen I, Mølhave L (1983) Controlled human studies with formaldehyde. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 155–165.

Ballantyne B (1995) *Toxicology of glutaraldehyde: Review of studies and human health effects*. Bound Brook, NJ, Union Carbide Corporation.

Ballarin C, Sarto F, Giacomelli L, Bartolucci GB, Clonfero E (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. *Mutation Research*, **280**:1–7.

Beauchamp ROJ, St Clair MB, Fennell TR, Clarke DO, Morgan KT, Kari FW (1992) A critical review of the toxicology of glutaraldehyde. *Critical Reviews in Toxicology*, **22**:143–174.

Berke JH (1987) Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. *Journal of Occupational Medicine*, **29**:681–684.

Boysen M, Zadig E, Digernes V, Abeler V, Reith A (1990) Nasal mucosa in workers exposed to formaldehyde: A pilot study. *British Journal of Industrial Medicine*, **47**:116–121.

Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma*, **48**:144–147.

Burgaz S, Erdem O, Cakmak G, Erdem N, Karakaya A, Karakaya AE (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, **7**:151–161.

Bushy Run (1983) *Glutaraldehyde vapour subchronic inhalation study on rats.* Export, PA, Bushy Run Research Center (Project Report 46-101).

Casanova M, Heck Hd'A (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [<sup>3</sup>H]- and [<sup>14</sup>C]formaldehyde in Fischer-344 rats: Effects of glutathione depletion. *Toxicology and Applied Pharmacology*, **89**:105–121.

Casanova M, Heck Hd'A, Everitt JI, Harrington WW Jr, Popp JA (1988) Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food and Chemical Toxicology*, **26**:715–716.

Casanova M, Deyo DF, Heck Hd'A (1989) Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundamental and Applied Toxicology*, **12**:397–417.

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# 参考文献

Andersen I, Mølhave L (1983) Controlled human studies with formaldehyde. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 155–165.

Ballantyne B (1995) Toxicology of glutaraldehyde: Review of studies and human health effects. Bound Brook, NJ, Union Carbide Corporation.

Ballarin C, Sarto F, Giacomelli L, Bartolucci GB, Clonfero E (1992) Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. *Mutation Research*, **280**:1–7.

Beauchamp ROJ, St Clair MB, Fennell TR, Clarke DO, Morgan KT, Kari FW (1992) A critical review of the toxicology of glutaraldehyde. *Critical Reviews in Toxicology*, **22**:143–174.

Berke JH (1987) Cytologic examination of the nasal mucosa in formaldehyde-exposed workers. *Journal of Occupational Medicine*, **29**:681–684.

Boysen M, Zadig E, Digernes V, Abeler V, Reith A (1990) Nasal mucosa in workers exposed to formaldehyde: A pilot study. *British Journal of Industrial Medicine*, **47**:116–121.

Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE (2001) Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma*, **48**:144–147.

Burgaz S, Erdem O, Cakmak G, Erdem N, Karakaya A, Karakaya AE (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, **7**:151–161.

Bushy Run (1983) *Glutaraldehyde vapour subchronic inhalation study on rats.* Export, PA, Bushy Run Research Center (Project Report 46-101).

Casanova M, Heck Hd'A (1987) Further studies of the metabolic incorporation and covalent binding of inhaled [<sup>3</sup>H]- and [<sup>14</sup>C]formaldehyde in Fischer-344 rats: Effects of glutathione depletion. *Toxicology and Applied Pharmacology*, **89**:105–121.

Casanova M, Heck Hd'A, Everitt JI, Harrington WW Jr, Popp JA (1988) Formaldehyde concentrations in the blood of rhesus monkeys after inhalation exposure. *Food and Chemical Toxicology*, **26**:715–716.

Casanova M, Deyo DF, Heck Hd'A (1989) Covalent binding of inhaled formaldehyde toDNAin the nasal mucosa of Fischer 344 rats: Analysis of formaldehyde andDNAby high- performance liquid chromatography and provisional pharmacokinetic interpretation. *Fundamental and Applied Toxicology*, **12**:397–417.

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck Hd'A (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Fundamental and Applied Toxicology*, **17**:409–428.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck Hd'A (1994) DNA–protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Fundamental and Applied Toxicology*, **23**:525–536.

Casanova-Schmitz M, Starr TB, Heck H (1984) Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [ $^{14}$ C]- and [ $^{3}$ H]formaldehyde. *Toxicology and Applied Pharmacology*, **76**:26–44.

Chang JCF, Steinhagen WH, Barrow CS (1981) Effects of single or repeated formaldehyde exposures on minute volume of B6C3F1 mice and F344 rats. *Toxicology and Applied Pharmacology*, **61**:451–459.

Chang JCF, Gross EA, Swenberg JA, Barrow CS (1983) Nasal cavity deposition, histopathology and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicology and Applied Pharmacology*, **68**:161–176.

CIIT (1999) Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation, rev. ed. Research Triangle Park, NC, Chemical Industry Institute of Toxicology.

Coggon D, Harris EC, Poole J, Palmer KT (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *Journal of the National Cancer Institute*, **21**:1608–1614.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F (2005) Meeting report: Summary of IARC Monographs on formaldehyde, 2-butoxyethanol and 1-*tert*-butoxy-2-propanol. *Environmental Health Perspectives*, **113**(9):1205–1208.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2004) Human respiratory tract cancer risks of inhaled formaldehyde: Dose–response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicological Sciences*, **82**:279–296.

Consensus Workshop on Formaldehyde (1984) Report on the consensus workshop on formaldehyde. *Environmental Health Perspectives*, **58**:323–381.

Crosby RM, Richardson KK, Craft TR, Benforado KB, Liber HL, Skopek TR (1988) Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and *E. coli. Environmental and Molecular Mutagenesis*, **12**:155–166.

#### Harmonization Project Document No. 4

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck Hd'A (1991) Covalent binding of inhaled formaldehyde toDNAin the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. *Fundamental and Applied Toxicology*, **17**:409–428.

Casanova M, Morgan KT, Gross EA, Moss OR, Heck Hd'A (1994) DNA–protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. *Fundamental and Applied Toxicology*, **23**:525–536.

Casanova-Schmitz M, Starr TB, Heck H (1984) Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [<sup>14</sup>C]- and [<sup>3</sup>H]formaldehyde. *Toxicology and Applied Pharmacology*, **76**:26–44.

Chang JCF, Steinhagen WH, Barrow CS (1981) Effects of single or repeated formaldehyde exposures on minute volume of B6C3F1 mice and F344 rats. *Toxicology and Applied Pharmacology*, **61**:451–459.

Chang JCF, Gross EA, Swenberg JA, Barrow CS (1983) Nasal cavity deposition, histopathology and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicology and Applied Pharmacology*, **68**:161–176.

CIIT (1999) Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route of inhalation, rev. ed. Research Triangle Park, NC, Chemical Industry Institute of Toxicology.

Coggon D, Harris EC, Poole J, Palmer KT (2003) Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *Journal of the National Cancer Institute*, **21**:1608–1614.

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F (2005) Meeting report: Summary of IARC Monographs on formaldehyde, 2-butoxyethanol and 1-*tert*-butoxy- 2-propanol. *Environmental Health Perspectives*, **113**(9):1205–1208.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ (2004) Human respiratory tract cancer risks of inhaled formaldehyde: Dose–response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. *Toxicological Sciences*, **82**:279–296.

Consensus Workshop on Formaldehyde (1984) Report on the consensus workshop on formaldehyde. *Environmental Health Perspectives*, **58**:323–381.

Crosby RM, Richardson KK, Craft TR, Benforado KB, Liber HL, Skopek TR (1988) Molecular analysis of formaldehyde-induced mutations in human lymphoblasts and *E. coli. Environmental and Molecular Mutagenesis*, **12**:55–166.

Dalbey WE (1982) Formaldehyde and tumors in hamster respiratory tract. *Toxicology*, 24:9–14.

Douglas MP, Rogers SO (1998) DNA damage caused by common cytological fixatives. *Mutation Research*, **401**:77–88.

Edling C, Hellquist H, Ödkvist L (1988) Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. *British Journal of Industrial Medicine*, **45**:761–765.

Feron VJ, Bruyntes JP, Woutersen RA, Immel HR, Appelman LM (1988) Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Letters*, **39**:101–111.

Feron VJ, Til HP, Woutersen RA (1990) Letter to the editor. *Toxicology and Industrial Health*, **6**:637–639.

Gaylor DW, Lutz WK, Connolly RB (2004) Statistical analysis of nonmonotonic doseresponse relationships: Research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. *Toxicological Sciences*, **77**:158–164.

Gross EA, Mellick PW, Kari FW, Miller FJ, Morgan KT (1994) Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. *Fundamental and Applied Toxicology*, **23**:348–362.

Hansen J, Olsen JH (1995) Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes and Control*, **6**:354–360.

Hardman JG, Limbird LE, Gilman AG, eds (2001) Goodman & Gilman's The pharmacological basis of therapeutics, 10th ed. The McGraw-Hill Companies, Inc., 2025 pp.

Hauptmann A, Lubin JH, Stewart PA, Hayes RB, Blair A (2004). Mortality from solid cancers among workers in formaldehyde industries. *American Journal of Epidemiology*, **159**:1117–1130.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H (1990) Mortality of U.S. embalmers and funeral directors. *American Journal of Industrial Medicine*, **18**:641–652.

He J-L, Jin L-F, Jin H-Y (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomedical and Environmental Sciences*, **11**:87–92.

Heck H, Casanova M (1995). Nasal dosimetry of formaldehyde: Modelling site specificity and the effects of pre-exposure. In: Miller JF, ed. *Nasal toxicity and dosimetry of inhaled xenobiotics: Implications for human health.* Washington, DC, Taylor & Francis, pp. 159–175.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Dalbey WE (1982) Formaldehyde and tumors in hamster respiratory tract. Toxicology, 24:9-14.

Douglas MP, Rogers SO (1998)D N A damage caused by common cytological fixatives. *Mutation Research*, **401**:77–88.

Edling C, Hellquist H, Ödkvist L (1988) Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. *British Journal of Industrial Medicine*, **45**:761–765.

Feron VJ, Bruyntes JP, Woutersen RA, Immel HR, Appelman LM (1988) Nasal tumours in rats after short-term exposure to a cytotoxic concentration of formaldehyde. *Cancer Letters*, **39**:101–111.

Feron VJ, Til HP, Woutersen RA (1990) Letter to the editor. *Toxicology and Industrial Health*, **6**:637–639.

Gaylor DW, Lutz WK, Connolly RB (2004) Statistical analysis of nonmonotonic dose– response relationships: Research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. *Toxicological Sciences*, **77**:158–164.

Gross EA, Mellick PW, Kari FW, Miller FJ, Morgan KT (1994) Histopathology and cell replication responses in the respiratory tract of rats and mice exposed by inhalation to glutaraldehyde for up to 13 weeks. *Fundamental and Applied Toxicology*, **23**:348–362.

Hansen J, Olsen JH (1995) Formaldehyde and cancer morbidity among male employees in Denmark. *Cancer Causes and Control*, **6**:354–360.

Hardman JG, Limbird LE, Gilman AG, eds (2001) Goodman & Gilman's The pharmacological basis of therapeutics, 10th ed. The McGraw-Hill Companies, Inc., 2025 pp.

Hauptmann A, Lubin JH, Stewart PA, Hayes RB, Blair A (2004). Mortality from solid cancers among workers in formaldehyde industries. *American Journal of Epidemiology*, **159**:1117–1130.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H (1990) Mortality of U.S. embalmers and funeral directors. *American Journal of Industrial Medicine*, **18**:641–652.

He J-L, Jin L-F, Jin H-Y (1998) Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay. *Biomedical and Environmental Sciences*, **11**:87–92.

Heck H, Casanova M (1995). Nasal dosimetry of formaldehyde: Modelling site specificity and the effects of pre-exposure. In: Miller JF, ed. *Nasal toxicity and dosimetry of inhaled xenobiotics: Implications for human health.* Washington, DC, Taylor & Francis, pp. 159–175.

Heck H, Casanova M (2004) The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. *Regulatory Toxicology and Pharmacology*, **40**:92–106.

Heck Hd'A, White EL, Casanova-Schmitz M (1982) Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomedical Mass Spectrometry*, **9**:347–353.

Heck Hd'A, Chin TY, Schmitz MC (1983) Distribution of [<sup>14</sup>C]formaldehyde in rats after inhalation exposure. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 26–37.

Heck Hd'A, Casanova-Schmitz M, Dodd PB, Schachter EN, Witek TJ, Tosun T (1985) Formaldehyde (CH<sub>2</sub>O) concentrations in the blood of humans and Fischer-344 rats exposed to CH<sub>2</sub>O under controlled conditions. *American Industrial Hygiene Association Journal*, **46**:1–3.

Heck Hd'A, Casanova M, Starr TB (1990) Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**:397–426.

Hedberg JJ, Strömberg P, Höög JO (1998) An attempt to transform class characteristics within the alcohol dehydrogenase family. *FEBS Letters*, **436**:67–70.

Hester SD, Benavides GB, Yoon L, Morgan KT, Zou F, Barry W, Wolf DC (2003) Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium. *Toxicology*, **187**:13–24.

Holmquist B, Vallee BL (1991) Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. *Biochemical and Biophysical Research Communications*, **178**:1371–1377.

Holmström M, Wilhelmsson B, Hellquist H, Rosén G (1989) Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Oto-laryngologica*, **107**:120–129.

Hopwood D (1975) The reactions of glutaraldehyde with nucleic acids. *Journal of Histochemistry*, **7**:267–276.

Horton AW, Tye R, Stemmer KL (1963) Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *Journal of the National Cancer Institute*, **30**:31–43.

IARC (2005) *Formaldehyde, 2-butoxyethanol and 1-*tert-*butoxypropan-2-ol.* Lyon, International Agency for Research on Cancer, 478 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88).

#### Harmonization Project Document No. 4

Heck H, Casanova M (2004) The implausibility of leukemia induction by formaldehyde: A critical review of the biological evidence on distant-site toxicity. *Regulatory Toxicology and Pharmacology*, **40**:92–106.

Heck Hd'A, White EL, Casanova-Schmitz M (1982) Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry. *Biomedical Mass Spectrometry*, **9**:347–353.

Heck Hd'A, Chin TY, Schmitz MC (1983) Distribution of [<sup>14</sup>C]formaldehyde in rats after inhalation exposure. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 26–37.

Heck Hd'A, Casanova-Schmitz M, Dodd PB, Schachter EN, Witek TJ, Tosun T (1985) Formaldehyde (CH<sub>2</sub>O) concentrations in the blood of humans and Fischer-344 rats exposed to CH<sub>2</sub>O under controlled conditions. *American Industrial Hygiene Association Journal*, **46**:1–3.

Heck Hd'A, Casanova M, Starr TB (1990) Formaldehyde toxicity—new understanding. *Critical Reviews in Toxicology*, **20**:397–426.

Hedberg JJ, Strömberg P, Höög JO (1998) An attempt to transform class characteristics within the alcohol dehydrogenase family. *FEBS Letters*, **436**:67–70.

Hester SD, Benavides GB, Yoon L, Morgan KT, Zou F, Barry W, Wolf DC (2003) Formaldehydeinduced gene expression in F344 rat nasal respiratory epithelium. *Toxicology*, **187**:13–24.

Holmquist B, Vallee BL (1991) Human liver class III alcohol and glutathione dependent formaldehyde dehydrogenase are the same enzyme. *Biochemical and Biophysical Research Communications*, **178**:1371–1377.

Holmström M, Wilhelmsson B, Hellquist H, Rosén G (1989) Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Otolaryngologica*, **107**:120–129.

Hopwood D (1975) The reactions of glutaraldehyde with nucleic acids. *Journal of Histochemistry*, 7:267–276.

Horton AW, Tye R, Stemmer KL (1963) Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. *Journal of the National Cancer Institute*, **30**:31–43.

IARC (2005) *Formaldehyde, 2-butoxyethanol and 1*-tert-*butoxypropan-2-ol.* Lyon, International Agency for Research on Cancer, 478 pp. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 88).

IPCS (2002) *Formaldehyde*. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document No. 40).

Kamata E, Nakadate E, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. *Journal of Toxicological Sciences*, **22**:239–254.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA (1983a) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Research*, **43**:4382–4392.

Kerns WD, Donofrio DJ, Pavkov KL (1983b) The chronic effects of formaldehyde inhalation in rats and mice: A preliminary report. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 111–131.

Kimbell JS, Gross EA, Richardson RB, Conolly RB, Morgan KT (1997) Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutation Research*, **380**:143–154.

Koivusalo M, Baumann M, Uotila L (1989) Evidence for the identity of glutathionedependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Letters*, **257**:105–109.

Liber HL, Benforado K, Crosby RM, Simpson D, Skopek TR (1989) Formaldehyde-induced and spontaneous alterations in human *hprt* DNA sequence and mRNA expression. *Mutation Research*, **226**:31–37.

Liteplo RG, Meek ME (2003) Inhaled formaldehyde: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health*, **B6**:85–114.

Luce D, Leclerc A, Begin D, Demers PA, Gerin M, Orlowski E, Kogevinas M, Belli S, Bugel I, Bolm-Audorff U, Brinton LA, Comba P, Hardell L, Hayes RB, Magnani C, Merler E, Preston-Martin S, Vaughan TL, Zheng W, Boffetta P (2002) Sinonasal cancer and occupational exposures: A pooled analysis of 12 case–control studies. *Cancer Causes and Control*, **13**:147–157.

Lutz WK (1998) Dose–response relationships in chemical carcinogenesis: Superposition of different mechanisms of action, resulting in linear–nonlinear curves, practical thresholds, J-shapes. *Mutation Research*, **405**:117–124.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR, Moss OR (1986) Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology*, **41**:253–266.

Merk O, Speit G (1998) Significance of formaldehyde-induced DNA-protein crosslinks for mutagenesis. *Environmental and Molecular Mutagenesis*, **32**:260–268.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

IPCS (2002) *Formaldehyde*. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document No. 40).

Kamata E, Nakadate E, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M, Kurokawa Y (1997) Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fischer-344 rats. *Journal of Toxicological Sciences*, **22**:239–254.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA (1983a) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Research*, **43**:4382–4392.

Kerns WD, Donofrio DJ, Pavkov KL (1983b) The chronic effects of formaldehyde inhalation in rats and mice: A preliminary report. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 111–131.

Kimbell JS, Gross EA, Richardson RB, Conolly RB, Morgan KT (1997) Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. *Mutation Research*, **380**:143–154.

Koivusalo M, Baumann M, Uotila L (1989) Evidence for the identity of glutathione- dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Letters*, **257**:105–109.

Liber HL, Benforado K, Crosby RM, Simpson D, Skopek TR (1989) Formaldehyde-induced and spontaneous alterations in human *hprtDNA*sequence and mRNA expression. *Mutation Research*, **226** 31–37.

Liteplo RG, Meek ME (2003) Inhaled formaldehyde: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health*, **B6**:85–114.

Luce D, Leclerc A, Begin D, Demers PA, Gerin M, Orlowski E, Kogevinas M, Belli S, Bugel I, Bolm-Audorff U, Brinton LA, Comba P, Hardell L, Hayes RB, Magnani C, Merler E, Preston-Martin S, Vaughan TL, Zheng W, Boffetta P (2002) Sinonasal cancer and occupational exposures: A pooled analysis of 12 case–control studies. *Cancer Causes and Control*, **13**:147–157.

Lutz WK (1998) Dose-response relationships in chemical carcinogenesis: Superposition of different mechanisms of action, resulting in linear-nonlinear curves, practical thresholds, J- shapes. *Mutation Research*, **405**:117–124.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR, Moss OR (1986) Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology*, **41**:253–266.

Merk O, Speit G (1998) Significance of formaldehyde-induced DNA–protein crosslinks for mutagenesis. *Environmental and Molecular Mutagenesis*, **32**:260–268.

#### Harmonization Project Document No. 4

Monticello TM (1990) Formaldehyde induced pathology and cell proliferation: A thesis. Durham, NC, Duke University.

Monticello TM, Morgan KT (1994) Cell proliferation and formaldehyde-induced respiratory carcinogenesis. *Risk Analysis*, **14**:313–319.

Monticello TM, Morgan KT, Everitt JI, Popp JA (1989) Effects of formaldehyde gas on the respiratory tract of rhesus monkeys. Pathology and cell proliferation. *American Journal of Pathology*, **134**:515–527.

Monticello TM, Miller FJ, Morgan KT (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subacute inhalation of formaldehyde. *Toxicology and Applied Pharmacology*, **111**:409–421.

Monticello TM, Swenberg JA, Gross EA, Leiniger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Research*, **56**:1012–1022.

Morgan KT, Monticello TM (1990) Formaldehyde toxicity: Respiratory epithelial injury and repair. In: Thomassen DG, Nettesheim P, eds. *Biology, toxicology, and carcinogenesis of the respiratory epithelium*. Washington, DC, Hemisphere Publishing, pp. 155–171.

Morgan KT, Jiang X-Z, Starr TB, Kerns WD (1986) More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicology and Applied Pharmacology*, **82**:264–271.

NICNAS (1994) *Glutaraldehyde. Full public report.* Canberra, Australian Government Publishing Service, National Industrial Chemicals Notification and Assessment Scheme, July (Priority Existing Chemical No. 3).

NTP (1993) *NTP technical report on toxicity studies on glutaraldehyde (CAS No. 111-30-8) administered by inhalation to F344/N rats and B6C3F1 mice.* Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Toxicity Report No. 25; NIH Publication No. 93-3348).

NTP (1999) Toxicology and carcinogenesis studies of glutaraldehyde (CAS No. 111-30-8) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 490; NIH Publication No. 99-3980).

Olsen JH, Asnaes S (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *British Journal of Industrial Medicine*, **43**:769–774.

Pinkerton L, Hein M, Stayner L (2004). Mortality among a cohort of garment workers exposed to formaldehyde: An update. *Occupational and Environmental Medicine*, **61**:193–200.

## Harmonization Project Document No. 4

Monticello TM (1990) Formaldehyde induced pathology and cell proliferation: A thesis.Durham, NC, Duke University.

Monticello TM, Morgan KT (1994) Cell proliferation and formaldehyde-induced respiratory carcinogenesis. *Risk Analysis*, **14**:313–319.

Monticello TM, Morgan KT, Everitt JI, Popp JA (1989) Effects of formaldehyde gas on the respiratory tract of rhesus monkeys. Pathology and cell proliferation. *American Journal of Pathology*, **134**:515–527.

Monticello TM, Miller FJ, Morgan KT (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subacute inhalation of formaldehyde. *Toxicology and Applied Pharmacology*, **111**:409–421.

Monticello TM, Swenberg JA, Gross EA, Leiniger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. *Cancer Research*, **56**:1012–1022.

Morgan KT, Monticello TM (1990) Formaldehyde toxicity: Respiratory epithelial injury and repair. In: Thomassen DG, Nettesheim P, eds. *Biology, toxicology, and carcinogenesis of the respiratory epithelium.* Washington, DC, Hemisphere Publishing, pp. 155–171.

Morgan KT, Jiang X-Z, Starr TB, Kerns WD (1986) More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicology and Applied Pharmacology*, **82**:264–271.

NICNAS (1994) *Glutaraldehyde. Full public report.* Canberra, Australian Government Publishing Service, National Industrial Chemicals Notification and Assessment Scheme, July (Priority Existing Chemical No. 3).

NTP (1993) NTP technical report on toxicity studies on glutaraldehyde (CAS No. 111-30-8) administered by inhalation to F344/N rats and B6C3F1 mice. Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Toxicity Report No. 25; NIH Publication No. 93-3348).

NTP (1999) Toxicology and carcinogenesis studies of glutaraldehyde (CAS No. 111-30-8) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 490; NIH Publication No. 99-3980).

Olsen JH, Asnaes S (1986) Formaldehyde and the risk of squamous cell carcinoma of the sinonasal cavities. *British Journal of Industrial Medicine*, **43**:769–774.

Pinkerton L, Hein M, Stayner L (2004). Mortality among a cohort of garment workers exposed to formaldehyde: An update. *Occupational and Environmental Medicine*, **61**:193–200.

Recio L (1997) Oncogene and tumor suppressor gene alterations in nasal tumors. *Mutation Research*, **380**:27–31.

Recio L, Sisk S, Pluta L, Bermudez E, Gross EA, Chen Z, Morgan K, Walker C (1992) *p53* mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. *Cancer Research*, **52**:6113–6116.

Rietbrock N (1965) [Formaldehyde oxidation in the rat.] *Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie*, **251**:189–190 (in German).

Rusch GM, Clary JJ, Rinehart WE, Bolte HF (1983) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicology and Applied Pharmacology*, **68**:329–343.

Schlosser PM, Lilly PD, Conolly RB, Janszen DB, Kimbell JS (2003) Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA–protein cross-link dosimetry model to estimate human equivalent doses. *Risk Analysis*, **23**:473–487.

Sewell BT, Bouloukos C, von Holt C (1984) Formaldehyde and glutaraldehyde in the fixation of chromatin for electron microscopy. *Journal of Microscopy*, **136**:103–112.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003) DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occupational and Environmental Medicine*, **60**:403–409.

Soffritti M, Maltoni C, Maffei F, Biagi R (1989) Formaldehyde: An experimental multipotential carcinogen. *Toxicology and Industrial Health*, **5**:699–730.

Speit G, Schutz P, Merk O (2000) Induction and repair of formaldehyde-induced DNA-protein crosslinks in repair-deficient human cell lines. *Mutagenesis*, **15**:85–90.

St Clair MB, Gross EA, Morgan KT (1990) Pathology and cell proliferation induced by intranasal instillation of aldehydes in the rat: Comparison of glutaraldehyde and formaldehyde. *Toxicologic Pathology*, **18**:353–361.

St Clair MB, Bermudez E, Gross EA, Butterworth BE, Recio L (1991) Evaluation of the genotoxic potential of glutaraldehyde. *Environmental and Molecular Mutagenesis*, **18**:113–119.

Stroup NE, Blair A, Erikson GE (1986) Brain cancer and other causes of deaths in anatomists. *Journal of the National Cancer Institute*, **77**:1217–1224.

Swenberg JA, Gross EA, Martin J, Popp JA (1983) Mechanisms of formaldehyde toxicity. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 132–147.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Recio L (1997) Oncogene and tumor suppressor gene alterations in nasal tumors. *Mutation Research*, **380**:27–31.

Recio L, Sisk S, Pluta L, Bermudez E, Gross EA, Chen Z, Morgan K, Walker C (1992) *p53* mutations in formaldehyde-induced nasal squamous cell carcinomas in rats. *Cancer Research*, **52**:6113–6116.

Rietbrock N (1965) [Formaldehyde oxidation in the rat.] *Naunyn-Schmiedebergs Archiv für* experimentelle Pathologie und Pharmakologie, **251**:189–190 (in German).

Rusch GM, Clary JJ, Rinehart WE, Bolte HF (1983) A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicology and Applied Pharmacology*, **68**:329–343.

Schlosser PM, Lilly PD, Conolly RB, Janszen DB, Kimbell JS (2003) Benchmark dose risk assessment for formaldehyde using airflow modeling and a single-compartment, DNA– protein cross-link dosimetry model to estimate human equivalent doses. *Risk Analysis*, **23**:473–487.

Sewell BT, Bouloukos C, von Holt C (1984) Formaldehyde and glutaraldehyde in the fixation of chromatin for electron microscopy. *Journal of Microscopy*, **136**:103–112.

Shaham J, Bomstein Y, Gurvich R, Rashkovsky M, Kaufman Z (2003) DNA–protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. *Occupational and Environmental Medicine*, **60**:403–409.

Soffritti M, Maltoni C, Maffei F, Biagi R (1989) Formaldehyde: An experimental multipotential carcinogen. *Toxicology and Industrial Health*, **5**:699–730.

Speit G, Schutz P, Merk O (2000) Induction and repair of formaldehyde-induced DNA- protein crosslinks in repair-deficient human cell lines. *Mutagenesis*, **15**:85–90.

St Clair MB, Gross EA, Morgan KT (1990) Pathology and cell proliferation induced by intra- nasal instillation of aldehydes in the rat: Comparison of glutaraldehyde and formaldehyde. *Toxicologic Pathology*, **18**:353–361.

St Clair MB, Bermudez E, Gross EA, Butterworth BE, Recio L (1991) Evaluation of the genotoxic potential of glutaraldehyde. *Environmental and Molecular Mutagenesis*, **18**:113–119.

Stroup NE, Blair A, Erikson GE (1986) Brain cancer and other causes of deaths in anatomists. *Journal of the National Cancer Institute*, **77**:1217–1224.

Swenberg JA, Gross EA, Martin J, Popp JA (1983) Mechanisms of formaldehyde toxicity. In: Gibson JE, ed. *Formaldehyde toxicity*. Washington, DC, Hemisphere Publishing, pp. 132–147.

Swenberg JA, Gross EA, Martin J, Randall HA (1986) Localization and quantitation of cell proliferation following exposure to nasal irritants. In: Barrow CS, ed. *Toxicology of the nasal passages*. Washington, DC, Hemisphere Publishing, pp. 291–300.

Takahashi M, Hasegawa R, Furukawa F, Toyoda K, Sato H, Hayashi Y (1986) Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine. *Japanese Journal of Cancer Research*, **77**:118–124.

Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE (1989) Two-year drinkingwater study of formaldehyde in rats. *Food and Chemical Toxicology*, **27**:77–87.

Titenko-Holland N, Levine AJ, Smith MT, Quintana PJ, Boeniger M, Hayes R, Suruda A, Schulte P (1996) Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutation Research*, **371**:237–248.

Tobe M, Naito K, Kurokawa Y (1989) Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology*, **56**:79–86.

Uotila L, Koivusalo M (1974) Formaldehyde dehydrogenase from human liver. Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *Journal of Biological Chemistry*, **249**:7653–7663.

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and *S*-formylglutathione hydrolase activities in different rat tissues. *Advances in Experimental Medicine and Biology*, **414**:365–371.

Van Miller JP, Hermansky SJ, Neptun DA, Loscoa PE, Ballantyne B (1995) Combined chronic toxicity/oncogenicity study with glutaraldehyde (GA) in the drinking water of rats. *Toxicologist*, **15**:203 (abstract).

Vargová M, Janota S, Karelová J, Barancokova M, Šulcová M (1992) Analysis of the health risk of occupational exposure to formaldehyde using biological markers. *Analysis*, **20**:451–454.

Vock EH, Lutz WK, Ilinskaya O, Vamvakas S (1999) Discrimination between genotoxicity and cytotoxicity for the induction of DNA double-strand breaks in cells treated with aldehydes and diepoxides. *Mutation Research*, **441**:85–93.

Walrath J, Fraumeni JF Jr (1983) Mortality patterns among embalmers. *International Journal of Cancer*, **31**:407–411.

Wolf DC, Gross EA, Lycht O, Bermudez E, Recio L, Morgan KT (1995) Immunohistochemical localization of p53, PCNA, and TGF- $\alpha$  proteins in formaldehydeinduced rat nasal squamous cell carcinomas. *Toxicology and Applied Pharmacology*, **132**:27–35.

#### Harmonization Project Document No. 4

Swenberg JA, Gross EA, Martin J, Randall HA (1986) Localization and quantitation of cell proliferation following exposure to nasal irritants. In: Barrow CS, ed. *Toxicology of the nasal passages*. Washington, DC, Hemisphere Publishing, pp. 291–300.

Takahashi M, Hasegawa R, Furukawa F, Toyoda K, Sato H, Hayashi Y (1986) Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with *N*-methyl-*N*<sub>0</sub>-nitro-*N*-nitrosoguanidine. *Japanese Journal of Cancer Research*, **77**:118–124.

Til HP, Woutersen RA, Feron VJ, Hollanders VHM, Falke HE (1989) Two-year drinking- water study of formaldehyde in rats. *Food and Chemical Toxicology*, **27**:77–87.

Titenko-Holland N, Levine AJ, Smith MT, Quintana PJ, Boeniger M, Hayes R, Suruda A, Schulte P (1996) Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutation Research*, **371**:237–248.

Tobe M, Naito K, Kurokawa Y (1989) Chronic toxicity study on formaldehyde administered orally to rats. *Toxicology*, **56**:79–86.

Uotila L, Koivusalo M (1974) Formaldehyde dehydrogenase from human liver. Purification, properties, and evidence for the formation of glutathione thiol esters by the enzyme. *Journal of Biological Chemistry*, **249**:7653–7663.

Uotila L, Koivusalo M (1997) Expression of formaldehyde dehydrogenase and *S*- formylglutathione hydrolase activities in different rat tissues. *Advances in Experimental Medicine and Biology*, **414**:365–371.

Van Miller JP, Hermansky SJ, Neptun DA, Loscoa PE, Ballantyne B (1995) Combined chronic toxicity/oncogenicity study with glutaraldehyde (GA) in the drinking water of rats. *Toxicologist*, **15**:203 (abstract).

Vargová M, Janota S, Karelová J, Barancokova M, Šulcová M (1992) Analysis of the health risk of occupational exposure to formaldehyde using biological markers. *Analysis*, **20**:451–454.

Vock EH, Lutz WK, Ilinskaya O, Vamvakas S (1999) Discrimination between genotoxicity and cytotoxicity for the induction of DNAdouble-strand breaks in cells treated with aldehydes and diepoxides. *Mutation Research*, **441**:85–93.

Walrath J, Fraumeni JF Jr (1983) Mortality patterns among embalmers. *International Journal of Cancer*, **31**:407–411.

Wolf DC, Gross EA, Lycht O, Bermudez E, Recio L, Morgan KT (1995) Immunohistochemical localization of p53, PCNA, and TGF-I proteins in formaldehyde- induced rat nasal squamous cell carcinomas. *Toxicology and Applied Pharmacology*, **132**:27–35.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron VJ (1989) Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *Journal of Applied Toxicology*, **9**:39–46.

Ying C-J, Yan W-S, Zhao M-Y, Ye X-L, Xie H, Yin S-Y, Zhu X-S (1997) Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomedical and Environmental Science*, **10**:451–455.

Zeiger E, Gollapudi B, Spencer P (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde—A review. *Mutation Research*, **589**:136–151.

Zissu D, Gagnaire F, Bonnet P (1994) Nasal and pulmonary toxicity of glutaraldehyde in mice. *Toxicology Letters*, **71**:53–62.

Zissu D, Bonnet P, Binet S (1998) Histopathological study in B6C3F1 mice chronically exposed by inhalation to glutaraldehyde. *Toxicology Letters*, **95**:131–139.

Zwart A, Woutersen RA, Wilmer JWGM, Spit BJ, Feron VJ (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. *Toxicology*, **51**:87–99.

#### IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans and Case-Studies

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A, Feron VJ (1989) Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. *Journal of Applied Toxicology*, **9**:39–46.

Ying C-J, Yan W-S, Zhao M-Y, Ye X-L, Xie H, Yin S-Y, Zhu X-S (1997) Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomedical and Environmental Science*, **10**:451–455.

Zeiger E, Gollapudi B, Spencer P (2005) Genetic toxicity and carcinogenicity studies of glutaraldehyde—A review. *Mutation Research*, **589**:136–151.

Zissu D, Gagnaire F, Bonnet P (1994) Nasal and pulmonary toxicity of glutaraldehyde in mice. *Toxicology Letters*, **71**:53–62.

Zissu D, Bonnet P, Binet S (1998) Histopathological study in B6C3F1 mice chronically exposed by inhalation to glutaraldehyde. *Toxicology Letters*, **95**:131–139.

Zwart A, Woutersen RA, Wilmer JWGM, Spit BJ, Feron VJ (1988) Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. *Toxicology*, **51**:87–99.

Reference	Target tissue	End- point	Response (control vs exposed)	Comments and exposures
Vargová et al. (1992)	PBL	CA	3.6% vs 3.08%	n = 20; high frequency in controls; wood splinter manufacture; formaldehyde 8-h TWA 0.55-10.36 mg/m <sup>3</sup> 5->16 years
Ballarin et al. (1992)	Nasal mucosa	MN	0.25 ± 0.22% vs 0.90 ± 0.47% ( <i>P</i> < 0.01)	Concurrent exposure to wood dust; no dose- response
Burgaz et al. (2001)	Nasal mucosa	MM	0.61 ± 0.27% vs 1.01 ± 0.62% ( <i>P</i> < 0.01)	Exposed, <i>n</i> = 23; non-exposed, <i>n</i> = 27; no dose-response
Burgaz et al. (2002)	Oral mucosa	NM	$0.33 \pm 0.30\%$ vs $0.71 \pm 0.56\%$ pathology laboratory ( $P < 0.05$ ) $0.33 \pm 0.30\%$ vs $0.62 \pm 0.45\%$ shoe factory ( $P < 0.05$ )	Exposed, $n = 22$ variable exposures; $n = 28$ exposed to formaldehyde; non-exposed, $n = 28$ ; correlation with duration of exposure
Titenko-Holland et al. (1996)	Oral mucosa	MN	0.6 ± 0.5% vs 2.0 ± 2.0% (P = 0.007)	Exposed, <i>n</i> = 28; pre- versus post-exposure; no details on smoking habits; formaldehyde
	Nasal mucosa	MN	2.0±1.3% vs 2.5±1.3% (NS)	concentrations: Oral: 1.2 mg/m³-h vs 18 mg/m³-h, 90 days Nasal: 2.4 mg/m³-h vs 20 mg/m³-h, 90 days
Ying et al. (1997)	Nasal mucosa Oral mucosa PBL	NM NM NM	1.20 ± 0.67 vs 3.84 ± 1.48 ( <i>P</i> < 0.001) 0.57 ± 0.32 vs 0.86 ± 0.56 ( <i>P</i> < 0.001) 0.91 ± 0.39 vs 1.11 ± 0.54 (NS)	Exposed, $n = 25$ ; pre-versus post-exposure; questions about controlling for age, sex, and smoking habits; formaldehyde concentrations 0.508 ± 0.299 mg/m <sup>3</sup> vs 0.012 ± 0.0025 mg/m <sup>3</sup>
He et al. (1988)	PBL	CA MN	3.40 ± 1.57% vs 5.96 ± 2.40% ( <i>P</i> < 0.01) 3.15 ± 1.46% vs 6.38 ± 2.50% ( <i>P</i> < 0.01)	Chromosomal aberrations included breaks and gaps, which renders interpretation difficult

# 付表:ホルムアルデヒドにばく露されたヒトにおける小核及び染色体異常に関する研究の 概要(IARC, 2005 年)

参考文献	標的組織	エンド ポイン ト	反応 (対照群vsばく露群)	コメント及びばく露
Vargováら (1992)	PBL	MN	3.6% vs 3.08%	n=20 ; 対照群では高頻度 ; 木材の破片製造 ホルムアルデヒド 8-h TWA 0.55-10.36 mg/m <sup>3</sup> 5- >16年
Ballarinら (1992)	鼻粘膜	MN	$\begin{array}{l} 0.25 \pm 0.22\% \ vs \ 0.90 \pm 0.47\% \\ (P < 0.01) \end{array}$	木粉の同時ばく露; 用量反応性なし
Burgazら (2001)	鼻粘膜	MN	$\begin{array}{l} 0.61 \pm 0.27\% \ vs \ 1.01 \pm 0.62\% \\ (P < 0.01) \end{array}$	ばく露、n=23; 非ばく露、n=27; 用量反応性なし
Burgaz රි (2002)	口腔粘膜	MN	0.33 ± 0.30% vs 0.71 ± 0.56% pathology laboratory (P < 0.05) 0.33 ± 0.30% vs 0.62 ± 0.45% shoe factory (P < 0.05)	ばく露、n=22の可変ばく露; ホルムアルデレドにばく露、n=28; 非ばく露、n=28; ばく露期間との相関性あり
Titenko-Holland ら (1996)	口腔粘膜 鼻粘膜	MN MN	$\begin{array}{l} 0.6\pm0.5\% \mbox{ vs } 2.0\pm2.0\% \\ (P=0.007) \\ 2.0\pm1.3\% \mbox{ vs } 2.5\pm1.3\% \mbox{ (NS)} \end{array}$	ばく驚、n=28; ばく驚前とばく露後の比較; 喫煙習慣の詳細は不明; ホルムアルデヒド濃度 口腔:1.2 mg/m <sup>3</sup> -h vs18 mg/m <sup>3</sup> -h、90日間 鼻腔:2.4 mg/m <sup>3</sup> -h vs20 mg/m <sup>3</sup> -h、90日間
Yingら் (1997)	鼻粘膜 口腔粘膜	MN MN	1.20 $\pm$ 0.67 vs 3.84 $\pm$ 1.48 (P < 0.001) 0.57 $\pm$ 0.32 vs 0.86 $\pm$ 0.56 (D < 0.001)	ばく露、n=25; ばく露前とばく露後の比較; 年齢、性及び喫煙習慣のコントロールに関する質問; ホルムアルデヒド濃度
	PBL	MN	(P < 0.001) 0.91 ± 0.39 vs 1.11 ± 0.54 (NS)	$0.508 \pm 0.299 \text{ mg/m}^3 \text{ vs. } 0.012 \pm 0.0025 \text{ mg/m}^3$
Heら (1988)	PBL	CA	$3.40 \pm 1.57\%$ vs $5.96 \pm 2.40\%$ (P < 0.01)	染色体異常には破壊や断片化が含まれており、 釈が難しくなっている
		MN	$\begin{array}{l} 3.15 \pm 1.46\% \ vs \ 6.38 \pm 2.50\% \\ (P < 0.01) \end{array}$	

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CA、染色体異常;MN、小核;NS、有意差なし;PBL、末梢血リンパ球;TWA、時間加重平均

# 第2部

非発がん MOA のヒトへの関連性を解析するための IPCS フレームワーク

# **PART 2**

# IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS

# PREFACE

Following completion of the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (see Part 1), an expert meeting was convened in Geneva in 2006 to explore the question as to whether the IPCS framework could be applied in chemical risk assessment generally (i.e. to develop a non-cancer framework). The participants at this expert meeting concluded that the framework should be applicable to all end-points and proceeded to author a draft publication out of session. The draft was sent for peer review by the members of the Harmonization Project Steering Committee and subsequently revised by the authors, taking into account the peer review comments received.

ヒトに対するがん MOA の妥当性を解析するための IPCS フレームワーク(第1部参照)の完成 後、2006 年にジュネーブで専門家会議が開催され、化学物質リスク評価に IPCS フレームワーク を一般的に適用できるか否か(すなわち、がん以外のフレームワークを開発すること)という問 題が議論された。この専門家会議の参加者は、フレームワークはすべてのエンドポイントに適用 可能であるべきであると結論付け、会期外に出版物の草案を作成した。この草案は、ハーモナイ ゼーションプロジェクト運営委員会のメンバーによって査読され、その後、受領した査読コメン トを考慮して著者によって修正された。

IPCS Framework for Analysing the Relevance of a Non-Cancer Mode of Action for Humans

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# LIST OF ACRONYMS AND ABBREVIATIONS

ACE	angiotensin-converting enzyme
CSAF	chemical-specific adjustment factor

- EMS eosinophilia-myalgia syndrome
- HBOC haemoglobin-based oxygen carriers
- HRF Human Relevance Framework
- ILO International Labour Organization
- ILSI International Life Sciences Institute
- IPCS International Programme on Chemical Safety
- MOA mode of action
- MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
- RSI Risk Science Institute (ILSI)
- SLE systemic lupus erythematosus
- UNEP United Nations Environment Programme
- WHO World Health Organization

# 頭字語と略語のリスト

ACE	アンジオテンシン変換酵素	
CSA	化学物質特異的調整係数	
EMS	好酸球增多筋痛症候群	
HBC	ヘモグロビン系酸素運搬体	
HRF	ヒト関連性フレームワーク	
ILO	国際労働機関	
ILSI	国際生命科学研究機構	
IPCS	国際化学物質安全性計画	
MOA	Mode of Action (作用モード)	
MPT	1-メチル-4-フェニル-1,2,3,6-テトラヒドロピリジ	$\sim$
RSI	リスクサイエンス研究所 (ILSI)	
SLE	全身性エリテマトーデス	
UNE	国際連合環境計画	

WHO 世界保健機関

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# IPCS FRAMEWORK FOR ANALYSING THE RELEVANCE OF A NON-CANCER MODE OF ACTION FOR HUMANS<sup>1</sup>

Alan R. Boobis, John E. Doe, Barbara Heinrich-Hirsch, M.E. (Bette) Meek, Sharon Munn, Mathuros Ruchirawat, Josef Schlatter, Jennifer Seed, & Carolyn Vickers

Structured frameworks are extremely useful in promoting transparent, harmonized approaches to the risk assessment of chemicals. One area where this has been particularly successful is in the analysis of modes of action (MOAs) for chemical carcinogens in experimental animals and their relevance to humans. The International Programme on Chemical Safety (IPCS) recently published an updated version of its MOA Framework in animals to address human relevance (cancer Human Relevance Framework, or HRF). This work has now been extended to noncancer effects, with the eventual objective of harmonizing framework approaches to both cancer and non-cancer end-points. As in the cancer HRF, the first step is to determine whether the weight of evidence based on experimental observations is sufficient to establish a hypothesized MOA. This comprises a series of key events causally related to the toxic effect, identified using an approach based on the Bradford Hill criteria. These events are then compared qualitatively and, next, quantitatively between experimental animals and humans. The output of the analysis is a clear statement of conclusions, together with the confidence, analysis, and implications of the findings. This framework provides a means of ensuring a transparent evaluation of the data, identification of key data gaps and of information that would be of value in the further risk assessment of the compound, such as on dose-response relationships, and recognition of potentially susceptible subgroups, for example, based on life stage considerations.

The framework described in this paper, a non-cancer Human Relevance Framework (HRF), was prepared by the International Programme on Chemical Safety (IPCS) (WHO/ILO/UNEP) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. This global "Harmonization Project" aims to harmonize global approaches to chemical risk assessment through both increased consistency of risk assessment methodologies and development of international guidance documents. The project enables the achievement of commitments on harmonization of chemical risk assessment (United Nations Conference on Environment and Development (United Nations, 1992), the Intergovernmental Forum on Chemical Safety (1994), the World Summit on Sustainable Development (UNEP, 2002), and the Strategic Approach to International Chemicals are assessed, and hence the documents produced can be applied in the assessment of industrial chemicals, biocides, pesticides, veterinary chemicals, pharmaceuticals, cosmetics, natural toxicants, food additives, and environmental contaminants in food, water, air, and consumer products.

A main outcome of the Harmonization Project is the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (Sonich-Mullin et al., 2001) and

# IPCS フレームワーク<sup>1</sup>

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体系化されたフレームワークは、化学物質のリスク評価の透明性と調和のとれたアプローチを促 進する上で非常に有用である。これが成功している分野の一つに、発がん化学物質の MOA の実験 動物とヒトの間の関連性の解析がある。最近、国際化学物質安全性計画(International Programme on Chemical Safety: IPCS)はヒトとの関連性に対処するために、動物における MOA フレームワーク の更新版を発表した(がんヒト関連フレームワーク、または HRF)。この作業は現在、発がん以外 の影響にまで拡大されており、最終的には、発がんと非発がんの両方のエンドボイントに対するフ レームワークの適用を調和させることを目的としている。発がん HRF と同様に、最初のステップ は、実験的観察に基づくエビデンスの重み付けが、仮説 MOA を確立するのに十分であるか否かを 判断することである。これは、毒性影響に因果関係のある一連の key events からなり、Bradford Hill 基準に基づくアプローチを用いて同定される。実験動物とヒトの間でこれらの事象を、先ずは定性 的に、それから定量的に比較する。解析により、結果の信頼性、解析及び帰結とともに、明確な結 論が記述される。このフレームワークは、データの透明性のある評価、主要なデータギャップの特 定、用量反応関係などの化合物のリスク評価に価値のある情報の特定、ライフステージの考慮など に基づく潜在的に感受性の高いサブグループを認識するための手段を提供している。

本論文に記載されているフレームワークは、非発がんヒト関連性フレームワーク(HRF)であ り、国際化学物質安全性計画(IPCS)(WHO/ILO/UNEP)の化学物質へのばく露によるリスク評価 のアプローチの調和プロジェクトによって作成されたものである。この世界的な「調和プロジェ クト」は、リスク評価手法の一貫性の向上と国際的なガイダンス文書の構築を通じて、化学物質 のリスク評価に対する世界的適用を目的としている。このプロジェクトは、環境と開発に関する 国際連合会議(1992年、国際連合)、化学物質の安全性に関する政府間フォーラム(1994年)、持 続可能な開発に関する世界首脳会議(2002年、UNEP)、国際的化学物質管理に関する戦略的アプ ローチ(2006年、WHO)などで合意された化学物質のリスク評価手法の調和に関する公約を達成 できるようにするものである。このプロジェクトには、化学物質の評価が行われる様々な分野の 専門家が参加しているため、作成された文書は、工業用化学物質、殺生物剤、殺虫剤、動物用化 学物質、医薬品、化粧品、天然毒物、食品添加物ならびに、食品中、水中、大気中及び消費者製品 中の環境汚染物質の評価に適用することができる。

調和プロジェクトの主な成果として、化学発がんに対する MOA 評価のための IPCS フレームワ

<sup>&</sup>lt;sup>1</sup> This article, to which WHO owns copyright, was published in 2008 in *Critical Reviews in Toxicology*, Volume 38, pages 87–96. It has been edited for this WHO publication.

<sup>&</sup>lt;sup>1</sup>本論文は、WHO が著作権を有するもので、2008 年に Critical Reviews in Toxicology, Volume 38, pages 87-96 に掲載された。本 論文は WHO の出版物のために編集された。

its subsequent development into an IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (IPCS cancer HRF) (Boobis et al., 2006; see also Part 1 of this document). The mode-of-action (MOA) analysis utilizes a weight-of-evidence approach based on the Bradford Hill criteria for causality (Hill, 1965). It aims to determine whether it is possible to establish an MOA for a carcinogenic response observed in an experimental animal study, through application of a weight-of-evidence approach that requires identification of key events along the causal pathway to cancer. When an MOA has been established in experimental animals, the cancer HRF provides an analytical tool to enable the transparent evaluation of the data in order to consider the human relevance of the MOA.

Following on from this, IPCS decided to consider whether the framework for cancer could be applied, with modifications, if necessary, to other end-points and their associated MOAs. Recognizing the work that the Risk Science Institute (RSI) of the International Life Sciences Institute (ILSI) had conducted in parallel to develop a similar framework and apply it to non-cancer risk assessment, IPCS convened an international meeting in Geneva in March 2006 to review and consider the ILSI publication (Seed et al., 2005), along with the IPCS cancer HRF (Boobis et al., 2006; see also Part 1 of this document), in order to explore the question as to whether the IPCS framework could be applied in chemical risk assessment generally. In summary, this IPCS meeting recognized that the framework should be applicable to all endpoints, both cancer and non-cancer, and recommended further work to put this into practice, including documenting the rationale for application of the framework more generally, which appears in the present paper, and steps to facilitate uptake and use of the framework.

The IPCS meeting recognized that the non-cancer HRF would have multiple uses in chemical risk assessment:

- It would provide an internationally harmonized approach to the establishment of an MOA in experimental animals and its relevance to humans.
- It would generate criteria for the MOA against which subsequent cases could be considered—that is, to show whether a compound shares an established MOA.
- It would enable clarification of key information relating to the human relevance of the MOA, and this would inform the assessment of other chemicals that share the MOA.
- In general, application of the framework would enable critical data deficiencies and research needs to be identified and inform qualitative and quantitative assessment.

## THE NEED FOR A NON-CANCER HUMAN RELEVANCE FRAMEWORK

The non-cancer HRF is a tool that provides a structured approach to the assessment of human relevance of a postulated MOA in animals in a weight-of-evidence context. Subsequently, it includes explicit consideration of the relevance of the proposed MOA to humans, often based on consideration of more generic information, such as anatomical, physiological, and biochemical variations among species. In this manner, the framework encourages maximum use of both chemical-specific and more generic information in a transparent and analytical fashion.

ーク (Sonich-Mullin ら、2001 年) と、それに続くがん MOA のヒトへの関連性を解析するための IPCS フレームワーク (IPCS cancer HRF) (Boobis ら, 2006 年;本文書の第1部も参照) がある。 MOA 解析は、因果関係の Bradford Hill 基準 (Hill、1965 年)に基づくエビデンスの重み付け (weight of evidence) アプローチを利用している。これは、腫瘍の原因に沿った一連の key events の特定を 必要とするエビデンスの重み付けアプローチを適用することで、動物実験で観察された発がん作 用に対する MOA を確立できるか判断することを目的としている。実験動物で MOA が確立され た場合、がん HRF は、MOA のヒトへの関連性を検討するために透明性のあるデータ評価を可能 にする解析ツールを提供できる。

これを受けて、IPCS は、必要に応じて修正を加えながら、他のエンドポイントとそれに関連す る MOA にもがんのフレームワークを適用できるか否かを検討することにした。国際生命科学研 究機構(ILSI)のリスクサイエンス研究所(RSI)は、がん以外のリスク評価に適用するために、 同様のフレームワークを開発した。また、IPCS は 2006 年 3 月にジュネーブで国際会議を開催し た。そこで、ILSI のフレームワークが一般的に化学物質のリスク評価に適用できるかという問題 を探るために、ILSI の出版物(Seed ら、2005 年)と、ILSI のがん HRF(Boobis ら、2006 年 ;本 文書の第1部も参照)を評価、検討した。要約すると、この IPCS 会議では、フレームワークが発 がんと非発がんの両方のエンドポイントに適用されるべきであり、これを実践に移すための更な る作業を推奨している。そしてこれには、本稿にも記載しているが、フレームワークのより一般 的適用のための根拠の文書化及びフレームワークの利用と利用を促進するための手順が含まれて いる。

IPCS 会議では、化学物質リスク評価において非発がん HRF は下記のような複数の有用性を持つことが認識された。

- 実験動物における MOA の確立とヒトとの関連性について、国際的に調和のとれたアプローチ を提供する。
- MOA の基準が作成され、後続のケースを考慮することができるようになる。すなわち、ある 化合物に確立された MOA を適合できるかを示すことができるようになる。
- MOAのヒトとの関連性に関する重要な情報が明らかになり、MOAを共有する他の化学物質の 評価に情報を提供できるようになる。
- 一般的に、このフレームワークを適用することで、重要データの不足や必要な研究が明らかになり、定性的・定量的な評価に役立てることができるようになる。

#### 非発がんとト関連性フレームワークの必要性

非発がん HRF は、動物 MOA のヒトとの関連性を評価するための体系化されたアプローチを提 供する手段であり、エビデンスの重み付けの文脈の中で、動物において推定される MOA のヒト への関連性を評価する。その後、推定される MOA のヒトへの外挿性を明確に検討することが含 まれており、多くの場合、種間の解剖学的、生理学的及び生化学的な差異などの一般的情報の検

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Pivotal to transparency in determining human relevance using the framework are the delineation and consideration of the nature of evidence in various species of key events—that is, those in a postulated MOA that are measurable and critical to the induction of the toxicological response. Evaluation of the concordance of key events based on explicit consideration of variations between experimental animals and humans constitutes the principal basis of transparency in consideration of weight of evidence for human relevance.

While principally relevant to hazard characterization, the non-cancer HRF additionally contributes more generally to transparency in risk assessment through explicit delineation and consideration of data on appropriate key events that are also relevant to subsequent dose–response analysis for MOAs deemed relevant to humans. If the MOA in experimental animals is judged to be qualitatively relevant to humans, a more quantitative assessment is required that takes into account any kinetic and dynamic information that is available from both the experimental animals and humans in order to determine whether human relevance might be precluded on this basis.

These same data are critical to subsequent dose–response analysis for MOAs considered relevant in considering the adequacy of, for example, available information as a basis for replacement of default uncertainty factors in the development of chemical-specific adjustment factors (CSAFs) (IPCS, 2005). This information could, for example, constitute an adequate basis to consider interspecies variation in rates of formation of reactive metabolites in the target tissue, for replacement of the default subfactor for interspecies differences in toxicokinetics with a CSAF (IPCS, 2005).

Use of this non-cancer HRF also promotes harmonization of approaches to risk assessment for all end-points, bridging previously distinct approaches on, for example, cancer and noncancer effects. Harmonization in this context refers to a biologically consistent approach to risk assessment for all end-points, for which exploration of biological linkages is critical to ensuring maximal use of relevant information. Often, for example, organ toxicity is a critical key event in postulated MOAs for induction of tumours at the same site. The non-cancer HRF, then, sets the stage for identification of critical precursor non-cancer key events for which subsequent quantification of interspecies differences and interindividual variability in dose–response analysis is relevant. In other cases, a postulated MOA may lead to toxic effects in multiple organs, and these would be considered in the same non-cancer HRF analysis.

In addition, consideration in a transparent framework may identify factors that, while not themselves essential for the toxicological effect (and hence not key events), may modulate key events and, as a result, contribute to differences between species or individuals. Such factors include genetic differences in pathways of metabolism, competing pathways of metabolism, and cell proliferation induced by concurrent pathology.

Such an analysis may also provide an indication of those components of a proposed MOA that may operate only over a certain dose range. If a high experimental dose of a given compound is needed to result in an obligatory step in an MOA, then the relevance to human

討に基づいている。このようにして、フレームワークは、透明性のある解析方法で、化学物質に 特異的な情報と一般的な情報の両方を最大限に利用することを奨励している。

フレームワークを用いてヒトへの関連性を決定する際の透明性を確保するために極めて重要な のは、key events の様々な種におけるエビデンスの性質を明確にし、検討することである。すなわ ち、推定される MOA における、測定可能で毒性学的反応の誘発に重要であると想定される key events に関するエビデンスの性質を明らかにし、考慮することである。実験動物とヒトの差異を 明確に考慮した上での key events の一致性の評価は、ヒトとの関連性に関するエビデンスの重み 付けを考慮する上での透明性を保つための基礎となる。

主にハザード評価において、非発がん HRF は、その後のヒトに関連すると考えられる MOA の 用量反応解析に関わる適切な key events の明確な定義とデータの検討を通じて、リスク評価の透 明性を高めることにも貢献している。実験動物における MOA がヒトと定性的に関連があると判 断された場合には、ヒトとの関連性が否定されるか否かを判断するために、実験動物とヒトの双 方からの動態学的及び薬力学的情報を考慮に入れた、より定量的な評価が必要である。

これらのデータは、その後の関連があると考えられる MOA の用量反応解析に極めて重要である (IPCS、2005年)。例えば、化学物質特異的調整係数 (CSAFs)の定義づけにおいて、デフォルトの不確実係数を置き換えるための基礎として、利用可能な情報の妥当性を考慮する場合である。この情報は、標的組織における反応代謝物の形成速度の種差を考慮して、毒物動態の種間差に対するデフォルトのサブファクターを CSAF で置き換えるための適切な根拠となり得る (IPCS、2005年)。

また、この非発がんHRFの使用はすべてのエンドポイントのリスク評価適用の調和を促進する。 例えば、発がん影響と非発がん影響に関するこれまでの異なるアプローチの架け橋となる。ここ でいう調和とは、関連情報を最大限に活用するために生物学的関連性を探ることが重要であり、 すべてのエンドポイントのリスク評価における一貫したアプローチを意味する。例えば多くの場 合、臓器毒性は同一部位に腫瘍を誘発すると推定される MOA において key events となる。非発が ん HRF は、その後の用量反応解析における種間差及び個体間のばらつきの定量化に関連する、重 要な前がん病変の key events を特定するための段階を設定する。他にも、推定される MOA が複数 の臓器に毒性効果をもたらす可能性があれば同じ非発がん HRF 解析で検討されることになる。

さらに、透明性のあるフレームワークで検討することで、それ自体は毒性学的影響に必須では ない(すなわち key events ではない)が、key events を修飾し、種や個体間の差異に寄与する因子 を特定できる可能性がある。そのような要因には、代謝経路の遺伝的差異、競合する代謝経路、 及び同時進行する病理学的要因により誘発される細胞増殖が含まれる。

このような解析は、特定の用量範囲でのみ、作動する可能性のある推定される MOA の構成要

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risk becomes a matter of exposure. Thus, the exposure assessment step of the risk assessment is critical to a comprehensive evaluation.

Importantly, then, application of the non-cancer HRF contributes to identification of any specific subpopulations (e.g. those with genetic predisposition) who are at increased risk and provides information relevant to consideration of relative risks at various life stages. In many cases, this is based not on chemical-specific information but rather on inference, based on knowledge of the MOA, as to whether specific age groups may be at increased or decreased risk. This requires explicit consideration of comparative developmental and ageing processes and events in humans and animal models. These considerations are critical to determination of focus in the remaining stages of risk assessment, such as dose–response analysis.

The transparent delineation of the weight of evidence for postulated MOAs and their relevance to humans (requiring explicit consideration of the strengths and weaknesses of the available database, as well as highlighting qualitative and quantitative similarities and differences among species and related uncertainties) also identifies any inconsistencies in the available data and defines critical data gaps and research needs. This derives from the requirement in each step to explicitly assess confidence in the quality and quantity of data underlying the analysis, consistency of the analysis within the framework, consistency of the database—that is, that studies are not contradictory of each other—and the nature and extent of the concordance analysis.

Iterative application of the non-cancer HRF, even before all of the data are available, to the analysis of a postulated MOA and its relevance to humans are beneficial as a basis for developing and refining research strategies as additional information becomes available. In this context, the framework should prove helpful in facilitating discussion between risk assessors and research scientists in jointly understanding the nature of data that would support human relevance analysis of a postulated MOA in animals and defining next steps in data acquisition. Iterative consideration of MOA in designing research strategies is also expected to increase efficiency by focusing resources in critical areas in more tiered and targeted approaches.

As knowledge advances, MOAs will become less chemical specific and based even more on the key biological processes involved, allowing greater generalization of human relevance from one compound to another. The need for chemical-specific data for established MOAs will be less, although it will always be necessary to establish rigorously that the key events comprising the MOA occur.

The transparency in the human relevance of a postulated MOA that results from application of the non-cancer HRF should promote confidence in the conclusions reached, through the use of a defined procedure that encourages clear and consistent documentation supporting the analysis and reasoning, highlights inconsistencies and uncertainties in the available data, and identifies critically important data gaps that, when filled, would increase confidence in outcome. This transparency not only is anticipated to facilitate discussion between the risk assessment and research communities, but may also contribute to greater convergence among different regulatory agencies. 素に関する指標も提供するかもしれない。MOA に必須な事象を引き起こすのに、対象の化合物の 高用量ばく露が必要とされる場合、ヒトのリスクとの関連性はばく露量に依存する問題となる。 このように、リスク評価におけるばく露評価は、包括的な評価を行う上で非常に重要である。

重要なことは、非発がん HRF を適用することで、リスクが高い集団(例えば遺伝的素因を持つ 人)を特定し、様々なライフステージにおける相対リスクに関連する情報を提供できるようにな るということである。これは多くの場合、化学物質固有の情報に基づくのではなく、MOA の知識 に基づき特定の年齢層でのリスクが増加しているか減少しているかを推論することに基づいてい る。このためには、ヒト及び動物モデルにおける発達及び老化過程を比較し、事象を明確に検討 する必要がある。これらの検討事項は、後続のリスク評価過程において用量反応解析など、どこ に重点を置くべきか決定する上で重要となる。

推定される MOAs のエビデンスの重み付けと透明性のあるヒトとの関連性を示すこと(利用可 能なデータベースの長所と短所を明確に検討し、種間の質的・量的な類似性と相違点及び関連す る不確実性を強調することを必要とする)は、利用可能なデータの矛盾を特定し、重要なデータ ギャップと研究の必要性を明らかにすることにもなる。これは、各ステップにおいて、解析の基 礎となるデータの質と量、フレームワーク内での解析の一貫性、データベースの一貫性(つまり 研究が互いに矛盾していないこと)、そして一致解析の質的・量的な信頼性を明確に評価する必要 があることに由来している。

すべてのデータが利用可能になる前であっても、推定される MOA 及びヒトとの関連性の解析 に、非発がん HRF を繰り返して適用することは、追加情報の獲得とともに研究戦略を策定・改良 するための基礎として有益である。すなわち、フレームワークは動物における推定される MOA の ヒトへの関連性解析を支援するデータの性質について、リスク評価者と研究者が共に理解を深め ること、データ取得に向け次の一手を決める手助けとなるに違いない。また、研究戦略を立案す る際に MOA を繰り返し検討することで、重要な分野に資源を集中させ、より階層的で集中的な アプローチを行うことができ、効率性を高めることが期待される。

知識の進歩に伴い、MOA は化学物質に特化したものから、関連する主要な生物学的プロセスに 基づいて、ある化合物から別の化合物へとヒトとの関連性をより一般化させることになるだろう。 MOA を構成する key events が発生していることを厳密に立証することが常に必要であるものの、 MOA を確立する際に化学物質固有のデータを必要とすることは少なくなるだろう。

非発がん HRF を適用した推定 MOA の透明性は、解析と推論を裏付ける明確で一貫性のある文 書化を奨励している。そして、それを満たすことで結果の信頼性を高める重要なデータギャップ を特定し、データの矛盾や不確実性を明らかにし、到達した結論に対する信頼性を向上させる。 このような透明性は、リスク評価者と研究者との議論を促進させるだけでなく、異なる規制機関 間の意見合意にも貢献すると期待される。 The non-cancer HRF also provides the basis for improved process and content for scientific peer input and peer review, specifying minimum criteria of clarity and transparency as a basis to acquire input and acceptance of postulated MOAs and their relevance to humans. Adherence to these criteria enables others to determine the basis of the conclusions reached with respect to the key events, the exclusion of other MOAs, and the analysis of human relevance.

# WHEN WOULD THE NON-CANCER HRF BE APPLIED?

The non-cancer HRF provides a valuable tool to assess an MOA, but it requires significant amounts of effort and experimental work, so it is not something that would be used during the course of the assessment of every chemical. Its main purpose would be to determine whether to apply the default assumption that all effects seen in animals are relevant to humans. This question increases in importance when the application of the default assumption during the course of a risk assessment indicates that adverse effects are likely to occur—for example, where there is a low margin of exposure between the point of departure for the effect under consideration and the estimated human exposure, especially if the human exposure estimate has already been refined. It then becomes important to know whether risk management measures will be required. This is of most concern when new data emerge, such as those identifying a new effect, additional data on the dose–response relationship of the chemical, or changes in use pattern or exposure estimation, which change the risk assessment of a chemical that is already in use.

Use of the non-cancer HRF may also be of value in the situation where the effects in animals would have potentially serious consequences if they occurred in humans, such as neuro-toxicity or teratogenesis. These effects are subject to very rigorous risk assessment procedures, so they comparatively frequently suggest the need for risk management measures.

Another situation in which use of the non-cancer HRF should be considered is where there are interspecies differences in either the type of effect or the dose levels at which an effect occurs. In these cases, it will be important to understand which species is the most appropriate upon which to base extrapolation to humans. This indication would also apply to differences between sexes or strains in the same species.

These situations indicate that further consideration is required, and the non-cancer HRF provides a way of doing this. The framework can be applied at any stage in the process of considering an effect. It should be applied in an iterative way during the course of investigating an effect to help guide the scientist. When an effect has first been observed and gives rise to concern, the framework allows the investigator to structure the work programme by prompting the questions to be addressed. As the investigation develops, it guides the investigator in assessing the data as they are generated and provides pointers in deciding whether and what other data would be required.

In situations where there is a large body of data, the framework allows the evaluator to weight the evidence according to its significance as well as its volume.

また非発がん HRF は、専門家の関与やピアレビューのために改善されたプロセスや内容の基礎 を提供し、推定される MOA 及びそのヒトへの関連性についての助言や承認を得るための基礎と して、解析の明確性や透明性の最低限の基準を明らかにする。これらの基準に従うことで、他者 が key events や他の MOA の除外、対象 MOA のヒトへの関連性の結論の根拠を、明確に認識でき るようになる。

# 非発がん HRF はどのような場合に適用されるのか

非発がん HRF は MOA を評価するための貴重なツールを提供するが、かなりの労力と実験的作 業を必要とするため、すべての化学物質の評価に用いられるものではない。HRF の主な目的は、 動物でみられるすべての影響がヒトに関連するというデフォルトの仮定を適用するか否かを決定 することであろう。この疑問は、リスク評価の過程で上記の仮定を適用した場合に評価結果がヒ トに有害な影響が発生する可能性が高いことを示す場合に重要性が増す。例えば、検討中の影響 が発生する最低用量とヒトの推定ばく露量との差が小さい場合、特にヒトのばく露量の推定値が 既に精緻化されている場合である。そしてその場合、リスク管理対策が必要かを知ることが重要 になる。このことは、新たな影響を特定したデータ、化学物質の用量反応関係に関する追加デー タ、または使用パターンの変更やばく露推定値の変更など、既に使用されている化学物質のリス ク評価を変更するような新たなデータが出てきた場合に最も懸念されることである。

非発がん HRF の使用は、動物での影響が神経毒性や催奇形性など、ヒトで発生した場合に重大 な影響を及ぼす可能性がある場合にも有用である。これらの影響は非常に厳格なリスク評価プロ セスの対象となるため、比較的頻繁にリスク管理措置の必要性が示唆される。

がん以外にHRFの使用を検討すべきもう一つの状況は、影響の種類または影響が発生する用量 レベルのいずれかに種差がある場合である。このような場合には、どの種がヒトへの外挿におい て最も適切なものであるかを理解することが重要である。この指針は、同一種の性別又は系統間 の違いにも適用される。

これらの状況は、さらなる検討が必要であることを示しており、非発がん HRF はこれを行う方 法を提供している。このフレームワークは、影響を検討するプロセスのどの段階でも適用でき、 科学者の道しるべとなるよう、影響を調査する過程で反復的に適用されるべきである。最初に影 響が観察され、懸念が生じたとき、フレームワークは、調査者が対処すべき問いを示すことによ って作業プログラムを構成できるようにする。研究の進展とともに、HRF はデータが作成された ときには、データを評価する研究者を導き、また他のデータが必要かどうか、どのようなデータ が必要かを決定する際の指標となる。

大量のデータがある状況では、フレームワークにより、評価者はその重要性と量に応じてエビ デンスの重み付けが可能となる。

The non-cancer HRF can also be useful when a chemical is observed to cause an effect suspected of being caused by an MOA that has already been established using the framework or shares structural similarity to a chemical or class of chemicals with an established MOA. The earlier use of the non-cancer HRF to establish this MOA will have identified the key steps that need to be investigated in order to ascribe the MOA to the new chemical. This will prove valuable both in a prospective way in designing new research or testing programmes and retrospectively in evaluating a data set.

# **CONSIDERATION OF THE NON-CANCER HRF**

The non-cancer HRF is an analytical tool that enables a structured approach to the assessment of the overall weight of the evidence for the postulated MOA and its relevance to humans. The framework is not designed to provide an absolute answer on sufficiency of the information, as this will vary, depending on the circumstance. It must be emphasized that it is not a checklist of criteria but an approach to data evaluation and presentation. The output from the application of the framework serves as the basis for the continuation of the risk assessment of the compound.

It is envisaged that the non-cancer HRF will be applicable to a wide range of toxicological end-points, encompassing changes in structure and function of organs, tissues, and cells, including physiological and neurobehavioural effects. The types of toxicity that could be addressed using the framework include, but are not limited to:

- *Organ toxicity*: Examples include benzene-induced haematotoxicity (aplastic anaemia), paraquat-induced lung toxicity, chloroquine-induced ocular toxicity.
- *Reproductive toxicity:* Examples include phthalate-induced male infertility, dioxininduced dysregulation of female fertility.
- *Developmental toxicity*: Examples include methylmercury-induced developmental neurotoxicity, retinoid-induced teratogenesis.
- *Neurotoxicity*: Examples include lead-induced peripheral neuropathy, acrylamide-induced axonopathy, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson disease.
- Immunotoxicity: Examples include organotin-induced immunosuppression, isoniazidinduced systemic lupus erythematosus (SLE)-like syndrome, contaminated L-tryptophaninduced eosinophilia-myalgia syndrome (EMS).

# **Introduction to MOA**

Prior to embarking on a non-cancer HRF analysis, there needs to be careful evaluation of the weight of evidence for a toxicological response on exposure to a chemical in experimental animals. The nature of the non-cancer HRF is such that only one MOA is analysed at a time; hence, for example, different toxicological effects associated with chemical administration, even if observed in the same animals, will require separate framework analyses to discern the MOA for each effect. Consistent with species- and tissue-specific variation in metabolic activation and detoxication, there may be poor site concordance for some toxicants. This will need to be kept in mind when comparing animal and human data.

非発がん HRF は、既にフレームワークを用いて確立されている MOA によって、化学物質が影響を 起こすことが観察された場合や、化学物質が既に確立された MOA を持つ化学物質と構造的類似があ る場合にも有用である。MOA を確立するために非発がん HRF を早期に使用することで、MOA を新し い化学物質に適用させるために調査する必要がある重要なステップを特定することができる。これは、 新しい研究や試験を設計する際の前向きな方法としても、データを遡及的に評価する方法でも、価値 あるものになるだろう。

#### 非発がん HRF の検討

非発がん HRF は、推定される MOA とヒトとの関連性を評価するための全体的なエビデンスの重み 付けのための手段を体系化する解析ツールである。このフレームワークは、状況に応じて異なるため、 情報が十分であるか否かに対する絶対的な答えを提供するようには設計されていない。これは基準の チェックリストではなく、あくまでもデータの評価と提示の手段である。フレームワーク適用による 成果は、化合物のリスク評価を継続するための基礎となる。

非発がん HRF は、臓器、組織、細胞の構造と機能の変化(生理学的影響や神経行動学的影響を含む) を含む幅広い毒性学的エンドポイントに適用されることが想定されている。このフレームワークを用 いて対処できる毒性の種類には、以下のものが含まれるが、これらに限定されるものではない。

- ・臓器毒性:例)ベンゼンによる血液毒性(再生不良性貧血)、パラコートによる肺毒性、クロロ キンによる眼毒性など
- 生殖毒性:例)フタル酸による男性不妊、ダイオキシンによる女性の受胎能力障害など
- 発達毒性:例)メチル水銀による発達神経毒性、レチノイドによる催奇形性など
- 神経毒性:例)鉛による末梢神経障害、アクリルアミドによる軸索障害、1-メチル-4-フェニル
   -1,2,3,6-テトラヒドロピリジン(MPTP)によるパーキンソン病など
- 免疫毒性:例)オルガノチンによる免疫抑制、イソニアジドによる全身性エリテマトーデス(SLE) 様症候群、汚染されたL-トリプトファンによる好酸球増多症症候群(EMS)など

#### MOA の紹介

非発がん HRF 解析に着手する前に、実験動物における化学物質へのばく露による毒性学的反応のエ ビデンスの重みを慎重に評価する必要がある。非発がん HRF の特徴は一度に1つの MOA のみを解析 する点である。したがって、例えば、化学物質の投与に関連した異なる毒性学的影響は、たとえ同じ動 物で観察されたとしても、各影響の MOA を識別するために別のフレームワーク解析を必要とする。代 謝的活性化と解毒における種や組織の違いと同様に、一部の毒性物質では毒性発現部位の一致性が低 い場合がある。動物とヒトのデータを比較する際には、この点に留意する必要がある。

# 推定されるMOA(事例における仮説)

ここで、被験物質の毒性学的影響の原因となっていると推定される MOA における一連の事象 112

# Postulated mode of action (theory of the case)

This comprises a brief outline of the sequence of events in the MOA postulated to be responsible for the toxicological effect of the test substance. This description leads into the next section, which identifies the events considered "key" (i.e. necessary and measurable) in the MOA.

## Key events

The "key events" in the MOA are briefly identified and described. Key events are those events that are critical to the induction of the toxicological response as hypothesized in the postulated MOA and are also measurable. To support an event as key, there needs to be a body of experimental data in which the event is characterized and consistently measured. The types of information that might be relevant include, for example, toxicological response and relevant key events in the same cell type, sites of action logically related to the event(s), specific biochemical events, changes in the expression or activity of enzymes, receptor–ligand interactions, effects on cofactor levels, specific changes in histology, changes in cell proliferation (increased or decreased), perturbations in hormone homeostasis or other signalling pathways (either intracellular or extracellular), second messengers, or ion fluxes, increased degradation of macromolecules, and changes in membrane permeability or integrity.

# Concordance of dose–response relationships

The dose–response relationships for each of the key events and for the toxicological response should be characterized and their interrelationships discussed with respect to the Bradford Hill criteria (Hill, 1965). Ideally, it should be possible to correlate the dose dependency of the increases in the magnitude (or frequency) of a key event with increases in the severity (e.g. lesion progression) of other key events occurring later in the process and with the ultimate toxicological response. Comparative tabular presentation of the magnitude of changes in key events and toxicological response is often helpful in examining dose–response concordance.

It is important to consider whether there are fundamental differences in the biological response (i.e. dose transitions) at different parts of the dose–response curve (Slikker et al., 2004). If so, key events relevant to the different parts of the dose–response curve will need to be defined and used in the framework analysis.

## Temporal association

The temporal relationships for each of the key events and for the toxicological response should be characterized. Key events should be observable before toxicity is apparent and should be consistent temporally with each other; this is an essential step in deciding whether the data support the postulated MOA. Observations of key events at the same time as the toxicological response (e.g. at the end of a study) do not permit conclusions as to temporal association, but can contribute to the analysis described in the next section.

# Strength, consistency, and specificity of association of toxicological response with key events

The weight of evidence linking the key events, any precursor lesions, and the toxicological response should be addressed (see Weed [2005] for a discussion of what is meant by weight of evidence). Stop/recovery studies showing absence or reduction of toxicity when a key

の概要を簡単に説明している。この記述は次のセクションにつながり、MOAの中で「重要」と考 えられる事象(すなわち、必要かつ測定可能な事象)を特定するものである。

## Key events

MOAにおける「key events」を簡潔に説明する。key events とは、推定される MOA で仮定され た毒性学的反応の誘発に重要な事象であり、測定可能な事象である。ある事象を key events とし て支持するためには、その事象を特徴づける、一貫した測定実験データが必要である。関連する 可能性のある情報の種類には、例えば、類似の細胞における毒性学的反応と関連する key events、 理論上関連する作用部位、特定の生化学的事象、酵素の発現または活性の変化、受容体とリガン ドの相互作用、補因子レベルへの影響、組織学における特異的変化、細胞増殖の変化(増加また は減少)、ホルモンのホメオスタシスまたは他のシグナル伝達経路(細胞内または細胞外)、セカ ンドメッセンジャー、またはイオン流束、高分子の分解性の増加、さらには膜透過性または膜統 合性の変化などがある。

# 用量反応関係の一致

それぞれの key events と毒性学的反応の量的反応関係を特徴づけ、Bradford Hill 基準 (Hill、1965 年)を参考にして相互関係を検討すべきである。理想としては、key events の大きさ(または頻度) の増加の用量依存性と、その後に発生する他の key events の重症度(例えば、病変の進行)の増 加、最終的な毒性学的反応に関連づけられるべきである。key events と毒性学的反応の変化の大き さを比較することは、用量反応の一致を検討するのに役立つことが多い。

用量反応曲線の異なる部分において生物学的反応に根本的な違いがあるか否かを考慮すること が重要である (Slikker ら、2004 年)。もしあれば、用量反応曲線の異なる部分に関連する key events を定義し、フレームワーク解析に用いる必要がある。

# 時間的関連性

各 key events と毒性反応の時間的関係を特徴付ける必要がある。key events は毒性が明らかにな る前に観察可能であり、時間的に互いに一貫している必要がある。これは、データが推定される MOA を支持しているか否かを判断する上で不可欠なステップである。毒性反応と同時に(試験終 了時など) key events を観察しても、時間的関連性についての結論は出ないが、次のセクションで 説明する解析に貢献することができる。

# key events と毒性学的反応との関連の強さ、一貫性及び特異性

key events、前駆病変及び毒性学的反応を結びつけるエビデンスの重みに注目すべきである(エ ビデンスの重みの意味についての考察は Weed [2005 年]を参照)。key events を遮断または減少さ せた場合に毒性が発現しない、または減少したことを示す中止/回復試験は関連性の試験として特 に有用である。

様々な実験計画は未知のバイアスや交絡を減らすことができるため、実際デザインの異なる多

event is blocked or reduced are particularly useful tests of the association. Consistent observations in a number of studies, with different experimental designs, increase support for the MOA, since different designs can reduce any unknown bias or confounding. Consistency, which is the repeatability of the key events in the postulated MOA in different studies, is distinct from coherence, however, which addresses the relationship of the postulated MOA with observations more broadly (see next point). Observations that may be of value here include toxicological response and relevant key events in the same cell type, sites of action logically related to event(s), and results from stop/recovery studies.

# Biological plausibility and coherence

One should consider whether the MOA is consistent with what is known about the biology of the target process/site in general (biological plausibility) and also in relation to what is known specifically about the overall biological effects of the substance (coherence). For the postulated MOA and its associated key events to be biologically plausible, they need to be consistent with current understanding of biology. However, when using biological plausibility as a criterion against which weight of evidence is assessed, it is important to consider the potential for gaps in our knowledge. Coherence, which addresses the relationship of the postulated MOA with chemical-specific observations more broadly—for example, association of the MOA for the toxicological response with that for other end-points—needs to be distinguished from consistency (addressed in the preceding point). In assessing coherence, information on structural analogues may be of value (i.e. structure–activity analysis). Information from other compounds that share the postulated MOA may also be helpful, such as sex, species, and strain differences in sensitivity and their relationship to key events. Additionally, this section should consider whether the database on the agent is internally consistent in supporting the proposed MOA.

#### Other modes of action

Alternative MOAs that logically present themselves should be considered. If alternative MOAs are supported, they will need a separate non-cancer HRF analysis. These should be distinguished from additional components of a single MOA, since these would be addressed as part of the MOA under consideration.

### Uncertainties, inconsistencies, and data gaps

Uncertainties should be stated fully and explicitly. They should include those related to the biology of the toxicological response and those for the database on the compound being evaluated. Any inconsistencies should be noted and data gaps identified. It should be clearly stated whether the identified data gaps are critical in supporting the postulated MOA.

#### Assessment of postulated mode of action

There should be a clear statement of the outcome of the analysis, indicating the level of confidence in the postulated MOA—for example, high, moderate, or low. If a novel MOA is being proposed, this should be clearly indicated. However, if the MOA is the same as one previously described, the extent to which the key events fit this MOA needs to be stated explicitly. Any major differences should be noted and their implications for acceptance of the MOA discussed.

くの研究で一貫した結果が得られることは、MOA の支持を高める。一貫性(Consistency)は、異 なる研究において仮定された MOA の key events の再現性であるが、整合性(coherence)とは異な るものであり、仮定された MOA と観察との関係をより広く扱うものである(次のポイントを参 照)。ここで価値があると思われる観察には、類似の細胞における毒性学的反応や関連する key events、論理的に関連する作用部位、中止/回復試験の結果などが含まれる。

# 生物学的妥当性及び整合性

MOA が、ターゲットとなる順序/場所の生物学一般についての知見と一致しているかどうか (生物学的妥当性)、また物質の全体的な生物学的影響について具体的に知られていることとの関 連性(整合性)を考慮すべきである。推定される MOA とそれに関連する key events が生物学的に 妥当であるためには、現在の生物学的知見と一致している必要がある。しかし、生物学的妥当性 をエビデンスの重みを評価する基準として用いる場合には、我々の知識との間にギャップが生じ る可能性を考慮することが重要である。推定される MOA と化学物質固有の観察結果との関係を より広く、例えば、毒性学的反応の MOA と他のエンドポイントの MOA との関連性などは、整合 性(前項で述べた)とは区別する必要がある。一貫性を評価する際には、構造類似体に関する情 報に価値があるかもしれない(すなわち、構造活性解析)。また、推定される MOA を共有する他 の化合物から得た性差、種差、系統の違いや key events との関係などに関する情報も有用であろ う。さらに、このセクションでは、推定される MOA を支持するために、その物質に関するデータ ベースが内部的に一貫しているかどうかを検討すべきである。

#### その他のMOA

論理的に存在する代替 MOA を検討すべきである。代替 MOA が支持される場合、それらは 別の非発がん HRF 解析を必要とする。これらは、検討中の MOA の一部として扱われるため、 単一の MOA の追加的な構成要素とは区別されるべきである。

# 不確実性、矛盾及びデータギャップ

不確実性は完全かつ明示的に記載すべきである。不確実性には、毒性反応の生物学的性質に関 連するものと、評価対象の化合物のデータベースに関するものが含まれるべきである。矛盾する ものはすべて記載し、データギャップを特定すべきである。また特定されたデータギャップが、 推定される MOA を支持する上で重要であるか否かも明記すべきである。

# 推定されるMOA の評価

推定される MOA に対する高、中、低など信頼度を示す解析結果の明確な記述があるべきであ る。新規 MOA が推定されている場合は、これを明確に示すべきである。しかし、MOA が以前に 説明されたものと同じである場合は、key events がこの MOA に適合する程度を明示的に記載する 必要がある。主要な相違点はすべて記載し、MOA の適用が与える影響を議論すべきである。

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## Life stage considerations

Since the response of an organism to a chemical exposure may vary through its lifespan, consideration of life stage is important for the MOA analysis of all toxic end-points. This is particularly true for effects that result from developmental exposures, since organ susceptibility may be restricted to critical periods of development, may depend on the ontogeny of key metabolic enzymes, or may depend on the interaction of the developing organism with its mother (see Zoetis & Walls, 2003). In addition, disruption of developmental processes may have downstream consequences.

Consideration of the ageing process is also important, for several reasons. First, developmental exposures can result in toxicities that are not detected until much later in life. In addition, there can be species-specific patterns of ageing for different organ systems. For example, reproductive senescence has a different etiology in rodents and humans and can even differ among different strains of rodents.

#### Human relevance

If it is possible to establish an MOA in animals for a toxicological effect, the next stage is to evaluate its relevance to humans. The IPCS non-cancer HRF is presented as an approach to answering a series of three (or four) questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying the toxicological effect. The application of the guidance results in a narrative with four (or five) sections, which may be incorporated into the hazard characterization of a risk assessment.

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals? This question is addressed by performing an MOA analysis as described above, the steps of which are based on the Bradford Hill criteria for causality (Hill, 1965). The weight of evidence for possible alternative MOAs needs to be considered and a conclusion reached on the overall strength of evidence supporting the MOA under consideration. The approach also identifies any critically important data gaps that, when filled, would increase confidence in the proposed MOA. If the postulated MOA has already been described for other chemicals, its human relevance will already have been evaluated. If the proposed MOA is novel, human relevance will need to be assessed de novo.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? This step involves a qualitative assessment of the relevance of the MOA to humans. Listing the critical key events that occur in the animal MOA and directly evaluating whether or not each of the key events might occur in humans facilitate the evaluation and increase the transparency of the process. Presentation in tabular form, referred to as a concordance table, can be particularly useful. The information in such tables should be relatively brief, as a narrative explanation should always accompany the table. In one column, the effect on humans for each of the key events is evaluated. Another column for the results in a different strain, species, or sex or for a different route of administration that does not result in toxicity can be useful for comparative purposes. Factors may be identified that, while not key themselves, can modulate key events and so contribute to differences between species or individuals. Examples include genetic differences in pathways of metabolism, competing pathways of

#### ライフステージの考察

化学物質ばく露に対する生物の反応は、その一生を通じて変化する可能性があるため、すべて の毒性エンドボイントの MOA 解析において、ライフステージを考慮することが重要である。器 官の感受性は臨界期、主要な代謝酵素の発生時期、あるいは母体と胎児の相互作用に依存するか もしれないため、特に発生期のばく露で生じる影響において重要である(Zoetis & Walls、2003 年 を参照)。さらに、発生過程の障害はその下流に影響をもたらす可能性がある。

老化を考慮することも、いくつかの理由から重要である。第一に、発育期のばく露では、かな り後にならないと毒性が検出されないことがある。さらに、器官系ごとに種特異的な老化パター ンが存在する可能性がある。例えば、生殖器はげっ歯類とヒトでは病因が異なり、げっ歯類の系 統によっても異なることがある。

# ヒトとの関連性

動物において毒性学的影響の MOA を確立することができれば、次の段階でヒトへの関連性を 評価する必要がある。IPCS の非発がん HRF は、一連の 3 つの(または 4 つの)問いに答えるア プローチとして提示されており、毒性学的影響の根底にある MOA のヒトへの外挿性に関する文 書化された論理的な結論を導き出すことができる。このガイダンスを適用すると、4 つ(または 5 つ)のセクションで構成された説明文が作成され、リスクアセスメントのハザード評価に組み込 むことができる。

1. 動物における MOA を確立するのにエビデンスの重み付けは十分か。この問いは、因果関係 の Bradford Hill 基準 (Hill、1965 年)に基づいた、上記のような MOA 解析を行うことで解決され る。可能性のある代替 MOA におけるエビデンスの重みを考慮し、検討中の MOA を支持するエビ デンス全体の強さに基づいて結論を出す必要がある。このようなアプローチは、重要なデータギ ャップを特定する。このデータギャップが埋められることにより、提示された MOA はより信頼 性を高めるであろう。推定される MOA が他の化学物質について既に記述されている場合、その ヒトへの外挿性は評価されているだろう。推定される MOA が新規である場合、ヒトへの外挿性 は新たに評価する必要がある。

2. 実験動物とヒトとの間のkey events の根本的、質的な違いに基づいて、MOA のヒトとの関連 性を合理的に排除することができるか。この問いでは、ヒトに対する MOA の関連性の質的評価 を行う。動物の MOA で発生する重要な key events をリストアップし、各 key events がヒトで発生 する可能性があるかどうかを直接評価することで、評価が容易になり、プロセスの透明性が高ま る。一致表と呼ばれる表形式での提示は特に有用である。この表に従って証明するため、表の情 報は比較的簡潔でなければならない。1 つの列には、key events のそれぞれについてのヒトへの影 響が評価されている。毒性をもたらさない異なる系統、種、性、または異なる投与経路の結果に 関する列は、比較するのに有用である。それ自体は重要ではないが、key events を修飾し、種差ま たは個体差に寄与する因子が同定される場合がある。例としては、代謝経路における遺伝的差異、

metabolism, and effects induced by concurrent pathology. Any such factors identified should be noted in a footnote to the concordance table.

The evaluation of the concordance of the key events for the MOA for a given chemical in humans is an evaluation of the MOA in humans, rather than an evaluation of the specific chemical. In general, details of the initial key events are likely to be more chemical specific. Later events will be more generic to the MOA. While information for evaluating the key events in humans can come from in vitro and in vivo studies on the substance itself, basic information on anatomy, physiology, endocrinology, genetic disorders, epidemiology, and any other information that is known regarding the key events in humans can be of value.

In answering this question, a narrative describing the weight of evidence and an evaluation of the level of confidence for the human information should be prepared. Examples of specific types of information that can be useful include:

- where appropriate, background incidences of the effect at the anatomical site and cell type of interest, including age, sex, ethnic differences, and risk factors, including chemicals and other environmental agents;
- knowledge of the nature and function of the target site, including development, structure (gross and microscopic), and control mechanisms at the physiological, cellular, and biochemical levels;
- human and animal disease states that provide insight concerning target organ regulation and responsiveness;
- human and animal responses to the chemical under review or structural analogues following short-, intermediate-, or long-term exposure, including target organs and effects.

Obviously, a substantial amount of information is required to conclude that a given MOA is not relevant to humans. If such a conclusion is strongly supported by the data, exposure to chemicals producing toxicity only by that MOA would not pose a risk to humans, and no additional risk characterization for this end-point is required.

The question of relevance considers all groups and life stages. It is possible that the conditions under which an MOA operates occur primarily in a susceptible subpopulation or life stage—for example, in those with a pre-existing viral infection, hormonal imbalance, or disease state. Any information suggesting qualitative or quantitative differences in susceptibility is highlighted for use in risk characterization.

There are several aspects relating to life stage that should be considered in the non-cancer HRF analysis. First, the analysis should consider the comparative developmental processes and events that occur in humans and the animal model(s) (see Zoetis & Walls, 2003). This comparison will demonstrate the extent to which developmental processes are similar in humans and the animal model(s). In general, development is highly conserved; where this is the case, it would lead to a conclusion that the MOA in animals is also plausible in humans. However, there are some developmental processes that are unique to some species, which may therefore lead to a species-specific MOA that will not be plausible in humans.

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競合する代謝経路及び同時並行的な病態によって誘発される影響などが挙げられる。特定されたそのような要因はすべて、一致表の脚注に記載する必要がある。

代謝経路における遺伝的差異、競合する代謝経路及び同時並行的な病態によって誘発される影響などが挙げられる。特定されたそのような要因はすべて、一致表の脚注に記載する必要がある。 ある化学物質のヒトにおける MOA の key events の一致性の評価は、特定の化学物質の評価という よりは、ヒトにおける MOA の評価である。一般的に、初期の key events の詳細は、より化学物質 に特化したものになる可能性が高い。その後の事象は、MOA により一般的に当てはまる事象とな る。ヒトにおける key events を評価するための情報は、物質そのものに関する in vitro 及び in vivo 研究から得られるが、解剖学、生理学、内分泌学、遺伝的疾患、疫学、その他ヒトにおける key events に関して知られているあらゆる基本的な情報が重要となる。

この問いに答える際には、エビデンスの重みとヒトの情報における信頼度の評価を記述した説 明文を作成する必要がある。有用な情報の具体例としては、以下のようなものがある。

- 必要に応じて、年齢、性別、人種差、化学物質やその他環境因子などの危険因子を含む、対象 となる解剖学的部位や細胞種における発生率の背景値
- 生理的、細胞的、生化学的レベルの制御機構や発生、構造(肉眼、組織)を含む標的部位の性質と機能に関する知識
- 標的器官の制御と応答に関する考察をもたらすヒト及び動物の疾患状態
- 対象となる臓器や影響を含む、短期、中期、または長期ばく露後の、評価対象の化学物質また は構造類似体に対するヒト及び動物の反応

MOA がヒトには関連性がないと明確に結論づけるためには、相当量の情報が必要である。その ような結論がデータによって強く支持されている場合、その MOA のみで毒性を生じる化学物質 はヒトにリスクをもたらさず、このエンドポイントに関する追加のリスク評価は必要ない。

関連性の問題は、すべての集団とライフステージを考慮している。MOA が作動する条件が、主 に感受性の高い集団やライフステージにいる人に発生する可能性がある。例えばすでにウイルス に感染しているか、ホルモンバランスの不均衡を有する人または疾患を持っている人である。感 受性の質的または量的差異を示唆する情報は、リスクの特徴付けに使用するため強調される。

非発がんHRF解析では、ライフステージに関連して考慮すべきいくつかの側面がある。第一に、 解析ではヒトと動物モデルで起こる発生過程と事象を比較検討すべきである(Zoetis & Walls、2003 年参照)。この比較は、ヒトと動物モデルにおいて発生過程がどの程度類似しているかを示すもの である。一般的に、発生過程はそんなに変化しておらず、このような場合には、動物 MOA はヒト MOA にも当てはまるという結論が導き出される。しかし、いくつかの種には特有の発生過程があ り、その場合種特異的な MOA となり、ヒトにもあてはまるとは限らない。 Second, the analysis should consider the phase specificity or relative timing of the developmental processes or events in humans and the animal model(s). Critical developmental events may occur at different times during ontogeny. Some developmental events may occur early during the prenatal development of the animal and relatively late in human prenatal development. Other developmental events may occur prenatally in humans and postnatally in the animal, or vice versa. Differences in timing of the developmental events can have an impact on the dose metrics if there are substantial differences in placental versus lactational transfer. Similarly, a comparison of the ontogeny of key metabolic enzymes relative to the key developmental process may reveal substantial differences between humans and the animal model. Such considerations may lead to a conclusion that the animal MOA is not plausible in humans.

3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans? If the MOA in experimental animals cannot be judged to be qualitatively irrelevant to humans (no to question 2), a more quantitative assessment is undertaken, taking into account any kinetic and dynamic information that is available from experimental animals and humans. Such data will of necessity be both chemical and MOA specific and where possible should include the biologically effective doses required to produce the dynamic effects giving rise to the toxicity. Kinetic considerations include the rate and extent of absorption, tissue distribution, metabolism, and excretion. Differences in ontogeny can result in substantial species differences in placental and lactational transfers, which will affect the dose metrics. This may therefore result in a quantitative difference in the MOA between humans and experimental animals. Similarly, the differential ontogeny of key metabolic enzymes can result in substantial quantitative differences between humans and experimental animals. Dynamic considerations include the consequences of the interaction of the chemical with cells, tissues, and organs. Only infrequently is it likely that it will be possible to dismiss human relevance on the basis of quantitative differences. Since quantitative exposure assessment is part of the subsequent risk characterization rather than the HRF, the difference would have to be of such a magnitude that human exposure could not possibly be envisaged to reach such levels. In most cases, it will not be possible to reach such a conclusion without undertaking formal exposure assessment in the subsequent risk characterization. Hence, the answer to the question will be no, but it may still be concluded that the risk is negligible in the subsequent risk characterization. Melamine-induced urinary bladder carcinogenesis provides a useful case-study illustrating this point (Meek et al., 2003). Again, tabular comparison of the data from experimental animals and humans can help in the evaluation. Information from studies of other compounds acting by the same or a similar MOA can be of value. As understanding of the basis for differences in responses between experimental animals and humans improves, differences in key events thought to be qualitative may be shown to be due to specific quantitative differences.

While it may not be possible to conclude that the MOA for toxicity is not relevant to humans on the basis of quantitative differences, during the evaluation it may become apparent that the magnitude of those differences is sufficient to impact markedly on the risk assessment. Hence, it is particularly important that the narrative for the answer to this question be comprehensive and capture as much quantitative information as possible. 第二に、解析では、ヒトと動物モデルにおける発生過程や事象の相対的な時期や段階の特異性 を考慮する必要がある。重要な発生過程における事象は、発生期の異なる時期に生じる可能性が ある。いくつかの発生過程における事象は、動物では出生前の早い時期に発生し、ヒトでは出生 前の遅い時期に発生する可能性がある。その他発生過程における事象は、ヒトでは出生前に発生 し、動物では出生後に発生することもあれば、その逆もある。胎盤移行と乳汁移行に大きな差が ある場合には、発生時期の違いが線量基準に影響を与える可能性がある。同様に、発生過程に関 連する主要な代謝酵素の発生時期を比較すると、ヒトと動物モデルの間にかなりの差があること が明らかになる可能性がある。このような考察から、ヒトでは動物モデルの MOA は妥当ではな いという結論が導き出されるかもしれない。

3. 実験動物とヒトとの間の薬力学的または動態的要因のいずれかの量的差異に基づいて、MOA のヒトヘの関連性を合理的に排除することができるか。実験動物における MOA がヒトと定性的 に無関係であると判断できない場合(問い2に「No」)、実験動物とヒトから得られるあらゆる動 態学的及び薬力学的情報を考慮して、より定量的な評価が行われる。このようなデータは、必然的 に化学物質と MOA に固有のものであり、可能であれば、毒性を引き起こす薬力学的影響が発現 する用量を含むべきである。動態学的考察には、吸収の速度及び程度、組織分布、代謝及び排泄が 含まれる。胎生の違いは、胎盤移行及び乳汁移行において実質的な種差をもたらす可能性があり、 これは定量的な影響を及ぼす。したがって、これによりヒトと実験動物との間で MOA の量的差 異がもたらされる可能性がある。同様に、主要な代謝酵素の発現時期の違いにより、ヒトと実験 動物の間ではかなりの差が生じる可能性がある。薬力学的な考慮事項には、化学物質と細胞、組 織及び器官との相互作用が含まれる。量的差異に基づいてヒトへの外挿性を否定することができ るのは、ごくまれである。定量的ばく露評価は HRF よりもむしろその後のリスクの判定の一部で あるため、その差はヒトへのばく露がそのようなレベルに達するとは考えられないほどの大きさ でなければならない。ほとんどの場合、後続のリスクの判定で正式なばく露評価を行わなければ、 そのような結論に達することはできない。したがって、問いの答えは「No」であるが、その後の リスク評価では、リスクは無視できる程度であると結論づけられるかもしれない。メラミン誘発 性膀胱発がんは、この点を説明する有用な事例研究を提供している(Meek ら、2003 年)ここで も、実験動物とヒトからのデータを表形式で比較することが評価に役立つ。同様または類似の MOA によって作用する他の化合物の研究から得られた情報も価値がある。実験動物とヒトの間の 反応の違いの基礎的な理解が深まれば、定性的であると考えられていた key events の違いが、定 量的な違いによるものであることが示されるかもしれない。

量的差異に基づいて毒性 MOA がヒトと関連性がないと結論づけることはできないかもしれな いが、評価の中で、これらの差異の程度はリスク評価に顕著な影響を与えるのに十分である。し たがって、この問いへの回答の説明文は包括的であり、可能な限り定量的な情報を把握しておく ことが重要である。

As with question 2, if the response to this question is *ves*, then exposure to chemicals producing toxicity only by this MOA would not pose a risk to humans, and no additional risk characterization is required.

The preceding three questions comprise a decision tree (see Figure 1).

		Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?	$\mathbf{NO}$ $\rightarrow$	Continue with risk assessment
		$\downarrow$ YES		
MOA not relevant	YES ←	Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?		
		$\downarrow$ NO		
MOA not relevant	YES ←	Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?	$\mathbf{NO}$ $\rightarrow$	Continue with risk assessment

Figure 1. Decision tree for determining human relevance of an MOA for toxicity observed in experimental animals.

#### Potential implications for dose-response assessment

Should it not be possible to exclude human relevance of the MOA for toxicity prior to proceeding with the risk assessment, a further question should be addressed. This is: 4. Are there any quantitative differences in the key events such that default values for uncertainty factors for species or individual differences could be modified? Such information, on either kinetics or dynamics, could be used to calculate a CSAF, in which one or more of the default values for species or interindividual differences in kinetics or dynamics are replaced by a value based on chemical-specific information (IPCS, 2005). The other components of the adjustment factor would retain their default values. Such information may lead to either an increase or a decrease in the adjustment factor relative to the normal default.

#### Published case-studies

In developing a framework for assessing the human relevance of MOAs for non-cancer endpoints, ILSI/RSI also developed a series of illustrative case-studies. These were on molinateinduced inhibition of spermatogenesis (Kavlock & Cummings, 2005a), renal and developmental effects of ethylene glycol (Corley et al., 2005), developmental neurotoxicity of nicotine (Slikker et al., 2005), phthalate ester effects on male reproductive development (Foster, 2005), vinclozolin-induced malformations (Kavlock & Cummings, 2005b), developmental effects of valproic acid (Wiltse, 2005), haemoglobin-based oxygen carriers (HBOC)-related congenital malformations (Holson et al., 2005), developmental effects of angiotensinconverting enzyme (ACE) inhibitors (Tabacova, 2005), developmental ototoxicity of polyhalogenated aromatic hydrocarbons (Crofton & Zoeller, 2005), and propylthiouracilinduced effects on neurological development (Zoeller & Crofton, 2005). While these cases

問い2と同様に、この質問への回答が「Yes」であれば、この MOA のみで毒性を示す化学物質 へのばく露はヒトにリスクをもたらさず、追加のリスク特性評価は必要ない。

前述の3つの質問は、決定ツリーを構成している(図1参照)。

動物における MOA を立証するのに <u>NO</u> リスク評価を続ける エビデンスの重み付けは十分か?

1 YES

実験動物とヒトとの間の key events の YES MOA は関係ない 基本的な質的差異に基づいて MOA のヒトへの関連性を合理的に排除できるか?

↓ NO

MOA は関係ない YES

実験動物とヒトとの間の動態学的または薬力学的 要因のいずれかの量的な違いに基づいて MOA のヒ NQ リスク評価を続ける トへの関連性を合理的に排除することができるか?

# 図1.実験動物で観察された毒性に対する MOA のヒトへの関連性を判断するための 決定ツリー

# 用量反応評価への影響

リスク評価を進める前に、毒性に対する MOA のヒトへの関連性を除外することはできないが、 更なる問いに対処すべきである。それは以下の点である。これは、4. 種や個体差の不確実性因子 のデフォルト値を変更できるような、kev events に量的な違いがあるか。このような情報は、動態 学又は薬力学のいずれかに関するものである。これは CSAF を計算するために使用することがで き、その場合動態学又は薬力学における種又は個体差のデフォルト値の1 つ以上が、化学物質固 有の情報に基づく値に置き換えられる(IPCS、2005年)。調整係数の他の要素は変更しない。この ような情報は、通常のデフォルト値と比較して調整係数の増加または減少をもたらす可能性があ る。

## 発表された事例研究

がん以外のエンドポイントに対する MOAs のヒトへの関連性を評価するためのフレームワーク を決定するにあたり、ILSI/RSI はまた、一連の例示的な事例研究を実施した。これらの事例研究 は以下の通り。

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モリネートによる精子形成阻害(Kavlock & Cummings、2005 年 a)、

エチレングリコールの腎及び発育への影響(Corley ら、2005年)、

ニコチンの発達神経毒性 (Slikker ら、2005 年)

フタル酸エステルの男性の生殖発達に対する影響(Foster、2005年)、

ビンクロゾリン誘発性奇形 (Kavlock & Cummings、2005 年 b)、

バルプロ酸の発育への影響(Wiltse, 2005年)、

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IPCS Framework for Analysing the Relevance of a Non-Cancer Mode of Action for Humans

covered a range of end-points, most involved effects during development. Hence, there is a need for additional case-studies on other end-points, such as those indicated above. As experience is obtained in using this framework, some of the published cases could be further refined to provide valuable illustrative examples for training in the application of the framework.

In general, the cases have been very useful in highlighting a number of the key issues on which this non-cancer HRF is based. Examples include the importance of the concordance analysis, the value of quantitative information identified during the application of the framework when it is not possible to exclude human relevance, the need for a transparent and comprehensive narrative when reporting the conclusions of a framework analysis, the importance in identifying key data gaps (e.g. case-study on molinate and HBOC), identification of research needs (e.g. case-study on vinclozolin), the importance of understanding the formation of a specific metabolite, and the importance of establishing a robust MOA through the application of the Bradford Hill criteria (Hill, 1965) to the key events.

#### Statement of confidence, analysis, and implications

Following application of the non-cancer HRF and answering the three (or four) questions, a statement of confidence should be provided that addresses the quality and quantity of data underlying the analysis, the consistency of the analysis within the framework, the consistency of the database, and the nature and extent of the concordance analysis. Alternative MOAs should have been evaluated, when appropriate, with the same rigor. A critical outcome is the identification of specific data gaps that could be addressed experimentally to increase confidence in the analysis.

The output of the non-cancer HRF provides information that is useful for more than just determining whether or not the MOA for toxicity in experimental animals is relevant to humans. It can also provide much information that is critically important in subsequent steps in the risk characterization for relevant effects. For example, it may be possible to develop CSAFs on the basis of the information provided. Application of the framework can also provide information on relevant modulating factors that are likely to affect risk. In addition, it can identify those elements of a proposed MOA that operate only over a certain dose range. Where an obligatory step in an MOA occurs only following a high experimental dose of a compound, the relevance of the MOA to human risk is determined by the exposure. Thus, effective exposure assessment is particularly important to the evaluation of human risk from such toxicity.

The analysis also contributes to the identification of any specific subpopulations (e.g. those with genetic predisposition) who may be at increased risk and often provides information useful in considering relative risk at various life stages. This may be based not always on chemical-specific information but rather on inference, on the basis of knowledge of the MOA, as to whether the risk in specific age groups might be expected to differ.

The data and their analysis using the non-cancer HRF should be reported in a clear and comprehensive manner, so that others can determine the basis of the conclusions reached.

ヘモグロビン系酸素運搬(HBOC)関連先天性奇形(Holson ら、2005年)、

アンジオテンシン変換酵素 (ACE) 阻害剤の発生影響 (Tabacova、2005 年)、

ポリハロゲン化芳香族炭化水素の発達聴器毒性(Crofton & Zoeller、2005年)、

プロピルチオウラシルが神経発達に及ぼす影響(Zoeller & Crofton、2005 年)などがある。

これらの事例は、様々なエンドポイントを対象としているが、そのほとんどは発生段階の影響 を対象としている。したがって、上記のような他のエンドポイントに関する追加の事例研究が必 要である。このフレームワークの使用経験の積み重ねにより、公開された事例のいくつかは、フ レームワーク適用ための例示的事例を提供できるように、さらに洗練されていく可能性がある。

一般的に、これらの事例は、この非発がん HRF の基礎となっているいくつかの重要な問題を強 調する上で非常に有用である。例としては、一致解析の重要性、ヒトへの関連性を排除できない 場合のフレームワーク適用中に特定された量的情報の価値、フレームワーク解析の結論を報告す る際の透明性のある包括的な構成の必要性、重要なデータギャップの特定(例:モリネートと HBOC の事例研究)、研究ニーズの特定(例:ビンクロゾリンの事例研究)、特定の代謝物の形成を理解 することの重要性、Bradford Hill 基準(Hill、1965 年)を key events に適用して堅牢な MOA を確 立することの重要性などが挙げられる。

## 信頼性、解析及び帰結の記述

非発がん HRF を適用し、3 つ(または4つ)の質問に回答した後、解析の基礎となるデータの 質と量、フレームワーク内での解析の一貫性、データベースの一貫性及び一致性解析の性質と程 度に対応した信頼性を示す文書を提供すべきである。必要に応じて代替的な MOAs は、同様に厳 密に評価されるべきである。重要な成果は、解析の信頼性を高めるために実験的に対処できるデ ータギャップを特定することである。

非発がん HRF を行うことにより、実験動物における毒性 MOA がヒトに関連するかどうかを判 断するだけでなく、それ以上に有用な情報が得られる。また、関連する影響のリスクの判定の次 の段階で重要な情報を得ることができる。例えば、得られた情報に基づいて CSAFs を開発するこ とができるかもしれない。フレームワークを適用することで、リスクに影響を与える可能性のあ る調節因子の情報も提供できる。さらに、推定される MOA の要素のうち、特定の用量範囲での み作用するものを特定することができる。MOA で必須の段階が、ある化合物の高用量投与後にの み発生する場合、ヒトのリスクに対する MOA の関連性はばく露量によって決定される。したが って、効果的なばく露評価は、このような毒性によるヒトリスクの評価にとって特に重要である。

解析もまた、リスクが増加する可能性のある集団(例えば、遺伝的素因を持つ人々)の特定に 寄与し、多くの場合様々なライフステージにおける相対的なリスクを考慮するのに有用な情報を 提供している。これは必ずしも化学物質固有の情報に基づくものではなく、むしろ MOA の知識 に基づいて、年齢によってリスクが異なるかどうかについての推論に基づくものである。

Although the specific form of presentation will vary with the type of data available, a structured report, including the key headings from the framework, should be provided where possible. Presentation should include sufficient details on the context and thought processes to ensure transparency of the conclusions reached. The inclusion of concordance tables is strongly encouraged. This increases transparency and facilitates peer engagement.

# **USE OF THE FRAMEWORK AND ITS OUTPUTS**

The IPCS non-cancer HRF, which is based principally on robust concordance analysis of key events in postulated MOAs, is envisaged to be of value to both the risk assessment and research communities as a basis to contribute to harmonization in several areas, including:

- adequacy and nature of weight of evidence for postulated MOAs in animals and their relevance to humans;
- MOA integration across end-points;
- criteria for transparency to ensure sufficiency of peer input and review.

Among the strengths of the non-cancer HRF are its flexibility, transparency, and general applicability across end-points. This includes determination of the nature and shape of the dose–response curve, the identification and location of biological thresholds for individual key events, and their consequences. In addition, consideration of the kinetic and dynamic factors involved in each key event is informative regarding the relevance or not to specific subpopulations—for example, in early life, in those with particular diseases, or in those with specific polymorphisms. Alternatively, application of the framework can provide quantitative information on the differences between such groups. Human relevance analysis may also indicate that a species is inappropriate for evaluating a potentially relevant end-point because of dose limitations.

# **NEXT STEPS**

To ensure effective adoption of the non-cancer HRF, there will be a need to train individuals in its application and in the interpretation of its outputs. Experience is being gained in the use of the cancer HRF, and the expertise gained would be applicable in the training of others in the use of the non-cancer HRF. Training would be facilitated by the availability of a number of suitable case-studies. Those published to date would be a sound basis for further development for this purpose (Seed et al., 2005). In addition, cases on a wider range of end-points need to be developed. It would be helpful if organizations with experience in non-cancer HRF analysis could develop courses and make the materials available to others with suitable expertise to help in training.

A database of generally accepted MOAs should be compiled and maintained, together with informative case-studies. Such a database would be of particular importance as experience continues to evolve in the development of MOAs and in determining whether the MOA for a compound is novel or has been described previously for other compounds.

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非発がん HRF を用いたデータとその解析は、他の人が到達した結論の根拠を判別できるように 明確かつ包括的な方法で報告されるべきである。

提示の具体的な形式は利用可能なデータの種類によって異なるが、可能であれば、フレームワ ークの主要な見出しを含む形式的な報告書を提供すべきである。発表は、到達した結論の透明性 を確保するために、文脈と思考プロセスの詳細を含めるべきである。一致表を含めることが強く 推奨される。これにより、透明性を高め専門家同士の協力が容易になる。

# フレームワークとその成果の利用

IPCS の非発がん HRF は、主に推定される MOA の key events の堅牢な一致解析に基づいてい る。以下のようないくつかの分野で調和に貢献するための基礎として、リスク評価と研究コミュ ニティの双方に価値があると考えられている。

- 動物における推定 MOA の妥当性とエビデンスの重み付けの性質及びヒトへの関連性
- ▶ エンドポイント間の MOA の統合
- 専門家の参加とレビューを十分に確保するための透明性のある基準

非発がん HRF の強みは、その柔軟性、透明性及びエンドポイント間で適用できることである。 これには、用量反応曲線の性質と形状の決定、個々の key events に対する生物学的閾値と位置の 特定及びそれらの影響が含まれる。さらに、各 key events に関与する動態学的及び薬力学的要因 を考慮することは、特定の集団、例えば、若年期、特定の疾患を有する人々、または特定の多型 を有する人々への関連性の有無に関して有益である。あるいは、フレームワークを適用すること で、そのようなグループ間の差異に関する定量的な情報を提供することもできる。また、ヒトへ の関連性の有無についての解析において、ある種の生物が、用量制限のために潜在的に関連する エンドポイントの評価には不適切である場合もある。

# 次のステップ

非発がん HRF を効果的に適用するためには、その適用及びその利用の解釈において個人を訓練 する必要がある。発がん性 HRF の利用においては経験が得られており、その得られた専門知識は、 非発がん HRF 使用における訓練にも適用できる。訓練は、適切な事例研究がより多く利用可能と なることによって促進されるであろう。これまでに発表された事例は、この目的のための適切な 基盤となる (Seed ら、2005 年)。さらに、より広範囲のエンドボイントに関する事例を調査する必 要がある。非発がん HRF 解析の経験を持つ組織が指針を作り、訓練を支援するために適切な専門 家がその教材を利用できるようにすることは有用であろう。

一般的に受け入れられている MOA のデータベースを、有益な事例研究とともに編集し、持続 させるべきである。このようなデータベースは、MOA の開発において事例は更新され続けられ、 ある化合物の MOA が新規か、あるいは他の化合物について以前に記載されていたものかを判断 The current non-cancer HRF, which arose out of the IPCS cancer HRF, is focused on noncancer end-points. However, there are marked similarities in the philosophy and strategy to evaluating cancer and non-cancer effects. It is strongly recommended that one of the next steps in harmonization of risk assessment of chemicals should be the preparation of a unified HRF that is applicable to all toxicological end-points, including cancer. The integration of framework approaches into the risk assessment process should be further elaborated, in which illustrative examples would be of value. Some guidance on problem formulation before embarking on an HRF analysis should be included in such a framework document, as should guidance on the use of the outputs of HRF analysis in risk assessment. For example, during application of the framework, a much deeper understanding of dose–response relationships is often developed, which should be taken forward into hazard characterization. As indicated above, knowledge of any dose transitions is invaluable in interpreting exposure data. Identification of key events in the MOA can provide insight into the sources and magnitude of interspecies and interindividual differences.

# REFERENCES

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Critical Reviews in Toxicology*, **36**:781–792.

Corley RA, Meek ME, Carney EW (2005) Mode of action: Oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—Renal and developmental effects of ethylene glycol. *Critical Reviews in Toxicology*, **35**:691–702.

Crofton KM, Zoeller RT (2005) Mode of action: Neurotoxicity induced by thyroid hormone disruption during development—Hearing loss resulting from exposure to PHAHs. *Critical Reviews in Toxicology*, **35**:757–769.

Foster PM (2005) Mode of action: Impaired fetal Leydig cell function—Effects on male reproductive development produced by certain phthalate esters. *Critical Reviews in Toxicology*, **35**:713–719.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Holson JF, Stump DG, Pearce LB, Watson RE, DeSesso JM (2005) Mode of action: Yolk sac poisoning and impeded histiotrophic nutrition—HBOC-related congenital malformations. *Critical Reviews in Toxicology*, **35**:739–745.

Intergovernmental Forum on Chemical Safety (1994) *The International Conference on Chemical Safety—Final report.* Geneva, World Health Organization (http://www.who.int/ifcs/documents/forums/forum1/en/FI-report\_en.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in dose/concentration-response assessment.

#### する上でも重要なものとなるだろう。

IPCS の発がん性 HRF から生まれた現在の非発がん HRF は、がん以外のエンドポイントに焦点を 当てている。しかし、発がんと非発がんの効果を評価するための哲学や戦略には顕著な類似点が ある。化学物質のリスク評価の調和における次のステップの一つとして、がんを含むすべての毒 性学的エンドポイントに適用可能な統一的な HRF を作成することが強く推奨される。リスク評価 過程へのフレームワーク適用は、さらに精緻化されるべきであり、その際には、例示が価値ある ものとなるであろう。このようなフレームワーク文書には、リスク評価における HRF 解析の使用 に関するガイダンスと同様に、HRF 解析に着手する前の問題の定式化に関するガイダンスが含ま れるべきである。また、フレームワークを適用することで、用量反応関係の理解が深まることが 多いが、これはハザード評価に反映されるべきである。上述のように、用量の変化に関する知識 は、ばく露データを解釈する上で非常に重要である。MOA における key events を特定することで、 種差及び個体差の原因や程度に関する知見を得ることができる。

# 参考文献

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W (2006) IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Critical Reviews in Toxicology*, **36**:781–792.

Corley RA, Meek ME, Carney EW (2005) Mode of action: Oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—Renal and developmental effects of ethylene glycol. *Critical Reviews in Toxicology*, **35**:691–702.

Crofton KM, Zoeller RT (2005) Mode of action: Neurotoxicity induced by thyroid hormone disruption during development—Hearing loss resulting from exposure to PHAHs. *Critical Reviews in Toxicology*, **35**:757–769.

Foster PM (2005) Mode of action: Impaired fetal Leydig cell function—Effects on male reproductive development produced by certain phthalate esters. *Critical Reviews in Toxicology*, **35**:713–719.

Hill AB (1965) The environment and disease: Association or causation? *Proceedings of the Royal Society of Medicine*, **58**:295–300.

Holson JF, Stump DG, Pearce LB, Watson RE, DeSesso JM (2005) Mode of action: Yolk sac poisoning and impeded histiotrophic nutrition—HBOC-related congenital malformations. *Critical Reviews in Toxicology*, **35**:739–745.

Intergovernmental Forum on Chemical Safety (1994) *The International Conference on Chemical Safety—Final report.* Geneva, World Health Organization (http://www.who.int/ ifcs/documents/forum1/en/FI-report\_en.pdf).

IPCS (2005) Chemical-specific adjustment factors for interspecies differences and human variability:

Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document No. 2; http://whqlibdoc.who.int/publications/2005/9241546786\_eng.pdf).

Kavlock R, Cummings A (2005a) Mode of action: Reduction of testosterone availability— Molinate-induced inhibition of spermatogenesis. *Critical Reviews in Toxicology*, **35**:685–690.

Kavlock R, Cummings A (2005b) Mode of action: Inhibition of androgen receptor function— Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, **35**:721–726.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

Seed J, Carney E, Corley R, Crofton K, DeSesso J, Foster P, Kavlock R, Kimmel G, Klaunig J, Meek E, Preston J, Slikker W, Tabacova S, Williams G (2005) Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Critical Reviews in Toxicology*, **35**:663–672.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity. *Toxicology and Applied Pharmacology*, **201**:203–225.

Slikker W Jr, Xu ZA, Levin ED, Slotkin TA (2005) Mode of action: Disruption of brain cell replication, second messenger, and neurotransmitter systems during development leading to cognitive dysfunction—Developmental neurotoxicity of nicotine. *Critical Reviews in Toxicology*, **35**:703–711.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Tabacova S (2005) Mode of action: Angiotensin-converting enzyme inhibition— Developmental effects associated with exposure to ACE inhibitors. *Critical Reviews in Toxicology*, **35**:747–755.

UNEP (2002) *Plan of implementation of the World Summit on Sustainable Development.* New York, NY, United Nations Environment Programme (http://www.un.org/esa/ sustdev/documents/WSSD\_POI\_PD/English/WSSD\_PlanImpl.pdf).

#### Harmonization Project Document No. 4

Guidance document for use of data in dose/concentration-response assessment.

Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document No. 2; http://whqlibdoc.who.int/publications/2005/9241546786\_eng.pdf).

Kavlock R, Cummings A (2005a) Mode of action: Reduction of testosterone availability—Molinateinduced inhibition of spermatogenesis. *Critical Reviews in Toxicology*, **35**:685–690.

Kavlock R, Cummings A (2005b) Mode of action: Inhibition of androgen receptor function— Vinclozolin-induced malformations in reproductive development. *Critical Reviews in Toxicology*, **35**:721–726.

Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, Longfellow DG, Pastoor T, Seed J, Patton DE (2003) A framework for human relevance analysis of information on carcinogenic modes of action. *Critical Reviews in Toxicology*, **33**:591–653.

Seed J, Carney E, Corley R, Crofton K, DeSesso J, Foster P, Kavlock R, Kimmel G, Klaunig J, Meek E, Preston J, Slikker W, Tabacova S, Williams G (2005) Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Critical Reviews in Toxicology*, **35**:663–672.

Slikker W Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity. *Toxicology and Applied Pharmacology*, **201**:203–225.

Slikker W Jr, Xu ZA, Levin ED, Slotkin TA (2005) Mode of action: Disruption of brain cell replication, second messenger, and neurotransmitter systems during development leading to cognitive dysfunction—Developmental neurotoxicity of nicotine. *Critical Reviews in Toxicology*, **35**:703–711.

Sonich-Mullin C, Fielder R, Wiltse J, Baetcke K, Dempsey J, Fenner-Crisp P, Grant D, Hartley M, Knaap A, Kroese D, Mangelsdorf I, Meek E, Rice J, Younes M (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, **34**:146–152.

Tabacova S (2005) Mode of action: Angiotensin-converting enzyme inhibition— Developmental effects associated with exposure to ACE inhibitors. *Critical Reviews in Toxicology*, **35**:747–755.

UNEP (2002) Plan of implementation of the World Summit on Sustainable Development. New York, NY, United Nations Environment Programme (http://www.un.org/esa/sustdev/documents/WSSD\_POI\_PD/English/WSSD\_PlanImpl.pdf).

United Nations (1992) Agenda 21: United Nations Conference on Environment and Development. New York, NY, United Nations Division for Sustainable Development (http://www.un.org/esa/sustdev/documents/agenda21/english/Agenda21.pdf).

Weed DL (2005) Weight of evidence: A review of concept and methods. *Risk Analysis*, **25**:1545–1557.

WHO (2006) *Strategic Approach to International Chemicals Management (SAICM)*. Geneva, World Health Organization (http://www.who.int/ipcs/features/iccm\_crp.pdf).

Wiltse J (2005) Mode of action: Inhibition of histone deacetylase, altering WNT-dependent gene expression, and regulation of beta-catenin—Developmental effects of valproic acid. *Critical Reviews in Toxicology*, **35**:727–738.

Zoeller RT, Crofton KM (2005) Mode of action: Developmental thyroid hormone insufficiency—Neurological abnormalities resulting from exposure to propylthiouracil. *Critical Reviews in Toxicology*, **35**:771–781.

Zoetis T, Walls I, eds (2003) Principles and practices for direct dosing of preweaning mammals in toxicity testing and research. A report of the ILSI Risk Science Institute Expert Working Group on Direct Dosing of Preweaning Mammals in Toxicity Testing. Washington, DC, ILSI Press.

#### IPCS Framework for Analysing the Relevance of a Non-Cancer Mode of Action for Humans

United Nations (1992) Agenda 21: United Nations Conference on Environment and Development. New York, NY, United Nations Division for Sustainable Development (http://www.un.org/esa/sustdev/documents/agenda21/english/Agenda21.pdf).

Weed DL (2005) Weight of evidence: A review of concept and methods. Risk Analysis, 25:1545-1557.

WHO (2006) *Strategic Approach to International Chemicals Management (SAICM)*. Geneva, World Health Organization (http://www.who.int/ipcs/features/iccm\_crp.pdf).

Wiltse J (2005) Mode of action: Inhibition of histone deacetylase, altering WNT-dependent gene expression, and regulation of beta-catenin—Developmental effects of valproic acid. *Critical Reviews in Toxicology*, **35**:727–738.

Zoeller RT, Crofton KM (2005) Mode of action: Developmental thyroid hormone insufficiency— Neurological abnormalities resulting from exposure to propylthiouracil. *Critical Reviews in Toxicology*, **35**:771–781.

Zoetis T, Walls I, eds (2003) Principles and practices for direct dosing of preweaning mammals in toxicity testing and research. A report of the ILSI Risk Science Institute Expert Working Group on Direct Dosing of Preweaning Mammals in Toxicity Testing. Washington, DC, ILSI Press.

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3. (4) 残留農薬のリスク評価にあたっての毒性試験結果の解釈に係る知見の収集

① 各種毒性試験における毒性を解釈する上で共通する考え方(適応性変化等)

番号	著者名	文献名	雑誌:巻:ページ	参照箇所の該当ページ
1	World Health Organization (WHO)	Guidance Document for WHO Monographers and	2015	Part3: 1.1 (34), 1.2 (34), 1.3 (34-36), 1.4 (36),
		Reviewers		1.10 (42)
2	Lewis RW, Billington R, Debryune	Recognition of Adverse and Nonadverse Effects in	Toxicol Pathol. 2002; 30(1): 66-74	Abstract, Figure2
	E, Gamer A, Lang B, Carpanini F	Toxicity Studies		
3	Williams GM, Iatropoulos MJ	Alteration of Liver Cell Function and Proliferation:	Toxicol Pathol. 2002; 30(1): 41-53	Abstract
		Differentiation Between Adaptation and Toxicity		
4	Perry R, Farris G, Bienvenu JG,	Society of Toxicologic Pathology Position Paper on	Toxicol Pathol. 2013; 41(8): 1159-1169	Abstract, 1164
	Dean C Jr, Foley G, Mahrt C, Short	Best Practices on Recovery Studies: The Role of		
	В	The Anatomic Pathologist		
5	Hall AP, Elcombe CR, Foster JR,	Liver Hypertrophy: A Review of Adaptive (Adverse	Toxicol Pathol. 2012; 40(7):971-994	Abstract
	Harada T, Kaufmann W, Knippel A,	and Non-adverse) ChangesConclusions from the		
	Küttler K, et al	3rd International ESTP Expert Workshop		

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# 概要

ヒトの健康に対する化学物質の有害影響を判断する際に重要となる毒性試験から得られる量的成果 に対して、統計学的あるいはそれ以外の観点も含めて意義のある有害影響を起こさない最高用量/濃度 を決定することが重要である。そのような毒性試験からの所見に対する首尾一貫した解釈が重要であ り、それはこれらのデータがヒトの健康リスク評価、特に「安全な」ばく露レベルを決定する重要な意 味を有しているためである。したがって、毒性試験において観察された影響が毒性か否かを判断する ことはヒトの健康リスク評価において最も重要かつ、論争上の課題の一つである。観察された影響が一過 性、可逆性あるいは軽度な場合、恒常性を損なわない限り、毒性ではないと判断する。

肝臓の肥大の毒性学的解釈については,病理組織学的な障害性変化(壊死,炎症,線維化等)または 生化学的に肝障害を示す変化(AST, ALT, ALP, ビリルビンの高値等)を伴わない場合は適応性変化 と考える。肝臓の肥大が適応反応か毒性かを判断する情報が不十分な場合,あるいは追加的な負荷に 対応する機能障害をきたしうる場合(例:累積的影響)や他の化学物質の影響に対する感受性の増加を もたらす可能性がある場合は,その影響は有害影響と仮定することをデフォルト,すなわち標準的な 方針とする。設定された参照値(例:ADI)は薬物代謝酵素誘導のLOELを超えてはならない。

統計学的に有意差があっても生物学的に意義がない影響がある。一方で,軽微ではあるが生物学的 に意義のある変化あるいは低用量での毒性影響は検出力の低い試験法では検出できない可能性がある。 "統計的に有意"とされる変化は、その影響の重要性または生物学的関連性と必ずしも直接結びつくわ けではない。軽微,軽度,中等度,重度に分類された組織学的所見などの実験データを考察する場合, 程度を考慮した統計解析を行うことで,総発生数だけでなく,程度についても用量反応性の有無を調 べることが出来る。統計学的に有意な所見は、投与との関連を示唆するが、それをそのまま生物学的な 毒性影響とか、毒性学的に意義ある影響とは結論付けられない。実際,正常な生物学的変動の範囲内で あっても統計学的には有意差があると判定される可能性がある(例:再生反応の兆候がない赤血球数 の減少)。これに対して、生物学的あるいは毒性学的に検討することで統計学的な結果が覆ることがあ る(例:1群4匹のみで実施するイヌ試験の場合)。しかし、統計学的に有意でない結果に対して生物 学的意義を主張する場合には常に注意が必要である。統計学的な有意差はないが用量との関連性のあ る軽度な病理組織所見は、投与と関連があるか否か、毒性か否かを判断するのが難しい場合がある(例: 肝細胞内の空胞の軽度な増加、脾臓における髄外造血の軽度な増加は毒性学的に重要とはみなさなく てよい)。全ての毒性試験において LOAEL で認められる軽度な所見は、ケースバイケースで包括的な 議論が必要になる。

投与期間中に消失する影響は一過性の影響として一般的に認められ,次のようなことに起因しうる。 1) 投与に対する非特異的反応(例:投与開始直後の摂餌忌避),2) 強制経口投与によるストレスが原 因の体重減少,3)年齢や体重増加に伴う摂餌量の減少。

投与期間中に消失する一過性の影響と投与終了後に回復する影響とを区別する必要がある。前者に ついては、急性ばく露でみられ、投与期間中に消失した一過性の影響が有害影響である可能性がある ため急性参照用量(ARfD)の設定に重要であり、後者の投与期間終了後に回復する影響については、 少なくとも継続的ばく露の場合では、投与期間中に現れる影響が有害影響である可能性があるため、 慢性参照用量を設定するためにそれぞれ重要である。

体重に対する一過性の影響(例:餌の嗜好性の変化による摂餌量の低下,体重減少が起こるが実験動物 の適応とともに影響が消失する場合)は毒性とは考えない。しかし,この結論は動物の成長の抑制が餌 の嗜好性低下によって直接もたらされた影響であることを証明することが可能な場合にのみ導き出さ れるということが重要である。

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著者名	Lewis RW, et al
文献名	Recognition of Adverse and Nonadverse Effects in Toxicity Studies
巻, ページ, 発行年	Toxicol Pathol.2002; 30(1): 66-74
分類 (①~④)	
参照箇所の該当ページ	Abstract, Figure2

毒性試験から得られる最も重要な定量的アウトプットの一つは、ヒトの健康リスク評価に妥当であ る(relevant)と考えられる投与に関連した影響を引き起こさない最も高いばく露レベル(用量または 濃度)の特定である。しかし、規制当局及び他の科学文献ならびに現状の実務をレビューした結果、下 記のような頻繁に使用される用語の定義や適用に一貫性がないことが判明した。さらに、有害影響と 有害でない影響の評価や鑑別を含め、毒性試験結果の解釈の指針となるような首尾一貫した基準は認 められなかった。

そこで、まずは毒性試験の結果を記述するために頻繁に使用される主要用語の定義を提案する。 <u>無作用量(NOEL)</u>:適切な対照群と比較して、ばく露群において(有害・有害でない)影響が観察され ない最高のばく露量。

<u>無毒性量(NOAEL)</u>:適切な対照群と比較して,ばく露群において有害影響の頻度・重症度に統計学的 あるいは生物学的に有意な増加が認められない最高のばく露量。このばく露量において何らかの影響 が生じる可能性はあるが,それらは有害影響や有害影響の前駆状態(precursor)とは考えない。

<u>最小毒性量(LOAEL)</u>:適切な対照群と比較して,ばく露群において有害影響の頻度・重症度に統計学的あるいは生物学的に有意な増加が認められる最低のばく露量。

<u>有害影響</u>:単独あるいは複合的に生体全体のパフォーマンスに有害影響を及ぼす,あるいは環境変化 への生体能力を低下させる,(刺激に対する)生化学的,形態学的あるいは生理学的変化。反対に,有 害でない影響は,生体の全身状態,成長,発達,寿命に影響を与えるような生化学的,形態学的あるい は生理学的変化を引き起こさない生物学的影響と定義できる。

<u>生物学的・毒性学的に意義のある影響:</u>生体システムの健康に(正または負の)重要あるいは顕著な影響を与えると考えられる(刺激に対する)反応。この概念は、(生体の全身状態に対しては意義のある場合とない場合がある)統計学的に有意な影響・変化とは区別されるべきである。

次に,毒性学者が一貫性のある試験結果の解釈を行なうことを支援する首尾一貫したフレームワーク(図1参照)を概説する。このフレームワークのプロセスには以下の2つの主要なステップがあり, それぞれにおける一貫した判断を行なうための基準が記載されている。

- 対照群の値との差が投与の影響によるものか、偶発性のものかを判断する。
   次の場合は投与による影響の可能性は低い:明らかな用量反応関係性がみられない。差の原因が外れ値にある。測定精度が悪い。正常な生物学的変動の範囲内である。生物学的妥当性に欠ける。
- 2) 投与による影響であると判断された変化についてはさらに評価し、有害影響であるかどうかを判断 する。

次の場合は有害影響の可能性は低い:生体あるいは影響を受けた臓器・組織の機能障害がない。適応反応である。一過性のものである。重篤度が低い。影響が単独発現している(その影響に通常関

連する他のパラメータの変化を伴わない)。有害影響に進行する前駆状態ではない。他の有害影響 の二次的影響である。影響が投与方法に起因する。

複雑な試験結果を解釈する際には、これらの基準を組み合わせて総合的な判断を下す証拠の重み付け アプローチが最適な方法であると考えられる。そして、このようなスキームを使用することで、ハザー ドとリスク評価の基礎となる試験結果の解釈について一貫性を向上させることができると考えられる。

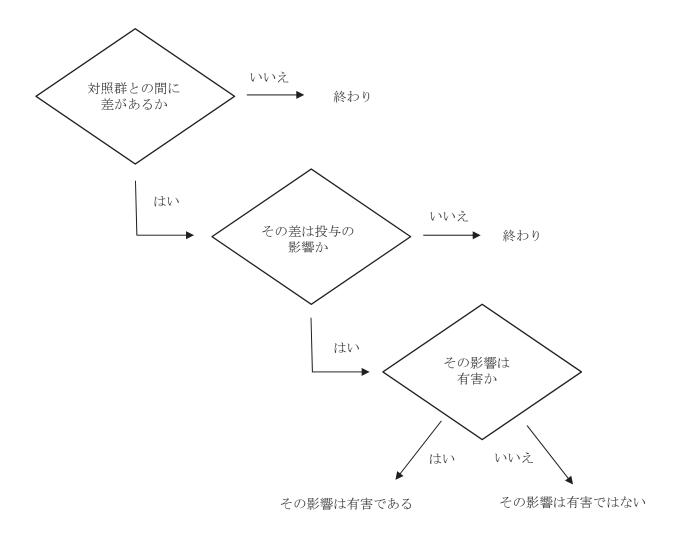


図1-毒性試験結果の解釈のためのフレームワーク

番号	1-03	
著者名	Williams GM, Iatropoulos MJ	
文献名	Alteration of Liver Cell Function and Proliferation: Differentiation Between	
又瞅石	Adaptation and Toxicity	
巻, ページ, 発行年	Toxicol Pathol. 2002; 30(1): 41-53	
分類 (①~④)		
参照箇所の該当ページ	Abstract	

実験動物が生物学的な影響の出るレベルの化学物質[内因性あるいは外因性(合成あるいは天然由 来)]にばく露されると、様々な組織単位で化学的摂動(変化またはかく乱)に対処するための反応が 誘発される。ある種の化学物質では、初期反応は適応反応を構成し恒常性を維持するが、どの組織レベ ルにおいてもそれが崩れると毒性(有害)影響が生じる。

実験動物やヒトの肝臓は,他の臓器と同様に,細胞の増殖に続く分化を特徴とするプログラムされ た段階的な成長,発達を経るが,分化した細胞は恒常性を維持するための機能及び生体のニーズに応 えるための特殊化した機能を持つようになる。成熟後の肝臓は,肝細胞(中間細胞に分類され,複製頻 度は低いもののシグナルに応答して複製することができる),複製細胞である幹細胞,内皮細胞,クッ パー細胞,星細胞(伊東細胞,周皮細胞),胆管上皮及び顆粒リンパ球(ピット細胞)で構成される。 これらの様々な細胞において,化学物質による分子レベルの定量可能な変化が細胞小器官レベルでの 変化の根底にあり,細胞小器官レベルでの変化は細胞レベルでの変化を誘導し,最終的には全身性の 変化につながる。この定量可能な変化には,細胞レベルで生じる複製,壊死・アポトーシス,分化のよ うに計数的(2値的,すなわち all or none)なものがある一方,細胞内あるいは細胞外レベルで生じる 酵素誘導,細胞小器官の肥大,細胞外基質の生成のように,段階的あるいは連続的(非2値的 nonbinary) なものもある。そして,あらゆるこのような変化によって反応や影響は構成される。肝臓におけるそれ ぞれの種類の細胞は,その局在と機能に応じて刺激に反応するが,一般的に再生を行なっている細胞 は,ほぼ静止している中間細胞と比較して化学的損傷に対しより脆弱である。

肝臓の適応反応では通常,化学物質が(多くの場合受容体を介して)細胞の制御経路に作用することで、遺伝子発現が変化し、最終的にメタボローム(metabolome)が変化する。この反応は、様々な細胞及び細胞外機能の調節を通じて、恒常性を維持しようとするためのものである。また適応反応は、あらゆる組織レベルで化学物質によるストレスへの応答能力を高め、可逆的であり、生存能力を維持するという点で有益である。毒性影響が生じる閾値を下回るばく露でのこのような適応反応は、ホルミシス(hormesis)とも呼ばれている。一方、肝臓における毒性(有害)影響では、化学物質が細胞の高分子と反応することで、恒常性の崩壊が起こる。このような影響は、ストレスへの応答能力を低下させ、非可逆的となり得、また生存能力を低下させ得る。通常は適応反応を誘導するレベルのばく露量でも、ばく露期間の長期化、もしくは高用量のばく露により閾値を上回った場合は、用量の変化につれて作用機序が変化し毒性影響が現れうる。従って、影響を及ぼす用量の変化とともに作用機序も変化していることになる。肝臓では様々な適応反応及び毒性影響が確認されており、前者を誘導するものとしてフェノバルビタールやシプロフィブラートが、後者を誘導するものとして*p*-ジクロロベンゼンや2-アセチルアミノフルオレンが挙げられる。肝臓への化学物質の影響は、例外はあるものの概して実験

動物とヒトで類似しているため,適応反応と毒性影響の鑑別とモニタリングは安全性評価上不可欠で ある。

番号	1-04	
著者名	Perry R, et al	
文献名	Society of Toxicologic Pathology Position Paper on Best Practices on Recovery	
	Studies: The Role of the Anatomic Pathologist	
巻, ページ, 発行年	Toxicol Pathol. 2013; 41(8): 1159-1169	
分類 (①~④)		
参照箇所の該当ページ	Abstract, 1164	

病理学者は、毒性試験において認められた病理組織学的所見の回復性を予測するためには、組織の 種類、細胞外基質の関与及び病変形成過程の性質ならびに重症度を考慮すべきである。

組織は、その構成細胞の増殖能に応じて不安定組織、安定組織及び永久組織のいずれかに分類され る。不安定組織は高い回復力を有し、細胞外基質(細胞を囲む間質基質と基底膜で構成されるネットワ ークであり、水分とミネラルを隔離する機能を持ち、細胞が付着する足場を提供し、さらに増殖因子の 貯蔵庫としての役割を果たす)が無傷である限り、同種の細胞の複製及び再増殖による再生を行なう。 安定組織は、不安定組織と同様に反応及び再生ができるものの、その程度は低い。また、正常な機能や 修復のためには、細胞外基質が正常・無傷であることが必要である。細胞外基質の間質フレームワーク が損傷した場合、再生した実質細胞が臓器内に不規則に散乱し、効果的な修復ができず、再生がうまく いかない。永久組織が致命的な傷害を受けた場合、同種の細胞による置換は行なわれず、通常は結合組 織で修復される。これらの組織の増殖(再生)能とその例を以下にまとめた。

- 1. 不安定組織(絶えず細胞分裂している組織)
  - a. 細胞は生涯にわたって増殖する
  - b. 傷害後, 容易に再生する
  - c. 幹細胞を含んでいる
  - d. 例:骨髄,造血組織,表面上皮(皮膚,消化管上皮)
- 2. 安定組織(静止状態の組織)
  - a. 低レベルの複製
  - b. 元の組織を再生・再構築することができる
  - c. 刺激に反応して急速に分裂することができる
  - d. 例: 肝臓, 腎臓, 膵臓の実質細胞; 線維芽細胞, 平滑筋, 内皮, リンパ球, 骨細胞, 筋衛 星細胞, 軟骨細胞などの間葉系細胞
- 3. 永久組織(細胞分裂しない組織)
  - a. 出生後,通常は細胞分裂しない
  - b. 再生できない
  - c. 例:神経細胞,心筋

再生過程における細胞外基質と細胞の関連性や相互作用は極めて複雑であり,例外もあるものの,

回復性の予測においては一般的に以下のことが適用できる。

- 1. 細胞外基質が影響を受けず、組織傷害が不安定性組織あるいは安定性組織に生じた場合、回 復の見込みがある。
- 組織傷害によって細胞外基質が損傷あるいは損失した場合は、その損傷や損失は回復せず、 無秩序で機能を喪失する再生、線維化あるいは鉱質沈着が生じる可能性が高い。

番号	1-05
著者名	Hall AP, et al
文献名	Liver Hypertrophy: A Review of Adaptive (Adverse and Non-adverse) Changes
又瞅石	Conclusions from the 3rd International ESTP Expert Workshop
巻, ページ, 発行年	Toxicol Pathol. 2012; 40(7): 971-94
分類 (①~④)	0
参照箇所の該当ページ	Abstract

概要

げっ歯類を用いた亜慢性(亜急性)及び慢性の毒性試験において薬物や化学物質によって引き起こ される肝臓の腫大は、同程度の用量での生涯試験における肝毒性や発がん性、あるいはヒトとの関連 性についての認識の点で長年にわたり毒性学の専門家を悩ませてきた。

肝肥大とは、病理組織学者にとっては明確に認識され組織学的に十分定義されている一方、毒性学 者にとっては器官重量の増加、肝細胞の平均サイズの増加(肝細胞の肥大)、そして(時に機能的な「亢 進性」肥大とも言われる)肝酵素の誘導さえも含まれる意味の言葉として捉えられている。前臨床毒性 試験において、実験動物に長期間(終身)肝酵素誘導剤をばく露した結果、肝重量の増加、肝細胞の肥 大、細胞増殖、そして長期試験における高頻度の肝発がんといった特徴を示す毒性学的変化が生じる ことが報告されている。近年の進歩により、多くの外因性化学物質は、CAR、PXR、PPARa といった 核内ホルモン受容体の活性化を介した共通の作用機序により、これらの変化が誘導される可能性があ ることが明らかになってきた。これらについて改変された核内ホルモン受容体を発現する遺伝子組み 換えマウスを作製し、他の研究とも複合的に検討したところ、これらの作用の多くがげっ歯類に特異 的であることが明らかになった。これらのデータは、ヒトについて得られた信頼できる利用可能な疫 学的及び経験的なエビデンスと一致しており、これらの変化はヒトではほとんど起こらないという科 学的見解を裏付けるものである。そのため ESTP は、肝細胞肥大に関連して何が有害と考えられるかを 毒性病理学者がより明確に定義するために、国際的な専門家パネルを招集し、エビデンスについて議 論した。このワークショップの結果、肝毒性を示す組織学的または臨床病理学的変化を伴わない肝細 胞肥大の結果としての肝肥大は適応性であり、有害反応ではないと結論づけられた。

肝重量の増加によってもたらされる組織学的変化を評価する際,その変化が有害か否かを判断する ために多くの段階を経て検討する必要がある。まず肝細胞の壊死,線維化,炎症や脂肪性の空胞変性, 胆管/卵形細胞の増殖,変性,線維化,胆汁うっ滞,他の肝臓構成細胞の壊死や変性といった構造的変 性や壊死性変化の組織学的証拠があるかを検討する。組織学的変化がない場合,ALT の少なくとも 2 倍から 3 倍の増加や他の肝胆管傷害の指標(ALP,AST,γGT,GLDH等)の生物学的に有意な変動。 さらに別の肝臓の異常を示す臨床検査マーカー(アルブミン,ビリルビン,胆汁酸,凝固因子,コレス テロール,過酸化脂質等)の生物学的に有意な変動のうち,少なくとも 2 つの肝臓の検査項目が用量 依存的であり生物学的に有意かつ継続的に変動しているとして特徴付けられる肝細胞傷害の臨床病理 学的証拠があるかを検討する。以上述べた有害作用の基準に達する変化が認められない場合,臓器重 量の増加や肝細胞肥大が薬物代謝酵素誘導に関連した適応反応であり,ヒトには毒性学的意義がない と判断しうる段階となる。その発現メカニズムにAhR\*や既知の毒性メカニズムが関与している場合は 有害作用と判断され, CAR, PXR, PPARa の活性化\*\*あるいは未知のメカニズムであってもその他の 毒性メカニズムを示す証拠がない場合,有害作用とはみなされないと判断する。

\*げっ歯動物に遺伝子毒性がないダイオキシンを与えると、AhRの活性化を通じた肝肥大や肝細胞の変 異増殖巣,及び肝発がんが認められる。AhR介在性の発がんのヒトへの外挿性は否定できない。

\*\*CAR, PXR, PPARa の活性化はげっ歯動物の肝細胞を増殖に向かわせる。ヒトでこれら核内受容体の活性化が起こったとしてもヒトの肝細胞の増殖性は惹起され難い。

海外のリスク評価機関における評価結果等に関する調査-

3.(4)残留農薬のリスク評価にあたっての毒性試験結果の解釈に係る知見の収集

② 毒性判断に用いる統計学的手法、背景データの利用

番号	著者名	文献名	雑誌:巻:ページ	参照箇所の該当ページ
1	World Health Organization (WHO)	Guidance Document for WHO Monographers and	2015	Part 2: 6.2.6 (16), Part 3: 1.5 (36,37), 1.6 (37,38)
		Reviewers		
7	OECD	Guidance Document 116 on The Conduct and	2012	4.4 (115,116)
		Design of Chronic Toxicity and Carcinogenicity		
		Studies, Supporting Test Guidelines 451,452 and		
		4532nd Edition. ENV/JM/MONO(2011)47		
8	Mann PC, Vahle J, Keenan CM,	International Harmonization of Toxicologic	Toxicol Pathol. 2012; 40(4 Suppl):7S-13S	9S-11S, Table 1
	Baker JF, Bradley AE, Goodman	Pathology Nomenclature: An Overview and Review		
	DG, Harada T, Herbert R, et al	of Basic Principles		
9	Elmore SA, Peddada SD	Points to Consider on the Statistical Analysis of	Toxicol Pathol. 2009; 37(5): 672-676	Abstract
		Rodent Cancer Bioassay Data When Incorporating		
		Historical Control Data		
19	OECD	Guidance Notes for Analysis and Evaluation of	2002	27,28,36, Appendix V
		Repeat-Dose Toxicity Studies		

番号	2-01	
著者名	World Health Organization (WHO)	
文献名	Guidance Document for WHO Monographers and Reviewers	
巻, ページ, 発行年	2015	
分類(①~④) ②		
参照箇所の該当ページ Part 2: 6.2.6 (16), Part 3: 1.5 (36, 37), 1.6 (37, 38)		

背景データ(Historical control data)は、腫瘍や発生異常の評価に利用することが多いが、それらの 結果が正常な生物学的範囲内に収まることを示すことが目的である。背景データは、比較対象とする 試験と同じ系統の動物を対象とし、ほぼ同時期に、同じ試験施設で実施した試験から得られたもので なければならない(理想的には、試験開始2年前から試験終了後2年までの間)。これらの基準に合わ ない場合は、その旨を本文中に記載する。可能なら、各試験の結果を別々に見ることが出来るような形 式で提供する必要がある。最低限、試験数、平均値、最小値~最大値の範囲を記載する。当該試験と背 景データの間で、解釈上または検査上の方法に違いがないことも確認する必要がある。提出された背 景データがこれらの基準を満たさないなら、その不足部分を補うよう、対応を依頼する必要がある。

長期試験では、当該試験の対照動物における非腫瘍性あるいは腫瘍性病変の発生率が正常より低い 場合、投与群におけるそれら病変の発生率が有意な高値を示すことがある。逆に、正常より高い場合は 投与群における毒性あるいは発がん性を隠してしまう。背景データは、同じ試験施設の同じ条件下で 飼育された同じ種及び系統の動物を使って行われた試験から得られたものでなければならない。背景 データには次の情報が示される必要がある。

1)動物種,系統の同定,生産者名,繁殖地の識別(生産者が複数の地理的場所にある場合)

2) 試験施設の名称及び試験実施日

- 3)動物の一般飼育条件(飼料の種類及び銘柄,可能なら消費量)
- 4) 試験開始時及び計画解剖時または死亡時の対照動物の大体の年齢(日数)及び体重
- 5) 試験中または試験終了時に観察された対照群動物の死亡パターン及び他の関連する所見(例:病気, 感染)
- 6) 試験からの病理学的データの収集及び解釈を担当した試験施設名及び検査担当科学者の氏名
- 7) 発生率データを作成するために合計された可能性のある腫瘍の種類の記載

背景データは,試験ごとに絶対値や相対値など評価に役立つ値が示されるべきである。集計値には, 値の範囲,平均値,中央値及び標準偏差を含むべきである。げっ歯類の稀な腫瘍の評価には文献データ が適用できる場合もある。肝臓,下垂体及び副腎の腫瘍のように通常みられる腫瘍の場合は,発生率の 範囲が広いために,投与群も含めた腫瘍の発生率が背景データの範囲内に収まることが多く,範囲だ けでは評価が困難であり平均や分布が有用である。被験物質と類似の構造または作用機序を示す他の 化学物質に関する組織学的データも有用である。もし,背景データの範囲内だが腫瘍発生数の軽度な 増加が被験物質の標的臓器でみられた場合,前腫瘍性病変やその標的臓器への連続的な障害の有無に ついて注意深く評価する必要がある。 (胎児の異常)対照群のデータは試験ごとに必要である。これに対し、複数の試験から得られた陰性 (溶媒)対照群のデータで構成される背景データは、日常的に必要とされるものではないが、試験の所 見を解釈するために有用で適切と考えられている。投与群のデータと比較する場合、背景データより も当該試験の対照群のデータが優先されるべきである。胎児の異常に関する背景データも長期試験の 背景データで上述した基準1)から6)に加え、7)奇形や変異の評価基準が示される必要がある。

番号	2-07	
著者名	OECD	
Guidance Document 116 on the Conduct and Design of Chronic Toxicity		
文献名	Carcinogenicity Studies, Supporting Test Guidelines 451,452 and 4532nd	
	Edition. ENV/JM/MONO(2011)47	
巻, ページ, 発行年	2012	
分類 (①~④)	2	
参照箇所の該当ページ	4.4 (115, 116)	

## OECD TGs における統計学的手法の記載

- ・OECD TGs 451, 452, 453 の共通項(275)
  - ・数値結果は適切で一般的に許容される統計的方法で評価されるべき
  - ・統計的手法及び分析すべきデータについては、試験計画の段階で選択されるべき
  - ・必要に応じて、生存率で調整する規定を設けるべき
- ・TG451及び453の共通項(276)
  - ・実験計画と目的を考慮し、最も適切な統計的方法は、試験開始前に確立されるべき
  - ・生存率の補正,生存期間に対する累積腫瘍リスクの分析,腫瘍発生時期の分析,及び1つまたは 複数の群が途中で終了した場合の解析を統計手法に含めるか考慮する
  - 適切な統計分析に関するガイダンス及び国際的に認められた統計手法の主な参照事項は、OECDの試験ガイドラインの公開サイトで入手可能な慢性毒性及び発がん性試験の設計及び実施に関するガイダンス文書、ならびに慢性毒性及び発がん性試験の分析及び評価に関するガイダンス文書No.35に記載されている
- OECD TGs 452 (276)
  - ・生存率の補正及び1つまたは複数の群が途中で終了した場合の解析を統計手法に含めるかを考慮 する

### EPA 発がん性物質リスクアセスメントのガイドラインにおける統計学的手法の記載

- ・長期試験の統計解析は、腫瘍の種類ごとに個別に行うべき
- ・同じ細胞由来の良性病変と悪性病変の発生率は、それぞれ別々に検討された後、科学的に説明可能な場合には組み合わせて検討される(McConnell ら、1986年)
- ・傾向検定及び pairwise 比較検定は、腫瘍発生率の明らかな増加が、被験物質投与に関連した影響というよりはむしろ偶発性であることを証明するために推奨される検定である
- ・Cochran-Armitage 検定(Snedecor & Cochran, 1967 年)のような傾向検定は、すべての投与群の結果 が投与量の増加に伴って増加するかどうかを問うものである
- ・Fisher exact 検定(Fisher, 1950年)のような pairwise 比較検定では、ある1つの投与群の発生率が対 照群よりも増加しているかどうかを問うものである
- ・統計的に有意な差とは、発生率の増加が偶発性であることを示すpが 0.05 未満のものである
- どちらの種類の検定でも、結果が偶然によるものであるという仮説を棄却するには、有意性があれば

+分である。統計的に有意な反応は、生物学的に有意である場合もあれば、そうでない場合もあり、そ の逆もある。有意水準の選択は、偽陽性と偽陰性のリスクの間のトレードオフに基づいている。有意水 準が5%以上または以下(最も一般的な有意水準)の場合、他の科学的情報を確認できるかどうかを検 討する。評価が単純な5%レベルから逸脱する場合は、リスク判定の際に強調する必要がある。両側検 定または片側検定を使用することは可能であるが、いずれの場合も根拠を示すことか必要である。

番号	2-08	
著者名	Mann PC, et al	
	International Harmonization of Toxicologic Pathology nomenclature: An Overview	
文献名	and Review of Basic Principles	
巻, ページ, 発行年	Toxicol Pathol. 2012; 40(4 Suppl): 7S-13S	
分類 (①~④)	2	
参照箇所の該当ページ	9S-11S, Table 1	

## グレーディングシステム/程度評価

- ・病変の程度は、変化の度合い(その所見に付随する変化がどれくらい存在するか)、分布(局所~び まん性)、及び実際の重症度の組み合わせを反映して選択されるべきである。
- ・グレーディングシステムは、研究施設により様々であり、病変の分布、病期、及び病変の範囲をどの ように組み入れるかで異なる場合があるため、普遍的なグレーディングシステムを構築することは 困難である。
- ・最も一般的に使用されているグレーディングシステムは、4~5 段階の明確な半定量的評価法(表) である。
- ・被験物質の影響及び用量相関性の判断は,統計学的分析よりも担当病理医が実施することが適切で ある。
- ・同一化合物に関する試験内又は試験間での程度付の整合性は、病理学的評価の品質保持のため重要であり、試験内での程度付の不整合性又は「ドリフト(drift)」(診断基準が経時的に変化する傾向)は、被験物質の影響を検出する感度が損なわれるか、または実際には被験物質の影響がないにもかかわらず、それがある(偽陽性)ことにつながる可能性がある。
- ・投与に関連した影響や用量反応性を伝えるために、毒性病理医は試験内のスコアの基準を変更したり、「分割(split)」したりすることがあるが、この場合は病理報告書の方法のセクションでそのプロ セスと基準を明記すべきである。
- ・Peer review において, reviewer は, 試験内でのグレーディングの一貫性を評価すべきである。

## 程度のない病変

- ・病変によっては、付加的な情報が得られないことから、程度付けを行わないことがある。
- ・これらの病変は通常,重症度の評価ではなく「存在する (P)」として記録される。
- ・例としては、腫瘍、嚢胞、自己融解、先天性異常などが挙げられる。
- ・一方で、病理データ入力システムの中には、すべての病変について等級を入力することを要求する ものもある。

表1 4 点採点システムの説明

数値スコア	説明	定義		
0	正常の	試験の条件下で,関係する動物の週齢,性別,系統を考慮して,正常であ		
	範囲内	ると考えられる組織。他の状況下では正常からの逸脱と考えられる変化が		
		存在する場合がある。		
1	極軽度	正常範囲内であると考えられるものからわずかに超えた変化。		
2	軽度	一般的に,病変は容易に同定できるが,限局的である。		
3	中等度	病変は顕著であるが、さらに重症する可能性がある。		
4	高度	変化の程度は可能な限り最大である(臓器の大部分を占めている)。		

番号	2-09	
著者名	Elmore SA, Peddada SD	
文献名	Points to Consider on the Statistical Analysis of Rodent Cancer Bioassay Data	
又顺泊	When Incorporating Historical Control Data	
巻, ページ, 発行年	Toxicol Pathol. 2009; 37(5): 672-676	
分類 (①~④)	2	
参照箇所の該当ページ	Abstract	

- ・被験物質の投与の影響を判断する最も関連性の高い対照データは、同時対照群のデータではあるが、 特定の状況においては背景データ Historical control data (HCD)の評価も有用である。これには、自 然発生腫瘍、希少腫瘍、一般的な腫瘍、発生数が激しく変動する腫瘍、同時対照群と比較して発生数 がわずかに増加している腫瘍、または対照動物における腫瘍発生数の予期せぬ増減などの評価が含 まれる。
- ・HCD は化合物の潜在的な発がん性を評価する際に,「根拠の重み」付けを補足できる多くの情報源の 1つとして使用されるべきである。考慮すべき他の情報としては,類似の細胞由来の他の病変,体重, 生存率,腫瘍の発生時期,その腫瘍が両種または雌雄に発生している場合,用量相関性がある場合, または両側性臓器の両側に発生している場合である。
- ・腫瘍発生数を HCD と比較する方法は 2 つあり、一方はデータの探索的(非公式)分析(exploratory analysis)、他方はより正式な統計的アプローチ(more formal statistical approach)分析である。
- ・箱ひげ図は,HCD の分布に関して現在のデータを説明する探索的なツールとして用いる。平均値, 標準偏差,範囲などの標準的な方法ではわからない,潜在的な異常値を特定することができる。
- ・ここで述べられている HCD の統計解析に関する様々なオプションは、必ずしも標準的な手法を示す ものではなく、これら以外にも種々の統計手法が存在している。

番号	2-19	
著者名	OECD	
文献名	Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity	
<b>又</b> 瞅石	Studies	
巻, ページ, 発行年	2002	
分類 (①~④) ②		
参照箇所の該当ページ	27, 28, 36, Appendix V	

### 主要試験項目の分析と評価

試験項目の分析と評価において,観察された被験物質の変化のすべてが必ずしも毒性作用ではない ことを念頭に置くべきである。肝酵素誘導による肝肥大のような適応反応や感染症の治療に使用され た抗生物質投与による白血球減少のような薬理作用などが例として挙げられる。

パラメータの中には加齢に伴って、計時的に値が変動するものがあるため、週齢の一致した対照群 と比較すべきである。背景データを用いる場合でも背景データとの統計学的な比較は適切ではない。 老齢げっ歯類に観察される慢性進行性糸球体腎炎やアミロイドーシスなどのような加齢性病変につい ては、被験物質投与に影響を受ける可能性があるとされている。

背景データは、当該試験の対照群に異常がないかの確認にも有用である。対照群の背景データから の逸脱がみられる場合には、報告書においてその解析結果に対して何らかの考察が必要である。背景 データとの間に一貫して差が認められる場合には、その動物の由来を含めて検討すべきである。

背景データを利用する場合には、以下の条件が必要である。

- ・試験から得られたデータは同じ試験実施場所で検査されたものであること。
- ・当該試験の動物は同じブリーダーからの同じ系統、年齢及び性別であること。
- ・当該試験の前後2~3年以内に実施されたものであること。
- ・試験条件(絶食・非絶食の条件,試験パラメータの想定方法,病理組織学的な判断基準,最終と殺 時の年齢)なども明らかにする。

溶媒を用いた場合には、溶媒対照群を投与しない対照群も設定することで溶媒の影響の有無を知る ことができる。また、動物に対する取扱い方法の違いによっても検査値に影響する場合がある(例え ば、マウスにおいて保定による血清ALT上昇が報告されており、対照群も同様に処置する必要がある)

基本的な検査項目(体重増加,食物消費量,変換効率など)は多くの場合,低用量で影響を受ける最 も重要なエンドポイントである。評価者は,さらに,臓器重量,病理組織学的検査結果,血液生化学的 検査値や神経毒性に関する項目も含め,さらに特定の生物学的または毒物学的影響に対する用量反応 関係を慎重に考慮して評価に重要なエンドポイントを判断すべきである。

### 統計解析の方法

毒性試験の目的は生物学的に重要な反応を示すことであることを念頭に置く必要があることから統 計結果を評価に用いる場合には以下の点に注意する。

- (1) 統計は質の悪い検査データを良くすることはできない。
- (2) 統計的有意性は生物学的な意義を意味するものではない。

- (3) 生物学的に意義のある影響であっても、統計的には有意でない場合がある。
- (4) 統計的有意性がないことが安全性の証明にはならない。

付録Vには、一般的に使用される統計的検定がいくつか記載されている。

なお,統計解析において以下の場合にはその旨を報告書に記載し,必要に応じて,データの再分析な どの他の措置をとる必要性がある。

- 統計的検定が使用されていない場合,
- ・不適切な検定が使用されていると思われる場合,
- ・一般的に使用されていない検定が使用されている場合には、その旨を報告書に記載する。
- 付録 V 適用可能な統計的検定の一覧

非正規性の検定

- カイ二乗
- Kolmogorov-Smirnov
- Shapiro-Wik

分散の同質性の検定

- バートレットの検定
- Levene の検定

正規分布データの仮定

- 1. 全体的な検定
- 分散分析 (ANOVA, 固定効果モデル, Model 1 ANOVA が最も一般的だが, 他のモデルもあり得る)
- 共分散分析(ANCOVA)
- Pearson の相関係数 (Pearson's Correlation Coefficient)
- 用量効果の傾向を調べるための線形回帰(従属データである2つのパラメータの関係をテストする。
   回帰スロープの有意性を検証する。例:男女間の反応は似ているか?)
- 2. ペアワイズ比較
- Duncan の多重範囲検定
- Dunnett の t-検定(コントロールと他のグループの平均値を比較する)
- Scheffeの検定(多重比較, Newman-Keulsの多重範囲検定よりも検出力が弱い)
- Williams の t-テスト
- Studentのt-テスト
- Fisher の最小有意差(LSD) 検定

ノンパラメトリック手続き(パーセント値,順位など)

- Kendallの順位相関係数
- Mann-Whitney U-Test (t-test に類するもの
- Wilcoxon signed-rank 検定 (ペア, マッチドペアデータ)
- Kruskal-Wallis ANOVA
- 分布によらない多重比較検定(例: Dunn の検定, Shirley の検定)
- Jonckheere の検定

定量的データ(死亡率,病理所見など)

- Fisher の正確検定
- R×Cカイ二乗検定
- Litchfield & Wilcoxon ED50 などの信頼限界

多変量解析法

- ホテリングの T<sup>2</sup>法
- 多変量分散分析(MANOVA)

海外のリスク評価機関における評価結果等に関する調査-

3.(4)残留農薬のリスク評価にあたっての毒性試験結果の解釈に係る知見の収集

③ 血液学的検査、血液生化学的検査及び尿検査に対する基本的な考え方及びこれらの検査において統計学的に得られる正常範囲

番号	著者名	文献名	雑誌:巻:ページ	参照箇所の該当ページ
1	World Health Organization (WHO)	Guidance Document for WHO Monographers and	2015	Part 3: 1.7 (38, 39)
		Reviewers		
5	Hall AP, et al	Liver Hypertrophy: A Review of Adaptive (Adverse	Toxicol Pathol. 2012; 40(7):971-94	976
		and Non-adverse) ChangesConclusions from the		
		3rd International ESTP Expert Workshop		
6	Yokoyama Y, Ono A, Yoshida M,	Toxicological Significance of Increased Serum	Regul Toxicol Pharmacol. 2019;	Abstract
	Mastumoto K, Saito M	Alkaline Phosphatase Activity in Dog Studies of	109:104482	
		Pesticides: Analysis of Toxicological Data		
		Evaluated in Japan		
10	Friedrichs KR, Harr KE, Freeman ASVCP Reference Interval Guidelines:		Vet Clin Pathol. 2012; 41(4):441-453	Abstract, 441, 446-447, Table 4
	KP, Szladovits B, Walton RM,	Determination of de novo Reference Intervals in		
	Barnhart KF, Blanco-Chavez j	Veterinary Species and Other Related Topics		

番号	3-01	
著者名	World Health Organization (WHO)	
文献名	Guidance Document for WHO Monographers and Reviewers	
巻, ページ, 発行年	2015	
分類 (①~④)	3	
参照箇所の該当ページ	Part 3: 1.7 (38, 39)	

血液学,血液生化学及び尿検査の毒性評価では,関連するパラメータが一貫して同じ方向(増加ある いは減少)に向かっているか,用量と関連しているか(又は,「用量依存的であるか」)を判断すること が重要である。血液学,血液生化学及び尿検査の背景データとしては,過去10年以内のデータや同一 試験施設で実施された毒性試験と同時期のものが適切である。背景データは,統計的範囲(±1標準偏 差)で表すことが望ましい。年齢,系統,取り扱いや給餌の技術,測定に使用する機器など,多くの要 因が背景データに影響を与える可能性がある。イヌでは,個体値のばらつきが大きいので,平均値より も投与開始前を含めた試験期間中の個々の値の変化の方が有用である。また,構造的に類似した化学 物質または類似の作用機序を示す化学物質の血液学,血液生化学及び尿検査パラメータに関する情報 も有用である。尿検査結果は,特に全身の栄養状態に影響するような高い濃度で投与する場合に,常に 他のエンドポイントと併せて解釈する必要がある。

毒性と考えらない影響には次のようなものがある。(血液検査)1)総白血球数の変化を伴わないリン パ球と好中球の数の変化,2)白血球の中のマイナーな細胞集団(好塩基球,好酸球,単球)の数の3 倍以内の変化,3)赤血球数,ヘマトクリット値またはヘモグロビン濃度の10%以内の増加または減少, 白血球数及び血小板数の20%以内の増加または減少,4)高用量で肝毒性によって二次的に起こった軽 度な貧血(血液生化学検査)1)組織・臓器に由来する酵素の僅かな増減(AST,ALT,ALP,γ-GTP, CK等):50%以上で毒性指標を考慮,2)若齢動物のγ-GTP活性を対照群との百分比で表した場合(誤 った解釈につながる),3)総タンパク濃度またはアルブミン濃度の変化を伴わないアルブミン/グロ ブリン比,4)10%以下の総タンパク濃度またはグルコース濃度(尿検査)1)排泄される酸性または塩 基性化合物に関連する pH の変化,2)水分消費量または体液減少(嘔吐または下痢など)の変化に関 連する尿量及び/または比重の変化。

軽度ではあるが毒性学的に有意な変化には次のようなものがある。(血液検査)1) 平均赤血球容積の 5%以上の増加は赤血球の形態学的異常を示す可能性がある、2) ヘモグロビン濃度が約 10%以上低下 した場合は貧血の指標となる、3) メトヘモグロビンは正常でも存在するが、投与群において、イヌで 5%以上、ラットで 1.5%以上の上昇は毒性と考えられる、4) スルファメトヘモグロビンは正常では存 在しないため、検出されれば異常である。(血液生化学検査) IgG 濃度の低下は免疫抑制の指標となる。

(尿検査)1)細胞(血液細胞,腎臓上皮細胞または膀胱上皮細胞)数の増加は腎臓・尿路系の障害の 指標となる,2)尿中の血液は、全身の出血や腎臓、尿路、雄性生殖器系における局所的な障害を示す ことがある。

番号	3-05	
著者名	Hall AP, et al	
	Liver Hypertrophy: A Review of Adaptive (Adverse and Non-adverse) Changes	
文献名	Conclusions from the 3rd International ESTP Expert Workshop	
巻,ページ,発行年	Toxicol Pathol. 2012; 40(7): 971-94	
分類 (①~④)	3	
参照箇所の該当ページ	976	

肝細胞  $\gamma$ GT ( $\gamma$ -glutamyltranspeptidase)の誘導は、フェノバルビタールナトリウムを含むいくつかの 化学物質でも報告されており、フェノバルビタールナトリウムは肝重量の増加を引き起こし、肝臓で の代謝亢進を誘導することが知られている。ワークショップで発表された事例研究では、ラットを用 いた反復投与(7日間)の経口毒性試験において、肝 $\gamma$ GT のメッセンジャーRNA (mRNA)が誘導され ることが示され、小葉中心性肝細胞肥大、肝重量の増加、CYPs(主に2B2と3A3)の増加、第2相酵 素活性(主にGSTA3とUGT1A6)の増加が観察された。しかし、これは循環血中の $\gamma$ GT活性の増加に のみ影響し、肝 $\gamma$ GT mRNA の顕著な誘導(50倍以上)が観察された。フェノバルビタールを5日間投 与したラットでは、肝 $\gamma$ GT 活性は増加したが、血清 $\gamma$ GT 活性は増加しなかったことから、実験動物の 肝胆道変化の指標としての血清由来 $\gamma$ GT は、肝 $\gamma$ GT の測定と比較して相対的に感度が低いことが示唆 された(Goldberg et al. 1981)

しかし, 肝内胆管の部分的な閉塞による胆汁閉塞も同様に γGT 酵素活性の変化を引き起こす可能性 がある。したがって, γGT の増加を酵素誘導によるものとする判断は, 肝重量の増加, 生物学的に意義 のあるトランスアミナーゼ活性の増加がないこと, 及び病理組織学的に有害な事象を示す変化がない ことを十分に考慮した上で, 証拠の重み付けに基づいて行われるべきである。

γGT と ALP は、 ラット、 マウス、 イヌにおいて異なる組織分布を有する (Clampitt and Hart 1978; Keller 1981)。例えば、 イヌでは、 肝型が血中の ALP 活性の主体となるが、 ラットでは比較的活性が低く、 このため、この種では診断感度が低い。さらに、 レギュラトリーな毒性試験に一般的に用いられる幼若 な動物では、 血清 ALP 活性は主に骨のアイソザイムに由来し、 肝臓には由来しない (Hoffmann et al. 1994)。したがって、 肝臓酵素の誘導に加えて、 他の臓器での変化も考慮した評価をする必要があるか もしれない。

前述の化学物質による酵素の誘導もまた,種特異性があると考えられる:イヌでは,コルチコイド誘発性 ALP アイソザイム活性がしばしば増加するのに対し(Gaskill ら 2005),マウスでは肝 ALP が増加する(Kawasaki, Mataki, and.Takano 1994)。ラットでは,肝酵素誘導剤に反応して肝  $\gamma$ GT が誘導される(Gallagher ら 1998; Satoh ら 1982)。妊娠 6 日目(GD6)から GD20 までの妊娠ラットでは,酵素誘導剤を投与すると, $\gamma$ GT 活性ではなく ALP 活性が上昇する(Strauss, pers.comm. 2011)。マウスでは,ラットとは対照的に,肝細胞毒性の病理組織学的徴候を伴わない肝酵素誘導剤を投与した場合には,ALT が増加することが多い(Strauss, pers. comm. 2011)。

また,市販のヒト用 γGT キットの検出限界は約 3 U/L であることに注意が必要である。(37℃ で 50 nkat/L,例として γGT, Szasz mod., 2010-06, V7:ロシュ取扱説明書)。正常なラット血清 γGT 活性は, 一般的にこの検出限界以下であるので,中等度の γGT 増加は,これらの検査では検出できない。

番号	3-06	
著者名	Yokoyama Y, et al	
	Toxicological Significance of Increased Serum Alkaline Phosphatase Activity in	
文献名	Dog Studies of Pesticides: Analysis of Toxicological Data Evaluated in Japan	
巻,ページ,発行年	Regul Toxicol Pharmacol. 2019; 109: 104482	
分類 (①~④)	3	
参照箇所の該当ページ	Abstract	

血清アルカリホスファターゼ (ALP) 活性の上昇は、ヒトや実験動物における肝障害の指標となる。 実際には、イヌを用いた農薬の毒性試験において、他の肝障害の変化を伴わないALPの上昇がしばしば 認められる。本研究では、食品安全委員会がリスク評価の過程で毒性評価報告書として評価した206種 の農薬について、ALP上昇の毒性学的意義を解析した。その結果、ラット (36/206)よりもイヌ (108/206) の方がALPの上昇が認められる試験の割合が高かった。108農薬中87農薬では、イヌで肝毒性を伴うALP 上昇が認められた。しかし、ALPの増加には、特定の種類の肝毒性との特異的な関連は認められず、肝 毒性のマーカーとしては高感度(または鋭敏)とはいえなかった。肝毒性を示した87種の農薬の約50% は、肝肥大も誘発した。残りの21種類の農薬では、肝重量の増加や肝肥大以外に肝毒性の変化はみられ なかった。これら21種類の農薬のほとんどは、齧歯類におけるフェノバルビタール様肝代謝酵素誘導 剤であった。これらの結果から、肝毒性所見がない場合、ALPの上昇はイヌにおける肝毒性の指標には ならないことが示唆された

解析の結果,イヌにおける ALP の上昇についてその毒性学的意義を決定する要因が明らかとなった。 まず ALP の上昇が肝毒性や肝肥大といった異常所見を伴っているかについて確認する。肝毒性に加え 肝肥大が認められる場合、ALP の増加が認められる用量と肝毒性が認められる用量を比較し、ALP 増 加が認められる用量が肝毒性のそれに比べ同等あるいはより高い場合,ALPの上昇は有害影響であり, 肝毒性の NOAEL には影響を与えない。ALP 増加がみられる用量が、肝毒性がみられる用量より低い 場合, さらにその用量について肝肥大を示す用量との比較を行う。ALP 増加がみられる用量と肝肥大 がみられる用量が同等であった場合, ALP の上昇は有害影響とはならず, 肝毒性の NOAEL に影響を 与える。同等でなかった場合には、期間の延長により毒性が増強されることを示す証拠があるかを考 慮し、証拠が認められる場合 ALP の上昇は有害影響であり、肝毒性の NOAEL には影響を与え、認め られない場合は ALP の上昇は有害影響とはならず, 肝毒性の NOAEL に影響を与える。肝毒性のみ認 められる場合, ALP の増加が認められる用量において肝毒性が認められる用量に比べ同等か高いこと, あるいは期間の延長により毒性が増強された証拠の有る、といった条件のいずれかに当てはまる場合 ALP の上昇は有害影響であり、肝毒性の NOAEL には影響を与えず、何れにも当てはまらないのであ れば、ALP の上昇は有害影響ではなく、肝毒性の NOAEL に影響を与える。肝肥大のみ伴っている場 合, 肝代謝酵素の誘導や肝肥大を示す証拠があれば ALP の上昇は有害影響ではなく, 肝毒性の NOAEL に影響を与える。そういった証拠がない場合、期間の延長によりさらに低い用量での毒性の増強を示 す証拠の有無を確認し、有る場合は ALP の増加はおそらく有害影響であり、肝毒性の NOAEL に影響 を与えることになる一方, 無い場合は ALP の上昇は有害影響ではなく, 肝毒性の NOAEL に影響を与 える。他の肝臓所見が見られない場合も同様の考察が可能であり、この解析はイヌにおける ALP の毒 性学的意義の新たな解釈を提供し、農薬の毒性学的評価に寄与する可能性がある。

番号	3-10	
著者名	Friedrichs KR, et al	
文献名	ASVCP Reference Interval Guidelines: Determination of de novo Reference	
又瞅石	Intervals in Veterinary Species and Other Related Topics	
巻, ページ, 発行年	Vet Clin Pathol. 2012; 41(4): 441-453	
分類 (①~④)	3	
参照箇所の該当ページ	Abstract, 441, 446-447, Table 4	

基準間隔Reference intervals (RI) は, 検査室での診断検査や臨床上の意思決定に不可欠な要素であり, 同等の個人の健康な集団からの基準値Reference values (RV)の推定分布を表す。診断の追求や治療開 始の意思決定は,しばしばRIに含まれない値に基づいて行われるため,RV値の収集と分析には細心の 注意を払う必要がある。

慣例上, RIはRVの中心95%を含み,基準値の上限と下限で区切られる。基準限界値を決定するため に選択された統計的方法は,RVの数と分布に基づく(表1)。ノンパラメトリック法は、少なくとも120 個のRVが利用可能な場合に推奨される。2.5分の1と97.5分の1のフラクタイルfractilesがそれぞれ基準値 の下限と上限として機能する。利用可能なサンプル数が120未満の場合,90%信頼区間を決定するため には、代替の方法(ブートストラップ等)が必要となる。39個は、95%ノンパラメトリックRIを決定で きる最小サンプル数であるが、この場合、極端な値が下限値と上限値となる。ノンパラメトリックRIを決定で きる最小サンプルサイズで使用しなければならない場合は、両極端の潜在的な外れ値をトリミングでき るように十分なサンプルを収集する必要がある。あるいは、ロバスト法またはパラメトリック法を使 用するべきである。ロバスト法は、≧40及び≦120の参照サンプルが利用可能な場合に推奨され、デー タの位置と広がりを推定するために反復プロセスを利用する。またガウス分布を必要としないが、デ ータが対称的に分布している場合、より良いパフォーマンスを発揮する。ブートストラップ法は90%信 頼区間を決定するために使用されるべきである。

データがガウス分布を持つか、またはガウス分布に変換できる場合、≧40及び≦120の基準サンプル が利用可能な場合、パラメトリック法を使用することができる。パラメトリック法は、値の中心95%よ りもわずかに多くの値を包含し、それぞれ平均+2SD及び平均-2SDで上限及び下限の基準限界を確立す る。パラメトリック法は、基準値の90%信頼区間を決定するために使用されるべきである。

獣医学では、特殊種や野生で捕獲された種、新生児など、基準サンプルの収集数が限られる場合があ る。20~40サンプルの基準サンプルの場合、RIはロバストまたはパラメトリックな方法で計算する必 要がある。小さなサンプルサイズに内在する不確実性を強調するために、90%信頼区間を計算すべきで ある。さらに、情報に基づいた臨床的意思決定を可能にするために、ヒストグラム、平均値や中央値、 最小値及び最大値を報告すべきである。あるいは、ヒストグラムと共にすべての値を列記した表の提 供に代替され得る。

10 人以上 20 人未満の基準サンプルが入手可能な場合は、ヒストグラム、平均値または中央値とともに昇順 値の表を報告すべきであるが、少ないサンプル数に基づく限界値の不確実性を考慮して、RI を決定すべきで はない。参照個体が非常に少ない場合には、被験者ベースの RI を考慮すべきである。一般に、利用可能な 参照個体が 40 人未満の場合, 適切な参照被験者の選択に細心の注意を払い, 標準化された収集技術と十分に管理された分析方法を遵守して, 外れ値のないサンプルを収集することに重点を置くべきである。 外れ値の存在を評価することは特に重要で, 単一の外れ値の存在は推定基準値に強い影響を与えるためである。 外れ値が識別された場合は, 代替サンプルを収集するためにあらゆる努力をすべきである。

<u> </u>		
	データ分布	
サンプルサイズ	(生または変換)	統計的手法
≥120	該当なし	参照限界の 90%信頼区間 (CI) を持つノンパラメトリック
$40 \le x < 120$	ガウス	参照限界の 90%CI でロバスト
		基準値の 90%CI を持つパラメトリック
	非ガウス	基準限界の 90%CI でロバスト(望ましい)
		ノンパラメトリック
$20 \le x < 40$	ガウス	参照限界の 90%CI を持つパラメトリック†
	非ガウス	参照限界の 90%CI でロバスト†
$10 \le x < 20$	該当なし	基準間隔を計算しない†
< 10	該当なし	参照値を報告しない

表 1. 参考サンプルサイズと分布に基づいた RI の設定のための推奨手順

\*120 個未満の参照サンプルでは、ノンパラメトリックに 90%信頼区間を決定できない; ブートストラ ップなどの代替方法が必要となる。

\* ヒストグラム,平均値または中央値,最小値及び最大値を含む;代わりに,ヒストグラムと一緒に すべての参照値の表を提供することができる。

# 海外のリスク評価機関における評価結果等に関する調査-

## 3.(4)残留農薬のリスク評価にあたっての毒性試験結果の解釈に係る知見の収集

## ④ 体重、摂餌量及び臓器重量に対する基本的な考え方及びこれらの重量につい統計学的に得られる正常範囲

番号	著者名	文献名	雑誌:巻:ページ	参照箇所の該当ページ
1	World Health Organization (WHO)	Guidance Document for WHO Monographers and Reviewers	2015	Part 3: 1.8 (40, 41)
	Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, Rosol TJ, Sellers T	Interpreting Stress Responses during Routine Toxicity Studies: A Review of the Biology, Impact, and Assessment	Toxicol Pathol. 2013; 41(4): 560-614	Abstract, 561-562, 563, 592, 595
12	Levin S, Semler D, Ruben Z	Effects of Two Weeks of Feed Restriction on Some Common Toxicologic Parameters in Sprague- Dawley Rats	Toxicol Pathol. 1993;21(1):1-14.	Abstract, 5, 13
13	Michael B, M, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K	Evaluation of Organ Weights for Rodent and Non- rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices	Toxicol Pathol.2007; 35: 742-750	742-743, 749, 750
14	Marino DJ (2012b)	Age-Specific Absolute and Relative Organ Weight Distributions for Fischer 344 Rats	J Toxicol Environ Health A. 2012; 75(24): 1484-1516	Abstract
15	Marino DJ (2012a)	Age-Specific Absolute and Relative Organ Weight Distributions for B6C3F1 Mice	J Toxicol Environ Health A. 2012; 75(2): 76-99	Abstract
16	Dorso L, Chanut F, Howroyd P, Burnett R	Variability in Weight and Histological Appearance of the Prostate of Beagle Dogs Used in Toxicology Studies	Toxicol Pathol. 2008; 36: 917-925	Abstract, 923, Figure 13
17	Bailey SA, Zidell RH, Perry RW	Relationships Between Organ Weight and Body/Brain Weight in the Rat: What Is the Best Analytical Endpoint?	Toxicol Pathol. 2004; 32: 448-466	Abstract, 461, 466, Table 3
18	Sellers RS, Morton D, Michael B, Room N, Johnson JK, yano BI, oerry R, Schafer K	Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies	Toxicol Pathol. 2007; 35: 751-755	752, Appendix 1
19	OECD	Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies	2002	30-31, 33-34

番号	4-01
著者名	World Health Organization (WHO)
文献名	Guidance Document for WHO Monographers and Reviewers
巻, ページ, 発行年	2015
分類 (①~④)	4
参照箇所の該当ページ	Part 3: 1.8 (40, 41)

正常な生物学的変動内での影響に関する一般的な結論

主に体重または体重増加, 臓器重量変化, 血液学的・生化学的・尿検査パラメータに関する影響であ る場合, 文献で公表されている正常範囲の値は, 当該試験で使われた方法と同等であることを保証す るために, それらが得られた方法が明記されている場合にのみ使用すべきである。それらの影響のパ ターンを考慮すると, 毒性学的影響の概算の変化の大きさは, 1) 10%の体重または体重増加, 2) 20% のアセチルコリンエステラーゼ阻害, 3) 臓器重量の変化がある。

臓器重量の変化は、毒性試験で観察された被験物質投与に関連した影響のうち、しばしば最も感度 の高い指標となる。臓器重量の解析は、絶対重量、相対重量の両方を基に行うべきである。臓器重量の 変化は, 絶対値及び相対値の両方とも, 対照群と投与群との差をパーセンテージで表したものである。 体重変化が大きい場合、相対重量は体重よりも脳重量との相対値で表す方が好ましい場合がある。臓 器重量の変化は, 絶対重量, 相対重量, またはその両方が変化しているかどうかという観点で評価すべ きである。その変化は、適切な対照群の平均値と比較した百分比(増加または減少)として表現すべき である。臓器重量の適切な評価には、個々の動物の値と群の平均値の両方が必要である。ラットにおけ る標的臓器毒性を最も正確に検出しやすいエンドポイント(臓器重量、臓器重量体重比、臓器重量脳 比)を決定するために実施された評価では、臓器重量体重比は、肝臓及び甲状腺、臓器重量脳比は卵巣 及び副腎への影響を評価するのにそれぞれ役立つ。脳、心臓、腎臓、下垂体及び精巣の重量はいずれを 選んでも適切に評価できないので,共分散分析などの代替方法を利用すべきである。マウスにおいて 投与に関連した臓器重量の変化を明らかにするためには、肝臓、腎臓、肺及び心臓の絶対重量の加齢に 伴う増加,及び脳と精巣の重量が比較的安定であることを考慮しなければならない。マウスにおける, 絶対重量のばらつきの一般的な傾向として,脳 < 精巣 < 腎臓 < 心臓 < 肝臓 < 肺 < 脾臓 < 胸腺 と報告されている。ラットについては、脳、肝臓、腎臓、肺、心臓、甲状腺及び精巣の加齢による絶対 重量の増加と相対重量の減少を考慮しなければならない。ラットにおける絶対重量のばらつきの一般 的な傾向として,脳 < 精巣 < 心臓 < 腎臓 < 肝臓 < 肺 < 胸腺 < 甲状腺と報告されている。

臓器重量データは、剖検所見、臨床検査結果及び病理組織所見とともに総合的に解釈しなければな らない。対応する肉眼所見や組織所見を伴わない臓器重量の変化は注意して解釈する必要がある。臓 器重量の変化が投与に関連したものかどうかを判断するためには以下の付加的な点を考慮すべきであ る。1)用量相関性、2)統計学的有意差があるか、3)両性で起こっているか、片性のみか、4)各投与 群での発生率はどうか、5)対照群の範囲外か、6)臓器重量の変化が体重の変動に依存しているか、7) 回復期間内で部分的または完全に回復するか、8)臨床検査結果、剖検所見、組織所見と関連があるか、 である。

番号	4-11
著者名	Everds NE, et al
文献名	Interpreting Stress Responses during Routine Toxicity Studies: A Review of the
又瞅泊	Biology, Impact, and Assessment
巻, ページ, 発行年	Toxicol Pathol. 2013; 41(4): 560-614
分類 (①~④)	4
参照箇所の該当ページ	Abstract, 561-562, 563, 592, 595

毒性試験の目的は、被験物質によって引き起こされた標的臓器の有害影響を特定することであり, 高用量で観察される被験物質投与に直接的に起因する影響(一次的影響)は二次的にストレスをもた らすのに十分な程度と持続時間をもつ。被験物質の一次的影響は一般的に投与用量に比例するため, ストレスに起因する変化も用量に比例すると考えられる。このため、毒性試験における被験物質の一 次的影響とストレスに関連した二次的影響とを区別することは困難である。ストレスが脳で感知され ると,主に交感神経-副腎髄質系の活性化によるカテコールアミンの放出及び視床下部-下垂体-副腎系 (HPA 系) を介した血清グルココルチコイドの上昇が起こる。これらの神経内分泌シグナルにより, 下流では総体重または体重増加量, 摂餌量ならびに活動量の減少, 臓器重量の変動 (胸腺及び脾臓重量 減少,副腎重量増加),胸腺及び脾臓のリンパ球減少,循環白血球数の変動(リンパ球数及び好酸球数 の減少を伴う好中球数の増加),生殖機能の変化など,多臓器にわたり幅広い影響がもたらされる(表 1)。これらの所見の一部またはすべてが発生した場合、それらはストレスに起因する可能性が高い。毒 性試験でストレスを示す最も感度の高いパラメータは、循環白血球数、リンパ球サブセット、副腎及び 胸腺重量であり、ストレスに対する感受性は動物種、ストレスの種類、動物の状態や個体差によって異 なる。また, 急性ストレスに関連した影響は臓器外観の変化ではなく, 臨床病理パラメータの変化(例: ストレス性の白血球像)及び/または感受性の高い細胞集団の組織学的変化(例:グルココルチコイド 誘発胸腺リンパ球のびまん性アポトーシス)として表れる。これに対し、慢性ストレスは体重及び臓器 重量に影響を与える可能性がある(体重、胸腺、副生殖器及び脾臓重量の減少ならびに副腎重量の増 加)。

毒性試験でみられる変化が被験物質ばく露に起因するものか、ストレスに起因するものかどうかを 区別するためにはデータを総合的に評価する必要があり、以下に示す証拠の重み付けアプローチ

(weight-of-evidence approach) が有用である。

・<u>被験物質の標的臓器を考慮する</u>:被験物質がストレスを受けやすい臓器に影響を及ぼす場合,その 変化がどちらの影響によるものなのかを特定することは難しいため,低用量での被験物質の影響から 判断材料を得られる可能性がある。

・<u>ストレスによる影響を受けやすいパラメータや臓器におけるストレス反応の大きさを評価する</u>: ストレス反応の大きさは与えられたストレスの大きさに見合ったものでなければならない(例:1ヵ月 程度の軽度の反復ストレスを与えられた動物は副腎の重量及び組織に軽微から軽度の影響がみられる 可能性がある)

・<u>ストレスに関連した変化の用量反応と被験物質に関連した変化の用量反応との比較</u>:ストレスに

関連した変化は一般的に被験物質の影響がみられない用量では起こりえない(例:中間用量及び高用 量群で投与用量に関連した循環リンパ球及び胸腺リンパ球の減少,副腎重量の増加及び体重減少が認 められ,低用量群では副腎重量の増加のみみられる試験では,低用量の変化は被験物質投与の直接的 な影響である可能性がある)。

・<u>他の解釈の可能性を考慮する</u>:ストレスに起因する変化のほとんどは、別の解釈をもつ。データの 解釈を行う前に、別の解釈とそれを支持または反論する証拠について十分に検討する必要がある(例: 副腎皮質の肥大/過形成はストレスまたはグルココルチコイドの産生を抑制する副腎毒性物質が原因で ある可能性が考えられるが、他にストレスに関連した変化が認められない場合は副腎皮質ホルモン合 成に対する影響である可能性がある)。

・<u>証拠の重み付けの強さを評価する</u>:一般的に感度の低いストレスの指標は,感度の高いストレスの 指標が認められない場合には起こりえない(体重やリンパ系への影響等がなく生殖器系の影響のみが みられる場合,ストレスの影響ではない可能性がある)。

試験段階	影響を受ける システム	パラメータ	起こり得るストレス影響 (影響がない場合もある)
In-life		体重または体重増加量	(影音//など/場合もめる) 減少
III IIIC		摂餌量	減少
	循環血液細胞	好酸球数	減少
		リンパ球数	増加または減少
		好中球数	增加
	免疫系	マクロファージの貪食作用	減少
臓器重量 a	内分泌系	副腎	増加
	免疫系	胸腺	減少
		脾臓	減少
	生殖器系	精巣 <sup>b</sup>	変化なし (ラット) または減少 (マウス)
		精巣上体	減少
		精嚢	減少
		前立腺	減少
		卵巣	減少
		子宮	減少
臓器組織学	消化器系	胃潰瘍	增加
	リンパ系	胸腺細胞数	減少
		脾臓細胞数	減少
	生殖器系	精巣	変化なし (ラット) または変性 (マウス)
		精巣上体, 前立腺, 精嚢	萎縮の可能性あり
		卵巣,子宮	機能低下
		膣	萎縮,粘液産生
	内分泌系	副腎皮質	肥大/過形成

表 1. 毒性研究でよく評価される In-life 及び病理学的パラメータ:ストレスに対する典型的な反応

注: ストレスの影響を受けるパラメータは,動物種,性別,週齢及びストレス因子の種類と持続時間に 依存する。種特異的反応については,該当部分を参照のこと。

a: 絶対重量,体重に対する相対量,及び/または脳重量に対する相対量

b: 絶対重量のみ

### ・免疫系とストレス

免疫系に対するストレスの生理学的及び形態学的影響は、血液、胸腺、脾臟、骨髄で見られる。リン パ系組織の中では、胸腺が最も感受性が高く、ストレスに対する反応は数時間以内に誘発される。グル ココルチコイドは、リンパ球の再分布、細胞応答の変化、及びリンパ球の破壊を引き起こす可能性があ る。臓器重量が約 20%以上減少した場合にリンパ球減少の組織学的所見と関連が認められる。未熟リ ンパ球はストレスの影響を最も受けやすいため、胸腺の皮質はリンパ球減少の結果、しばしばサイズ が減少するが、骨髄や脾臓では胸腺に比べて成熟リンパ球が優勢であるため、これらの臓器でのスト レス反応はそれほど明瞭ではない。ストレスに関連した変化は、他の二次リンパ系臓器ではあまり一 貫性が認められない。

### ・胸腺

胸腺では、ストレスは皮質リンパ球の減少に起因する皮質のサイズの減少をもたらし、未熟な皮質 リンパ球が最も影響を受ける。重度の急性ストレスは、多数の"tingible-body"マクロファージと細胞の 破片(すなわち, starry sky 像)の出現を特徴とするびまん性のアポトーシスを引き起こす可能性があ る。対照的に、軽度な慢性ストレスは皮質のサイズの明らかな減少をもたらすかもしれないが、アポト ーシスの証拠は限られているか、全くない。胸腺リンパ球のアポトーシスはラットの拘束ストレスの2 時間後に始まるが、胸腺重量は拘束後 8 時間まで変化しない。ラットの胸腺リンパ球数と重量は、毎 日の強制水泳ストレスを 21 日間受けると減少する。マウスの胸腺重量は、ケージラックを短時間移動 させた 12 時間後と 24 時間後にそれぞれ 12%と 15%減少した。胸腺に対する効果は可逆的であり得る。 例えば、マウスにデキサメタゾンを単回投与すると、胸腺萎縮が生じ(細胞数及び増殖の減少によって 示される)、これは処置後 3 日目までに最も顕著であるが、12 日目までに完全に回復する。

#### ・脾臓

毒性試験では、一般に脾臓に影響を及ぼすほどの十分に強いストレス因子は、リンパ球のアポトーシスの有無に関係なく、白脾髄領域の減少を伴う脾臓重量の減少をもたらす。ストレスに関連した変化は胸腺に比べて脾臓では一般的に一貫性がなく軽度である。1回の急性ストレスが脾臓重量に一過性の影響を与えることがマウスで報告されている。1日2時間のストレスに7日間連続でばく露されたラットでは、Tリンパ球領域の増殖が低下して白脾髄量が減少する。さらに、アポトーシスの増加によりBリンパ球領域が減少する。前述したように、循環Bリンパ球はストレス時にリンパ系器官に再分配し、その結果、ストレス下での脾臓のBリンパ球領域の可変的な変化に寄与している可能性がある。 脾臓重量を増加させる被験物質の薬理学的または毒性学的効果は、ストレスに関連した脾臓重量の減少を隠蔽する可能性がある。

番号	4-12
著者名	Levin S, et al
文献名	Effects of Two Weeks of Feed Restriction on Some Common Toxicologic
又顺泊	Parameters in Sprague-Dawley Rats*
巻, ページ, 発行年	Toxicol Pathol. 1993;21(1): 1-14.
分類 (①~④)	4
参照箇所の該当ページ	Abstract, 5, 13

本研究は味の悪い餌や食欲不振によって起こる短期の摂餌量減少が引き起こす変化を特定すること を目的とし、4-5 週齢の Sprague-Dawley SD ラット (Charles River) 各群雌雄 10 匹ずつの 4 グループに、 2週間にわたり異なる量の餌 [グループ1:自由摂取(対照群), グループ2:前日の対照群の摂餌量の 75%(軽度食餌制限),グループ3:前日の対照群の摂餌量の50%(中等度食餌制限),グループ4:前 日の対照群の摂餌量の25%(重度食餌制限)]を与え、血液学的検査、血液生化学的検査、臓器重量測 定(副腎,脳,心臓,腎臓,肝臓,精巣及び胸腺)及び組織学的検査(臓器重量測定を行った臓器に加 え,骨及び骨髄(胸骨),ならびに胃)を実施した。その結果,いずれの群にも動物の死亡は認められ ず、すべての食餌制限群において発育遅延以外の臨床症状は観察されなかった。試験終了時の最終体 重は、雄ではグループ2、3及び4においてそれぞれ対照群と比較して77%、62%、38%であり、雌で は82%,66%,42%であった。血液学的検査では、赤血球数はヘマトクリット値及び血色素量とともに 食餌制限の程度に比例して増加し、血小板数はすべての食餌制限群で減少した。白血球数はグループ4 で著しく減少し,特にリンパ球数の減少が目立った。血液生化学的検査では,グループ4の雌雄で血 清ビリルビンの増加,電解質異常及びコレステロールの減少(雌のみ)がみられた。臓器重量では、体 重への著しい影響に伴い、グループ 4 のすべての臓器の絶対重量が減少していた。胸腺はグループ 4 の雌雄及びグループ3の雄で、肝臓はすべての食餌制限群で絶対及び相対重量が減少した。その他の 臓器の相対重量については、変化しないか、もしくは増加していたが、これは減少した体重の大部分が 脂肪、筋肉及び水分の喪失によるものであったことを示唆していた。食餌制限に関連した組織学的所 見は骨髄, 胸腺, 胃及び精巣で観察された [発生頻度は雌雄合算し(n=20), 病変の程度は軽微(1), |軽度(2),中等度(3),高度(4),重度(5)の5段階で評価した]。食餌制限により最も生物学的に重 大な組織学的変化が観察されたのは骨髄であり、グループ2及び3では軽微から中等度の造血組織の 減少(骨髄腔の脂肪増加)、グループ4では脂肪がみられず、中等度から重度の造血及び間質成分の変 性及び壊死が観察され、造血組織は対照群の10~25%であった。胸腺ではグループ4のすべての動物 に退縮または萎縮が認められ、雌でより強い影響がみられた(重度の萎縮は雄1/10及び雌7/10に観察 された)。重度の胸腺萎縮では、ハッサル小体とわずかに残るリンパ球とともに泡沫状マクロファージ が充満する間質しか残っておらず,正常構造は失われていた。グループ 4 の残りの動物の胸腺には, マクロファージ (星空像を呈する可染体マクロファージ),皮質のリンパ球減少,ハッサル小体の増加 及び皮髄境界部の不明瞭が観察され、より程度の弱い萎縮像と考えられた。グループ2及び3では、 少数の動物に皮質における泡沫状マクロファージの軽微な増加が観察された。精巣の組織学的変化は, グループ4において4例にみられ、このうち2例では軽度な精上皮変性(精上皮の脱落及び巨細胞の

形成)が,残りの2例では精子数の減少が観察された。胃では,剖検時にすべての食餌制限群で粘膜の 局所的な赤色化もしくは血様内容物が認められ,組織学的に観察された軽度なびらんの発生頻度は食 餌制限群で高かった(グループ 1-4:1/20,5/20,2/20,5/20)。副腎,脳,心臓,腎臓,または肝臓で は,食餌制限に関連する組織変化は観察されなかった。

以上の結果より、生物学的に特に重要であると考える変化を以下に示す。

・摂餌量が対照群の75または50%の場合、胸骨骨髄における造血組織の減少及び脂肪の増加

・摂餌量が対照群の25%しかない場合,骨髄の壊死,胸腺萎縮(特に雌で強い),骨髄及びリンパ組織へ影響を反映した好中球,リンパ球及び血小板減少,脱水の影響による血液濃縮及び血清電解質の 異常,ならびに肝萎縮(絶対及び相対重量の減少)。その他の変化については,それほど重要ではない と考えられた。

(翻訳注:肝臓の萎縮については相対重量の変化は統計学的に有意ではなく,組織学的な変化は観察されていないが,生体異物の代謝に影響を及ぼすかもしれないので無視出来ないとしている。)

		副	腎	月	Ă	心	·臓	12 12	腎臓	月	干臓	胸	腺	米	青巣
	FRW	WT	ORG:FBW (=10*)	WT	ORG:FBW (×10-')	WF	ORG:FBW (*10-')	WT	ORG : FBW {×10-*}	WT	ORG:FBW (×10 <sup>-1</sup> )	WT	ORG:FBW (×10-')	WT	ORG:FBW (×10-7)
			1000				Mak	es							1.0
Group I	213.4 8.92 10	0.04 0.015 10	1.89 0.736 10	1.9 0.10 10	8.9 0.64 10	0.9 0.06 10	4,4 0,28 10	2.1 0.23 10	9,9 0,98 10	7.8 0.54 10	3.7 0.18 10	0.58 0.138 10	2.71 0.657 10	2.24 0.066 10	10.51 0.413 10
Group 2	180.0*** 7.82 10	0.04 0.008 9	2.21 0.466 9	1.8* 0.07 9	0.45	0.8*** 0.04 9	4.3 0.29 9	1.6*** 0.07 9	9.1 0.42 9	6.1*** 0.41 9	3.4 0.15 9	0.57 0.157 9	3.18 0.888 9	2.21 0.167 9	12.29*** 0.634 9
Group 3	151.2*** 6.96 10	0.04 0.007 9	2.57* 0.474 9	1.8* 0.09 10	0.52	0.6*** 0.05 9	3.9 0.42 9	1.4 *** 0.14 9	9.0 0.58 9	5.4*** 0.38 9	3.6 0.21 9	0.33*** 0.077 10	2.21** 0.495 10	2.22 0.192 10	14.69*** 0.915 10
Group 4	90.1*** 4.46 10	0.03** 0.005 8	3.21*** 0.580 8	1.7*** 0.06 10	1.31 10	0.4*** 0.05 10	4.9** 0.41 10	1.0*** 0.06 10	0.61 10	3.0*** 0.30 10	3.4 0.39 10	0.07*** 0.029 10	0.78*** 0.303 10	1.34*** 0.306 10	14.80 3.322 10
							Fema	les							
Group 1	144.4 16.36 10	0.05 0.010 10	3.62 0.741 10	1.8 0.10 10	12.2 1.05 10	0.7 0.13 10	5.0 0.54 10	1.4 0.16 10	10.0 0.48 10	5.3 0.73 10	3.7 0.26 10	0.48 0.123 10	3.33 0.679 10		
Group 2	131.5* 10.50 10	0.04 0.009 9	3.43 0.683 9	1.7 0.09 10	13.1* 0.78 10	0.6** 0.04 10	4.5 0.32 10	1.2** 0.10 10	9.4 0.51 10	4.8 0.42 10	3.7 0.13 10	0.44 0.089 10	3.35 0.723 10		
Group 3	108.7*** 6.06 10	0.04*** 0.006 10	3.29 0.483 10	1.7 0.09 10	15.3*** 0.64 10	0.5*** 0.08 10	4.6 0.68 10	1.1*** 0.10 10	10.0 0.68 10	3.7*** 0.40 10	3.4 0.27 10	0.36 0.133 10	3.35 1.227 10		
Group 4	64.7*** 5.85 10	0.02*** 0.007 9	3.65 1.072 9	1.6*** 0.06 10	24.3*** 1.43 10	0.3*** 0.06 10	5.2 0.58 10	0.8*** 0.12 10	12.4*** 1.04 10	2.3*** 0.21 10	3.6 0.23 10	0.03*** 0.024 10	0.50*** 0.320 10		

表 IV. 臓器重量(g) 及び臓器: 最終体重比; 算術平均, 標準偏差及び検査例数

略語: FBW = 最終体重,ORG: FBW = 臟器: 最終体重比,WT = 重量

\*, \*\*, または\*\*\* 5, 1, または 0.1%レベルでそれぞれ有意な傾向検定(フラグはそれぞれ平均値間の有意差を示す)

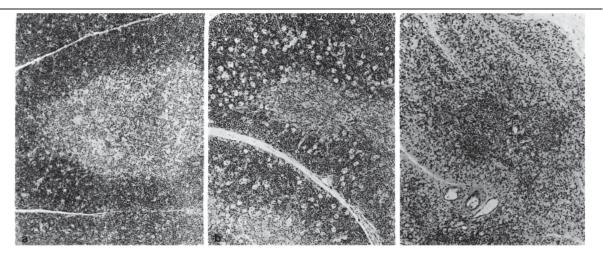


図 5-(a)対照群のラット正常胸腺。(b) 軽度のリンパ球減少と皮質の星空像を示すグループ4(重度食餌制限)のラット胸腺。(c) 重度にリンパ球が減少し、胸腺構造が失われているグループ4(重度食餌制限)のラット胸腺。HE 染色。130 倍

番号	4-13
著者名	Michael B et.al. (2007)
文献名	Evaluation of Organ Weights for Rodent and Non-rodent Toxicity Studies: A
又瞅石	Review of Regulatory Guidelines and a Survey of Current Practices
巻, ページ, 発行年	Toxicol Pathol.2007; 35: 742-750
分類 (①~④)	4
参照箇所の該当ページ	742-743, 749, 750

Society of Toxicologic Pathology (STP) は、各分野の規制ガイドラインの間には一定の違いがあるこ とを認識しており、その中で、臓器重量の現行の評価方法が大きく異なることを認識している。そのた め、STPは毒性試験における臓器重量に関する評価の方法を調査するためのワーキンググループを招集 した。調査は、ヨーロッパ、北米、日本の製薬、獣医、化学、食品/栄養、消費者製品の種々の分野の 企業にアンケートを配布し、意見を求めた。

調査では、げっ歯類及び非げっ歯類を使った様々な種類の毒性試験で日常的に測定されている臓器 を特定し、臓器重量の評価法を比較し、臓器重量測定の有用性についての見解をまとめた。結果とし て、脾臓及び胸腺重量は免疫毒性、ストレス及び生理学的なかく乱の鋭敏な指標であると考えられ、さ らに脾臓及び胸腺の病理組織学的変化は臓器重量の変化と相関があるとされた。一方、これらの重量 測定にはほとんど価値がないという見解もあり、その理由として、胸腺では解剖時の技術的な問題や 加齢に伴う退縮、ストレスに関連した影響などによるばらつきの大きさが挙げられた。脾臓では、動物 間の変動性、ストレス関連の影響及び非げっ歯類(特にイヌ)における安楽死に関連した脾臓のうっ血 のような投与とは無関係の生理的な要因のため、その価値は限定的であると考えられた。さらにその 価値が限定的であると考える人には、脾臓及び胸腺の重量変化はしばしば病理組織学的所見と相関し ないことが多く、病理組織学的検査は被験物質投与による影響のより鋭敏な指標と考えられていた。

今回の STP 調査の回答では、一般的には、臓器重量は毒性試験における被験物質投与との関連性を 評価するために有用なスクリーニングツールであると考えられていた。しかし、個別の臓器ごとの評 価に関しては、実施した試験の目的に応じてその有用性の重要度については意見が大きく異なってい た。また、臓器重量データは、体重変化、被験物質の薬理作用、臨床病理学的データ、動物の絶食状態 または絶食した場合の情報及び肉眼的・組織学的所見などのデータを含めた試験全体の流れの中で評 価すべきであると強調する意見もあった。

番号	4-14
著者名	Marino DJ (2012b)
文献名	Age-Specific Absolute and Relative Organ Weight Distributions for Fischer 344
又瞅石	Rats
巻, ページ, 発行年	J Toxicol Environ Health A. 2012; 75(24): 1484-1516
分類 (①~④)	4
参照箇所の該当ページ	Abstract

フィッシャー344 (F344) ラットは、米国国立がん研究所 (NCI) 及び米国国家毒性プログラム (NTP) による毒性試験に使用される標準的なラット系統である。臓器重量の影響は、規制当局がヒトの健康 リスク評価の毒性参照値 (TRV) を設定するために使用する重要なエンドポイントであり、加えて相対 臓器重量を利用する生理学的薬物動態 (PBPK) モデルが TRV の策定に使用されるようになってきて いる。このように F344 ラットの集計された臓器重量は TRV 設定と PBPK モデリングに有益なデータ になり得るため、本研究では、NCI/NTP の混餌、飲水、吸入試験から、被験物質非投与の対照群の F344 ラットの利用可能なすべての絶対及び相対臓器重量データを収集し、週齢別にまとめた。

得られた知見が示しているのは,月齢に応じて絶対重量が増加し,相対重量が減少するのは脳,肝 臓,右腎,肺,心臓,甲状腺,右精巣である。結果によると,絶対臓器重量における一般的な変動傾向 は、小さいほうから脳,右精巣,心臓,右腎,肝,肺,胸腺,甲状腺の順であることが示唆される。

番号	4-15
著者名	Marino DJ (2012a)
文献名	Age-Specific Absolute and Relative Organ Weight Distributions for B6C3F1 Mice
巻, ページ, 発行年	J Toxicol Environ Health A. 2012; 75(2): 76-99
分類 (①~④)	4
参照箇所の該当ページ	Abstract

B6C3F1 マウスは、米国国立がん研究所(NCI)及び米国国家毒性プログラム(NTP)が実施する毒 性試験に使用される標準的なマウス系統である。臓器重量データは、規制当局がヒトの健康リスク評 価の毒性参照値(TRV)を設定するために使用する重要なエンドポイントであり、加えて相対臓器重量 を利用する生理学的薬物動態(PBPK)モデルがTRVの設定に使用されるようになってきている。こ のようにB6C3F1マウスの集計された臓器重量はTRV設定とPBPKモデリングに有益なデータになり 得るため、本研究では、NCI/NTPで実施した試験から、被験物質非投与の対照群のB6C3F1マウスの 利用可能なすべての絶対及び相対臓器重量データを収集し、週齢別にまとめた。

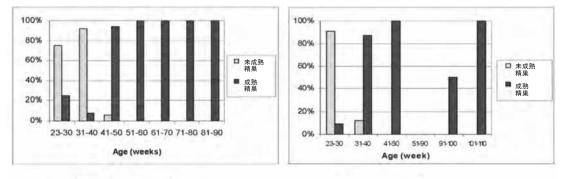
結果によると、月齢に応じて絶対重量が増加するのは、肝、右腎、肺、心臓であり、相対重量が安定 するのは、脳と右精巣であった。結果から示唆されるのは、絶対重量における一般的な変動傾向は、小 さいほうから脳、右精巣、右腎、心臓、肝臓、肺、脾臓、胸腺であった。

番号	4-16
著者名	Dorso L, et al
文献名	Variability in Weight and Histological Appearance of the Prostate of Beagle Dogs
又瞅泊	Used in Toxicology Studies
巻,ページ,発行年	Toxicol Pathol.2008; 36: 917-25
分類 (①~④)	4
参照箇所の該当ページ	Abstract, 923, Figure 13

2 つのブリーダーの生後 23 週齢から 108 週齢までの未処理の雄のビーグル犬の前立腺の重量と組織 学的所見を評価した。27 のガイドライン試験で得られた 125 頭の対照ビーグル犬のデータを使用した。 年齢,最終体重及び前立腺重量を測定した。前立腺について病理組織学的変化(腺房の発達,分泌量, 拡張や炎症のパターンなど)を観察し,必要に応じて等級付けを行った。前立腺の重量及び組織学的所 見に及ぼす年齢,最終体重及びブリーダーの影響,及び動物間の変動の程度を評価した。

前立腺は精巣から分泌されるテストステロンによって発達することから,精巣及び精巣上体の組織 学的検査により性成熟の評価を行った(図1)。その結果,前立腺の成熟度と精巣及び精巣上体の成熟 度との間には強い相関関係があることが明らかとなった。さらに,Harlan Franceの31-40週齢の動物は 90%が性成熟していたのに対し,Marshall Farms USA の同じ週齢の動物では10%しか性成熟しておら ず,ブリーダーにより性成熟期のずれがあることが示唆された。同じ週齢の平均最終体重はMarshall Farms USA より Harlan Franceの動物の方が統計学的に有意に高く,これは生産コロニーの遺伝的背景 が異なるためと考えられた。これらのことから,前立腺重量のばらつきの要因をまとめると以下のよ うになる。

- 性成熟期の個体差(Marshall Farms USAの動物は41-50週齢, Harlan Franceの動物では31-40週齢の 間に性成熟に達した)
- ② ブリーダーの違い(Harlan Franceの動物はMarshall Farms USAより同じ週齢でも体重が重く、早く性成熟を迎えていたが、同じ成熟段階の前立腺にブリーダー間の組織学的な差はなかった)



③ 同じ性成熟段階における個体差

## Marshall Farms USA

Harlan France

図 1. 週齢別の未成熟及び成熟精巣の割合: Harlan France のイヌでは,未成熟と診断された最高齢のイ ヌは 31 週齢であったのに対し, Marshall Farms USA のイヌでは 41 週齢であった。この結果から, Harlan France のイヌは Marshall Farms USA のイヌよりも早く性成熟を迎えることが確認された (Marshall Farms USA [n=97], Harlan France Harlan France [n=28])。

番号	4-17
著者名	Bailey SA, et al
文献名	Relationships Between Organ Weight and Body/Brain Weight in the Rat: What Is
义   江	the Best Analytical Endpoint?
巻,ページ,発行年	Toxicol Pathol. 2004; 32: 448-466
分類 (①~④)	4
参照箇所の該当ページ	Abstract, 461, 466, Table 3

本研究では 1992 年 1 月から 1999 年 7 月までの期間に Wyeth Research で実施された, Crl:CD<sup>®</sup>[SD]BR (Charles River Laboratories) ラットを用いた 26 本の 1 ヵ月間試験の対照動物(雄 307 匹,雌 266 匹) において,様々な臓器がどの程度,臓器重量と体重あるいは脳重量との間に比例関係があるかを調査 し,これらの結果に基づき,どのエンドポイント(臓器絶対重量,臓器重量/体重比または臓器重量/脳 重量比)が標的臓器毒性を正確に検出できるかを検証した。

その結果を表1に示す。雌雄の肝臓及び甲状腺重量は体重との間に比例関係が存在し、雌雄の副腎 及び雌の卵巣重量では脳重量との間に比例関係が認められた。従って、臓器重量/体重比は肝臓及び甲 状腺重量を評価するのに適しており、臓器重量/脳重量比は副腎及び卵巣重量を評価するのに適してい ると考えられた。脳、心臓、腎臓、下垂体及び精巣の臓器重量については絶対重量、臓器重量/体重比 または臓器重量/脳重量比を用いた解析に適合していないことから、共分散分析のような別の統計手法 を利用すべきであろう。

臓器	体重比	脳重量比	別の統計手法
副腎		$\checkmark$	
心臓			$\checkmark$
腎臓			$\checkmark$
肝臓	$\checkmark$		
下垂体			$\checkmark$
甲状腺	$\checkmark$		
精巣			$\checkmark$
卵巣		$\checkmark$	
旭			$\checkmark$

表1. 絶対臓器重量, 臓器重量/体重比及び臓器重量/脳重量比の最適な利用

✓統計解析で、このエンドポイントに相当するモデルがデータに適合していることを示す。
 このエンドポイントは、群間に体重差が存在する場合に臓器重量差の評価に推奨される。

✓統計解析で、モデルが絶対重量、臓器重量/体重比または臓器重量/脳重量比の解析に適合していないことを示す。臓器重量の解析には、別の統計手法を利用すべきである。

番号	4-18
著者名	Sellers RS, et al
文献名	Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies
巻, ページ, 発行年	Toxicol Pathol. 2007; 35: 751-755
分類 (①~④)	4
参照箇所の該当ページ	752, Appendix 1

毒性試験における臓器重量の評価は、医薬品、化学物質、医療機器の評価に不可欠である。米国毒性 病理学会は、7日間から1年間のGLPでの一般毒性試験において重量測定が推奨される臓器を提案す る(付表1)。

脾臓及び胸腺はげっ歯類を対象とした試験では測定すべきである。胸腺重量は非げっ歯類でも価値 があるが、生理的な胸腺の退縮は思春期以降のイヌやサル、特に期間が3ヶ月を超える試験では解釈 を複雑にする。さらに非げっ歯類の脾臓重量は麻酔の方法や放血の程度により影響を受けることがあ る。脾臓及び胸腺重量は、リンパ系臓器重量に固有のばらつきがあるため、常に病理組織学的結果と併 せて解釈すべきである。

ラット	マウス	非げっ歯類
肝臓	肝臓	肝臓
腎臓	腎臓	腎臓
心臓	心臓	心臓
副腎	副腎	副腎
月凶	月凶	月送
精巣	精巣	精巣
前立腺		
精巣上体		
脾臓	脾臓	
胸腺	胸腺	
甲状腺/上皮小体		甲状腺/上皮小体
下垂体		下垂体

付表1.GLPの一般毒性試験において重量測定が推奨される臓器(投与期間は7日間から1年間\*)

\*:発がん性試験は除く

番号	4-19
著者名	OECD (2001)
文献名	Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity
▲ 秋 秋 石	Studies
巻, ページ, 発行年	2002
分類 (①~④)	4
参照箇所の該当ページ	30-31, 33-34

### 体重変化、食物及び水の消費量

試験期間中の個々の動物や動物群の体重変化(増加または減少)を,対照群の変化と比較すること は,かなり重要な基準となる。体重変化は通常,飼料摂取量に関連しており,両者の評価を併せて行う ことが必要である。体重の減少は必ずしもそれ自体が毒性と関連しているとは限らない理由としては, 被験物質を含む飼料に対して動物の飼料に対する嗜好性が低下することにより,飼料摂取量に影響す ることがある。動物の忌避がみられた場合に餌の容器からの飼料のこぼしが増加することがあり,被 験物質摂取量について配慮が必要となる。被験物質を飲料水によって投与する場合も同様に考慮する 必要がある。

この忌避の作用は、多くの場合、最初の2~3週間の投与で明らかになるが、それ以降、これら動物 は被験物質を含む飼料に適応できるようになり、体重増加が徐々に回復することがある。その際、亜急 性毒性試験では、摂取した飼料1グラムあたりの個々の動物の増加量(食餌効率)が好転し、それに遅 れて投与群の体重増加が続く場合があり、被験物質の毒性とは無関係に被験物質投与群と対照群間に 投与初期に統計的に有意差を生じることがある。

被験物質の添加によって餌中の1つまたは複数の必須栄養素と相互作用することで、体重増加の減 少や毒性反応の変化が生じることもある。この現象が亜急性毒性試験で観察された場合には、それに 続く慢性毒性試験を開始する前に許容可能な手段(acceptable means)で克服することができる。まれに 対照群の体重増加が低いために、他の群の増加値が高いようにみえることがあります。

飼料組成,飼料及び水の摂取量,体重の増加は,代謝,ホルモン,恒常性維持機構の変化や病歴,成 熟などの動物の状態に重要な影響を与えることがあり,臓器やその他の重要なシステムに異常な所見 がないにもかかわらず,これらの悪影響が観察された場合には考慮すべきである。

### 臓器重量の絶対値と相対値

これまで, 亜急性毒性試験では, 肝臓, 腎臓, 体重の変化が毒性のエンドポイントであるとされてき た。一般的には, 臓器や体重の変化よりも病理組織学の方が最小中毒量を確定するのに適していると 考えられているものの, 臓器重量も通常, 絶対臓器重量及び相対臓器重量(体重ないし脳重量に対する 相対)が評価に用いられる。

実験者が制御可能なものと制御できない両方の要因(概日リズム,食物摂取量,脱水,食餌の性質,動物の年齢,臓器の作業量,ストレス,殺処分方法など)も,臓器重量や体重に影響する。

殺処分時に放血処理をしなかった場合には,肝臓や腎臓の絶対あるいは相対重量を減少させるが, 心臓,脳,脾臓重量には影響はないとされている。

被験物質が体重減少あるいは体重増加抑制に大きな影響を与えている毒性試験では、脳の相対量の

増加は評価上意味がなく、また、脳だけでなく他の臓器においても体重に依存して相対重量に影響した可能性があるため、これらは毒性影響による変化と区別するべきである。

また臓器重量変化に対する判断は,対照値との間の統計的に有意な差の有無だけでなく,病理組織 学的データや代謝・トキシコキネティクスデータを含めて考慮する必要がある。