

国内外の反復投与毒性に関するデータベース・毒性予測ツールの現状について

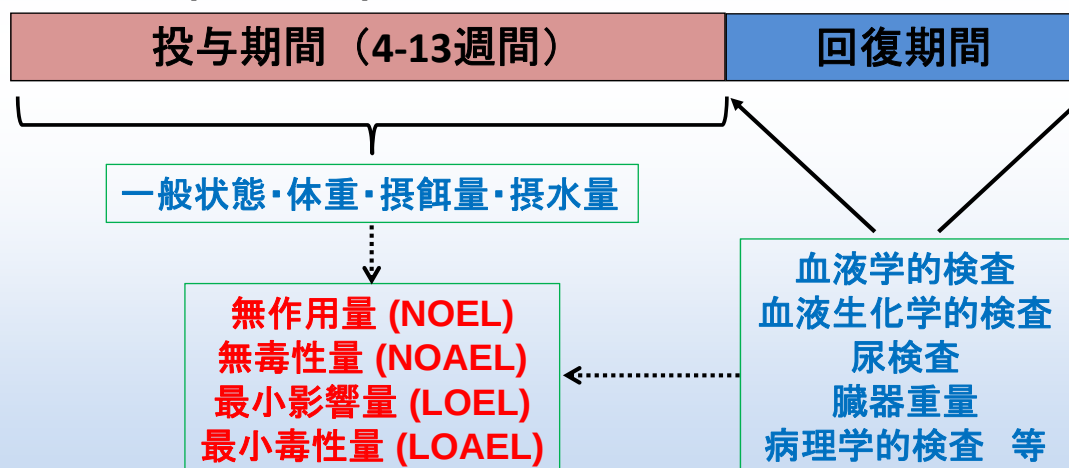
国立医薬品食品衛生研究所
安全性生物試験研究センター 安全性予測評価部
山田隆志

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反復投与毒性試験

動物に被験物質を一定期間反復投与したときに現れる生体の機能及び形態の変化を観察することにより、被験物質の毒性を明らかにすることを目的とする。

齧歯類(原則 ラット) 高・中・低用量群、対照群



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Challenge for future

Rodent Repeated Dose Toxicity

有害性評価支援システム 統合プラットフォーム

Hazard Evaluation Support System (HESS) Integrated Platform

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NEDO/METI委託事業 構造活性相関手法による有害性評価手法開発

- 実施期間: 2007-2011
- プロジェクトリーダー: 林 真 (食品農医薬品安全性評価センター)
- 参加機関(メンバー):
 - 国立医薬品食品衛生研究所
(広瀬明彦、本間正充、吉田緑、鎌田栄一、簾内桃子)
 - 製品評価技術基盤機構
(山田隼、櫻谷祐企、阿部武丸、西川智、小林克己、前川昭彦、張 慧琪、
田中雄四郎、長谷川隆一、山田隆志)
 - Bourges "Prof. Assen Zlatarov" Univ. (O. Mekenyan, S. Dimitrov)
 - 関西学院大学(岡田孝)
 - 富士通(株)(山下辰博、酒井宏太)
 - 東北大学(山添康、吉成浩一)

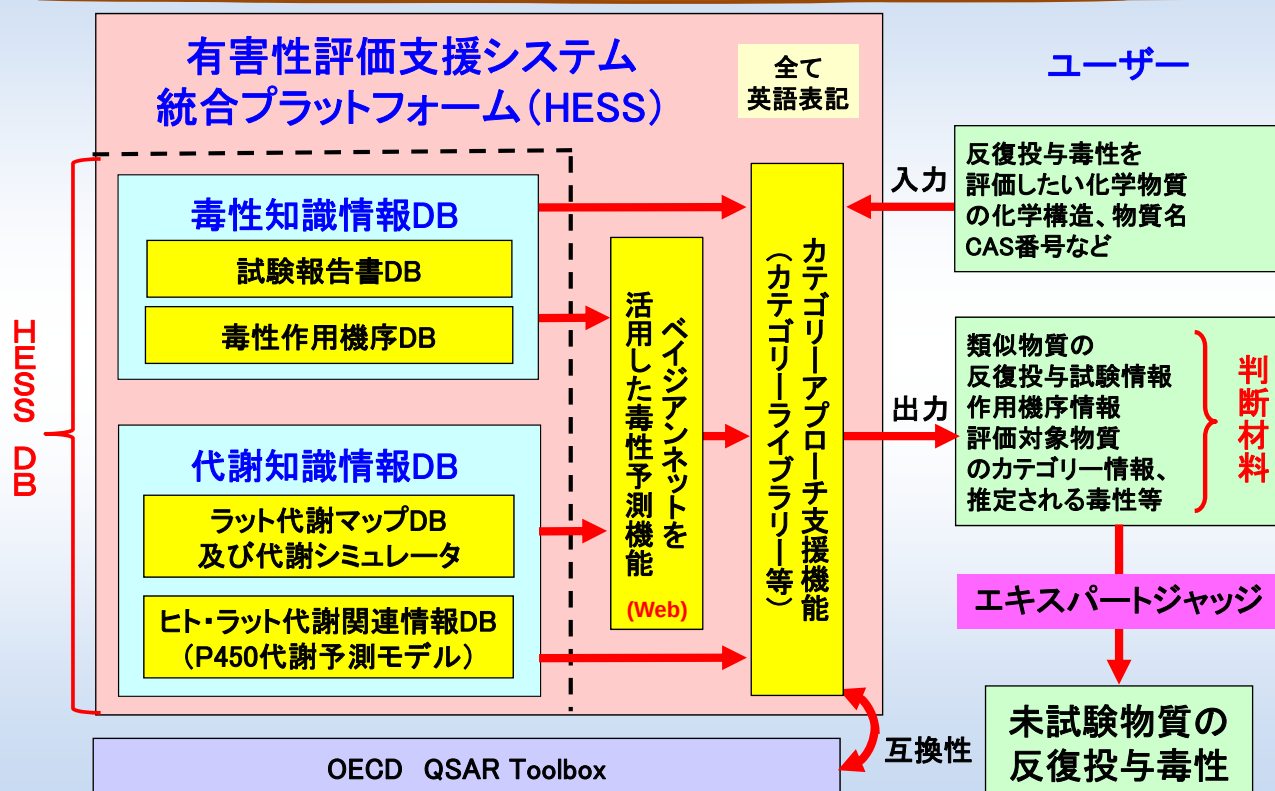
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開発方針

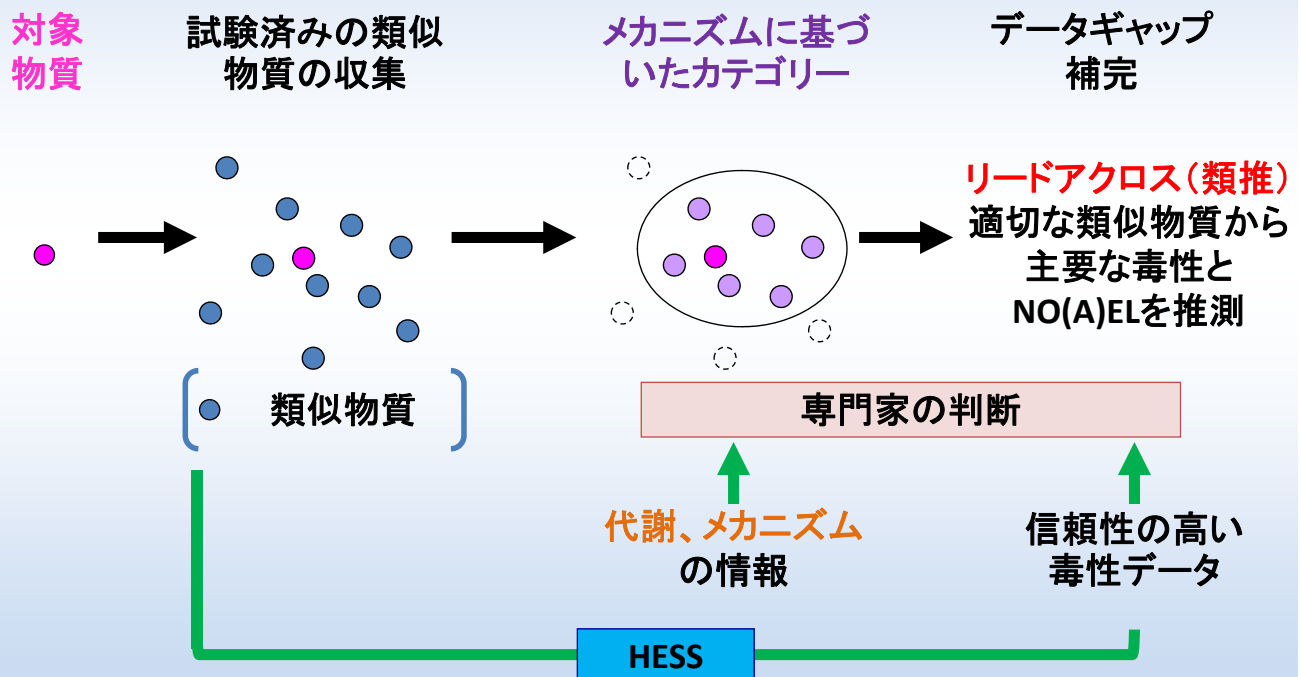
- 専門家の判断をサポートするためのシステムを構築する(システムが判断を下すのではない)。
- 毒性、病理そして総合的なリスク評価を行う専門家が主導的な役割を果たす(システムの専門家主導ではない)。
- 国際的に利用されるものを目指す(OECD QSAR Toolboxの開発者をメンバーに加え、OECDと連携しつつ研究開発を実施)。

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開発システムの構成



反復投与毒性のリードアクロス(類推)の ワークフロー



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1. 毒性知識情報 DB

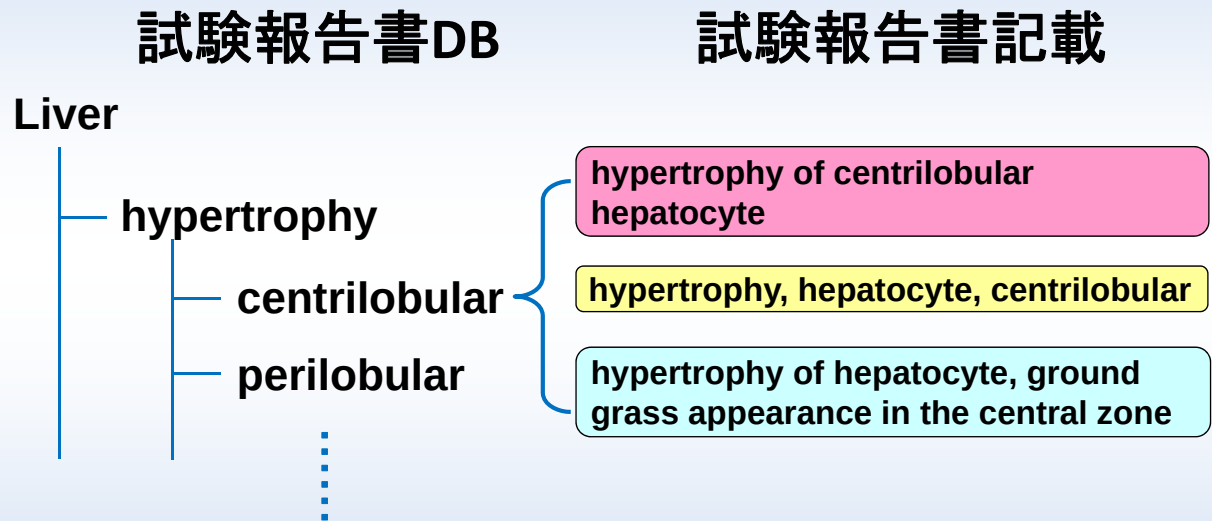
1-1. 試験報告書 DB

- 充実したデータセットを持つ我が国の化審法既存点検『28日反復投与毒性試験報告書』を中心に、GLP基準の試験報告書を採用(約800試験)
- 用量反応データを収載(血液学、血液生化学、臓器重量、病理所見など)
- 毒性・病理専門家の意見や化学物質審議会判定に基づく有意差マーク(フラグ)を付与して、毒性学的な注意喚起を図った。

Item	Unit	0 mg/kg		20 mg/kg				100 mg/kg				500 mg/kg			
		mean	SD	mean	SD	sgnif.	F	mean	SD	sgnif.	F	mean	SD	sgnif.	F
BUN	mg/dL	12.6	1.0	15.0	2.0			12.9	1.3			15.6	2.2	*	▲
Creatinine	mg/dL	0.52	0.04	0.53	0.02			0.48	0.04			0.47	0.04		
T.cholesterol	mg/dL	86	17	86	19			138	25	**	▲	212	15	**	▲
T.bilirubin	mg/dL	0.37	0.04	0.42	0.03			0.48	0.04	**	▲	0.59	0.08	**	▲

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病理所見用語の統一 (病理所見のシソーラス)



- ・ 83臓器・11302所見のシソーラスを作成
- ・ DBの検索エンジンに搭載

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1. 毒性知識情報 DB

1-2. 毒性作用機序DB

変性・壊死など重篤な毒性のメカニズム情報を収載
(標的: 赤血球、肝臓、腎臓、精巣、神経、膀胱、甲状腺)

<レファレンス情報>

- Reference

<メカニズム要約>

- Summary

<メカニズム情報>

- Possible chemical reaction /metabolism
- Possible toxicant
- Possible interaction with bio-molecule
- Effects
- Target cell/ tissue/ organ etc.

<試験情報>

- Cell line/ Species
- Experimental design
- *in vitro* / *in vivo* / *ex vivo*
- Concentration / dose employed

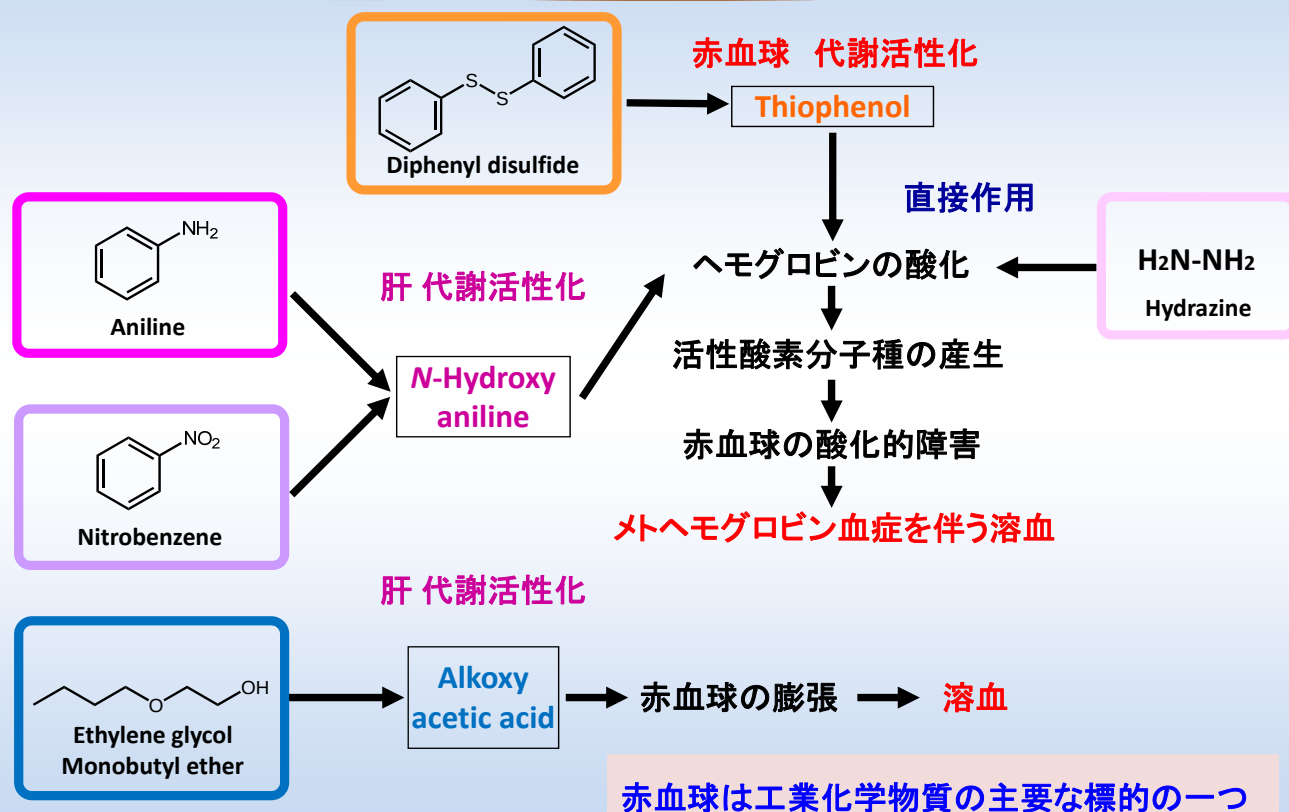
<その他>

- Effective concentration/ Dose
- Related compounds studied
- Additional information
- Authors' proposal (opinion)

(130物質、260 レファレンス)

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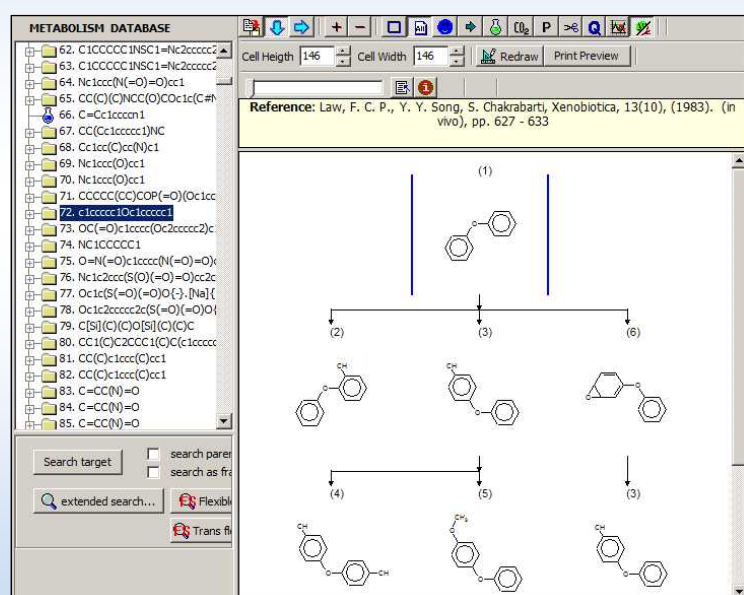
メカニズムに基づいたカテゴリー(溶血性物質)



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2. 代謝知識情報 DB

2-1. ラット代謝マップと代謝シミュレータ



ラット肝を中心とした
代謝マップのDB
(1000 化合物, 1200 マップ)

Liver Metabolism
Simulator

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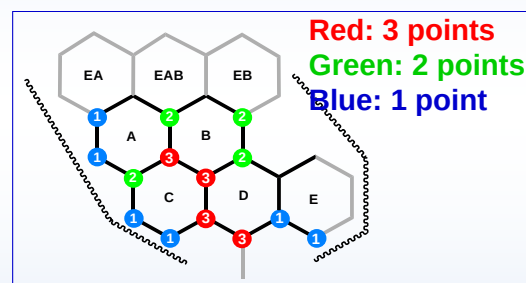
2. 代謝知識情報 DB

2-2. ヒト / ラット 代謝関連情報DB

- ABSORPTION** Absorption rate, Cmax, Tmax
Involvement of transporter
- DISTRIBUTION** Apparent volume of distribution
Time-dependent changes by repeated doses
Brain → Blood-brain barrier, Adipocyte → storage
Liver → Metabolism, Kidney → Urinary excretion
Kidney → Binding to protein
Organs with higher concentration of chemicals than blood
Involvement of transporter
- METABOLISM** Related enzyme and molecular information
Contribution ratio, Cellular fraction, Metabolite
Species differences, Strain differences
- EXCRETION** Excretion rate, Involvement of transporter
Species differences, Strain differences

Result of interaction, inhibition, enhanced of enzyme
Relationship of toxicity test

リガンド構造に基づいた
P450代謝予測モデル



ヒト CYP2E1 モデル
(Yamazoe et al, 2011)

(350 chemicals, 500 references)

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3. ベイジアンネット毒性予測モデル

Repeated Dose Toxicity Test Report

Analysis

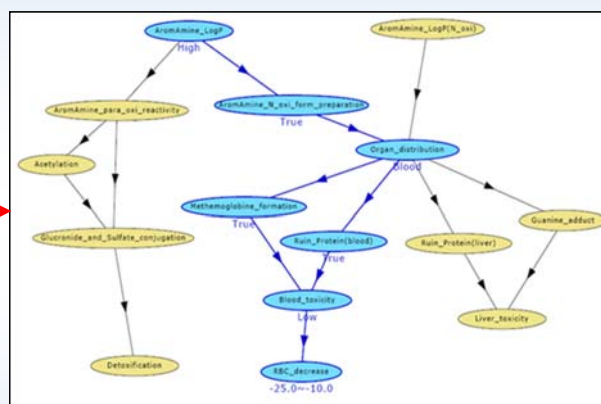
Cascade
Model

Basic Active
Structure
Knowledge
Base

BASiC

<http://www.dm-lab.ws/BASiC/>

e.g., Liver and blood toxicity model



Causality of Toxicity

- Knowledge of toxicologist
- Toxicity knowledge DB
- Metabolism knowledge DB

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各所見のNOELとLOELをデータベース化

Item	Unit	0 mg/kg		20 mg/kg				100 mg/kg				500 mg/kg			
		mean	SD	mean	SD	sgnif.	F	mean	SD	sgnif.	F	mean	SD	sgnif.	F
BUN	mg/dL	12.6	1.0	15.0	2.0			12.9	1.3			15.6	2.2	*	▲
Creatinine	mg/dL	0.52	0.04	0.53	0.02			0.48	0.04			0.47	0.04		
T.cholesterol	mg/dL	86	17	86	19			138	25	**	▲	212	15	**	▲
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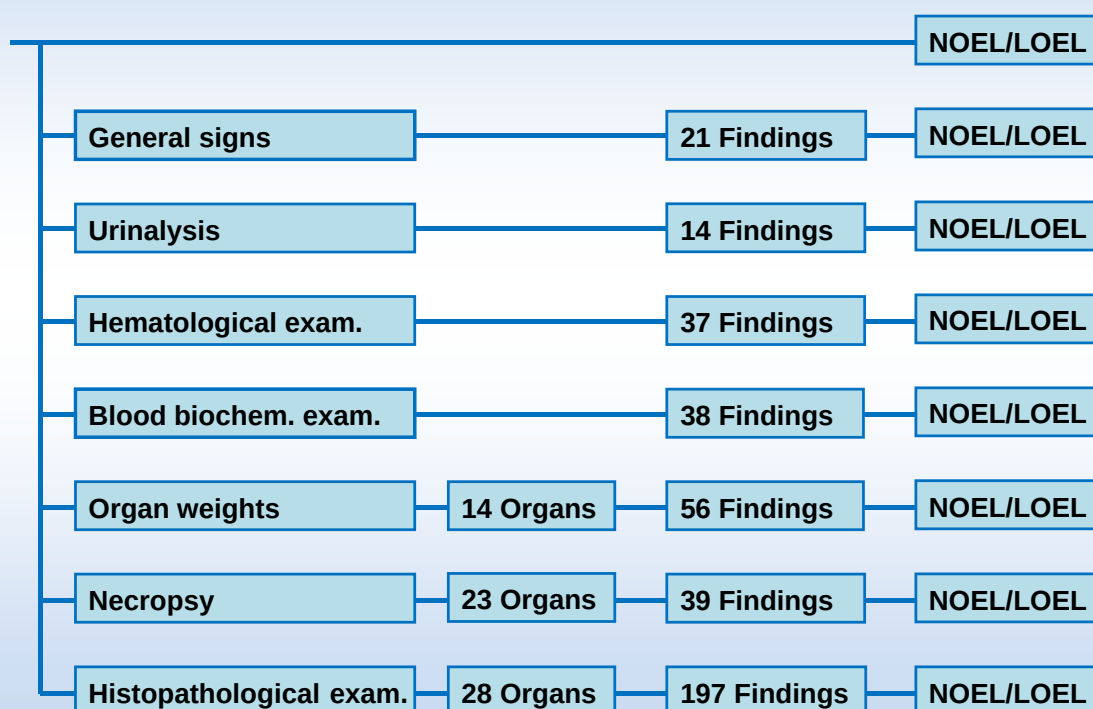


(試験報告書DB)

BUN・・・・・・・・ NOEL 100mg/kg, LOEL 500mg/kg
 Creatinine・・・・ NOEL 500mg/kg
 T. cholesterol・・・ NOEL 20mg/kg, LOEL 100mg/kg
 T. bilirubin・・・・ NOEL 20mg/kg, LOEL 100mg/kg

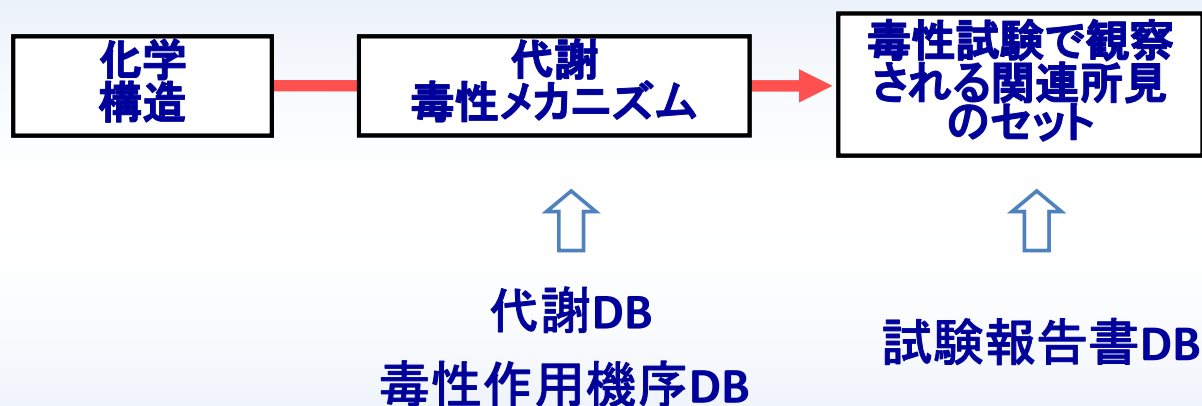
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反復投与毒性試験結果のデータ構造



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Mode of Action/Adverse Outcome Pathwayを ベースにした反復投与毒性のカテゴリーの構築

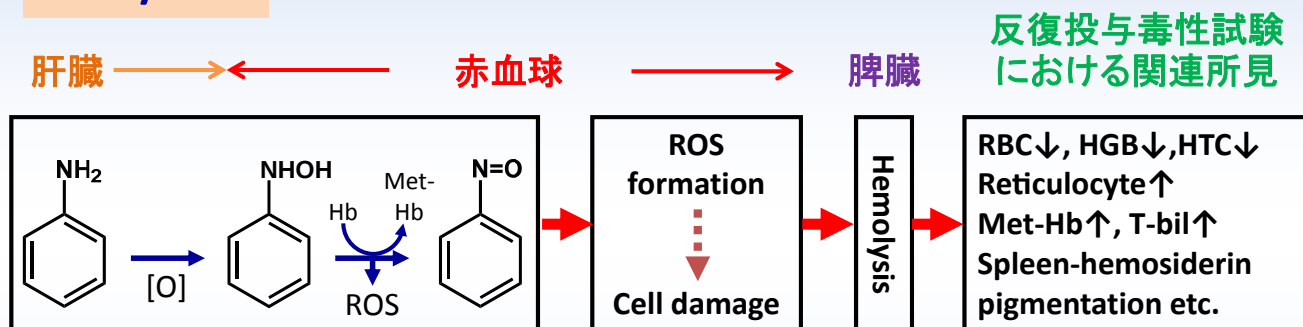


類似したメカニズムをもつ物質をカテゴリー化する。

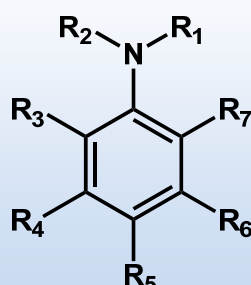
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アニリン溶血性貧血カテゴリー

MoA/AOP



Structural boundary



$R_1, R_2 = \text{H, methyl or ethyl.}$
 $R_3 \sim R_7 = \text{H, alkyl, halo, alkoxy, NO}_2 \text{ or NH}_2$

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Screenshot of HESS

Hazard Evaluation Support System

Chemical name: allyl valerate; pentanoic acid, 2-propenyl ester
CAS No. 6321-45-5
SMILES: C=C(C)CC(=O)OCC=C

対象物質 試験済みの類似物質

所見

試験データ (NOEL/LOEL)

Link

候補となるカテゴリー

4 Allyl esters (Hepatotoxicity) Rank A (Repeated dose (HESS))

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関連情報へのリンク

代謝

Metabolism profiling...

Reference: Kohn, C., Buchhorn, J., Lutz, J., (1973), (in press), pp. 1180-1181

メカニズムに基づいたカテゴリーの構築

適切な類似物質の試験データの確認

MoA/AOP

Summary of Mechanistic Information

Allyl alcohol is readily oxidized to allyl hydroperoxide (AHP) and allyl hydroperoxide (AHP) is a reactive intermediate. AHP is a reactive intermediate that can cause oxidative stress and cellular damage. AHP is a reactive intermediate that can cause oxidative stress and cellular damage. AHP is a reactive intermediate that can cause oxidative stress and cellular damage.

Figure 1. Hepatotoxic pathway induced by allyl acetate.

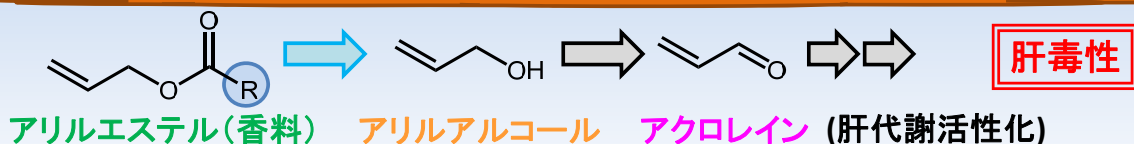
毒性試験結果

Study: HESS Search

Test Item	mg/kg	Actual	Control	Standard Error
DOSE	0	10	10	10
No. of animals	10	10	10	10
BUN	mg/dL	16.2	14.3	0.4
CAN	mg/dL	0.77	0.70	0.02
T-CHO	mg/dL	0.77	0.70	0.02
ALB	g/dL	4.5	4.4	0.1
AST (GPT)	U/L	88	82	5
ALT (GPT)	U/L	626	588	9
LDH	U/L	140	140	5

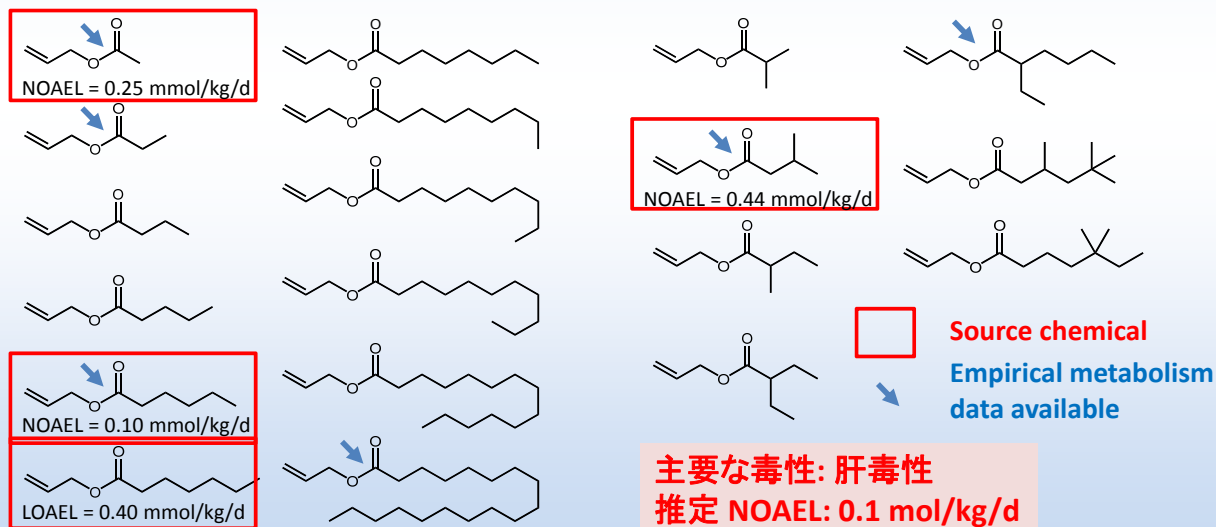
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ケーススタディ: カテゴリーの構築とリードアクロス



肝毒性

Metabolic hydrolysis: critical key event directly linked to the primary adverse outcome



Reviewed by OECD IATA Case Studies Project 2015

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データ更新・共有

厚労省: 化審法既存点検試験報告書
OECD SIDS 初期評価報告書等

追加

HESS RDT DB
(Core DB)



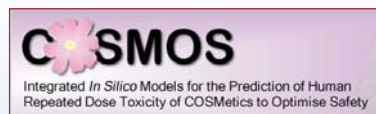
共有



DB/カテゴリー
提供



(後述)



(後述)

毒性試験
データ提供

厚労省 Toxicogenomics Project/
Toxicogenomics Informatics Project
経産省 ToxOmics

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RepDose : 化学物質の反復投与毒性データベース


Fraunhofer
ITEM



The database for the analysis of relationship between chemical function groups/categories and target organs in repeated dose studies.

[User guide](#)
[About RepDose](#)
[Contact](#)
[Impressum](#)
 Please enter your email and password :
 Email:
 Password:
[Log In](#)
[Password forgotten](#)
[New member](#)

Project partner





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物質名、動物種、雌雄、経路、期間、標的臓器、影響、LOEL...

工業化学物質
約1200物質、3400試験

(<http://fraunhofer-repdose.de/>)

CAS	Name	Species	Sex	Route	Duration (days)	study NOEL (mg/kg bw/d)	study LOEL (mg/kg bw/d)	Organ/Target	Effect	Sex (effect)	effect LOEL (mg/kg bw/d)
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	clinical symptoms	ataxia	male	36,000
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	clinical symptoms	hyperexcitability	male	36,000
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	clinical symptoms	moving uncoordinated	male	36,000
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	testes	degeneration	male	36,000
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	testes	sperm parameters	male	36,000
79-06-1	Acrylamide	mouse	male	gavage	56	36,000	36,000	testes	weight decreased	male	36,000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	brain	tumour	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	clinical symptoms	mortality increased	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	nervous system	degeneration	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	oral cavity	tumour	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	spinal cord	tumour	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	testes	tumour	male	0.500
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	thyroid gland	tumour	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.100	0.500	stomach	tumour	female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.500	1.000	clinical symptoms	mortality increased	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.500	1.000	mammary gland	tumour	female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.500	1.000	nervous system	degeneration	male & female	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.500	1.000	testes	tumour	male	2.000
79-06-1	Acrylamide	rat	male & female	drinking water	730	0.500	1.000	thyroid gland	tumour	male & female	1.000
79-06-1	Acrylamide	rat	male & female	drinking water	30	0.200	1.000	adipose tissue	weight decreased	male & female	20.000

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OECD QSAR Toolbox

■ カテゴリーアプローチによるデータギャップ補完を支援するためのシステム

(<http://www.qsartoolbox.org/>)。

■ 種々のエンドポイントの実測試験データや、カテゴリー化のための支援ツールが搭載されている。

QSAR Toolbox 3.2.0.103 [Document_1]

QSAR TOOLBOX

Input Profiling Endpoint Category Definition Data Gap Filling Report

Categorize Define Subcategorize Combine Clustering Delete Delete All

The OECD QSAR Toolbox for Grouping Chemicals into Categories
Developed by LMC, Bulgaria

Grouping methods

- General Mechanistic
 - Biodeg BioC half-life (Biowin)
 - Biodeg primary (Biowin 4)
 - Biodeg probability (Biowin 1)
 - Biodeg probability (Biowin 2)
 - Biodeg probability (Biowin 5)
 - Biodeg probability (Biowin 6)
 - Biodeg probability (Biowin 7)
- Defined Categories
 - Document_1
 - [457] SN1<AND>SN1 >> Nitrenium Ion forma
 - [271] Subcategorized: DNA binding by OE

Structure

1 [target]	2	3	4	5
<chem>CC1=CC=CC=C1</chem>	<chem>ClC1=CC=CC=C1</chem>	<chem>CC1=CC=CC=C1</chem>	<chem>Nc1ccccc1</chem>	<chem>Nc1ccccc1</chem>
TA 100 (236/236)	M: Negative	M: Negative	M: Negative	M: Negative
TA 100(1,8-DNP6) (1/1)				
TA 102 (16/16)		M: Negative		
TA 104 (5/5)		M: Negative		
TA 1535 (111/111)	M: Negative	M: Negative	M: Negative	M: Negative
TA 1537 (96/96)		M: Negative		M: Negative
TA 1538 (62/62)				M: Negative

271 Subcategorized: DNA binding by OECD

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QSAR Toolbox: ヒト健康影響のデータベース



化学物質規制に関連したエンドポイント:
細菌変異原性、発がん性、細胞形質転換、
生殖発生毒性、ECHA CHEM、エストロ
ゲン受容体結合親和性、遺伝毒性、ヒト
半減期、小核、反復投与毒性 など

反復投与毒性では2つのデータベース
が登録されている。



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カテゴリープロファイラー

カテゴリーのリスト

構造アラートとメカニズムの記述 (遺伝毒性の例)

Structural alert: Primary aromatic amine
 $R-NH_2$
 R = aromatic carbon atom

Mechanism
 Primary aromatic amines undergo metabolism to a reactive nitrenium ion. This ion can bind to DNA via an S_N1 mechanism (Kalgutkar et al 2005, Jones et al 2003).

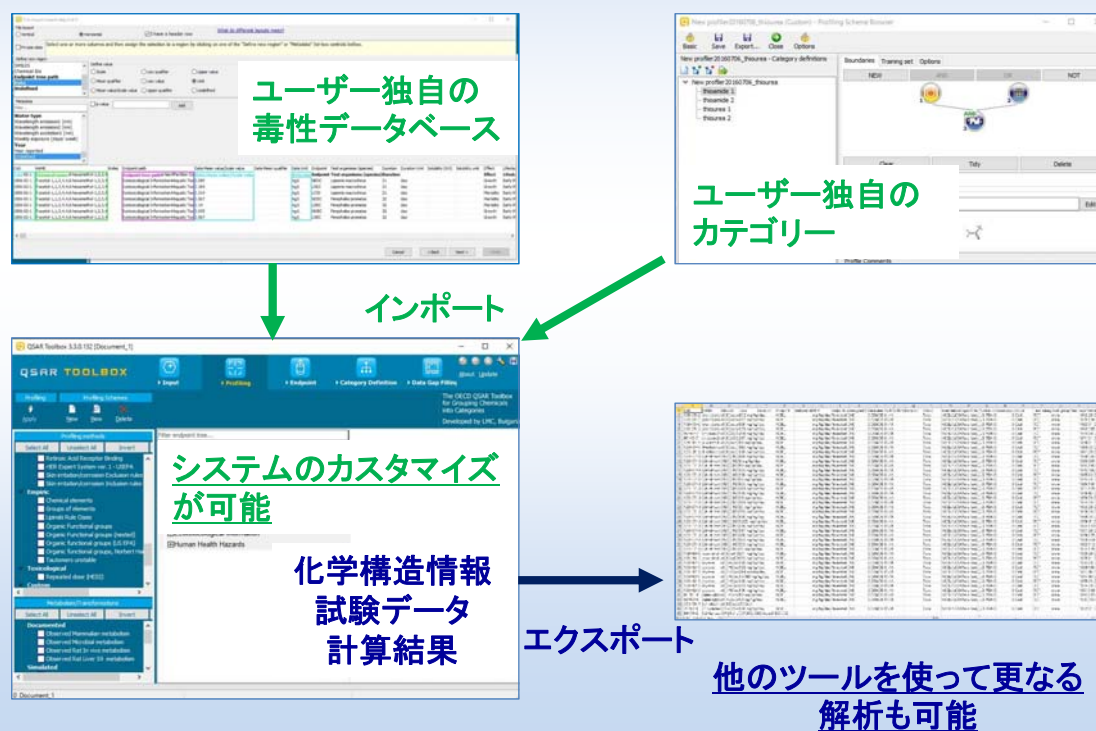
Structural alert mitigating factors
 The following mitigating factors have been identified (Benigni et al 2008). The presence of sulphate (SO_3H) groups in any ring position. This group probably causes increased solubility and thus leads to increased detoxification.

- Substituents in both the 2 and 6 position on the six membered aromatic ring. This causes steric hindrance which presumably prevents the initial metabolism step thus preventing the formation of the nitrenium ion.
- The presence of a carboxylate group (CO_2H) in the 2 position. This could be due to the formation of a six membered intramolecular hydrogen bonded ring which inhibits the initial metabolic transformation.

References
 Benigni R. et al (2008) Mutation Research, 659, p248-261
 Jones CR et al (2003) Chemical Research in Toxicology, 16, p1251-1263
 Kalgutkar AS (2005) Current Drug Metabolism, 6, p161-225
 Kalgutkar AS et al (2005) Current Drug Metabolism, 6, p161-225

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データのインポート・エクスポート



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COSMOS



Integrated *In Silico* Models for the Prediction of Human Repeated Dose Toxicity of COSMeTics to Optimise Safety

SEURAT-1: 反復投与毒性試験の
代替法開発プロジェクト

HOME ABOUT COSMOS RESULTS OVERVIEW PUBLICATIONS BACKGROUND USEFUL LINKS CONTACT

COSMOS - a European Union project developing methods for determining the safety of cosmetic ingredients for humans, without the use of animals, using computational models.

COSMOS was one of seven projects forming the SEURAT-1 cluster, SEURAT being a European research initiative with the long-term goal of achieving "Safety Evaluation Ultimately Replacing Animal Testing".

COSMOS was a unique collaboration addressing the safety assessment needs of the cosmetics industry, without the use of animals. The main aim of COSMOS was to develop freely available tools and workflows to predict the safety to humans following the use of cosmetic ingredients.

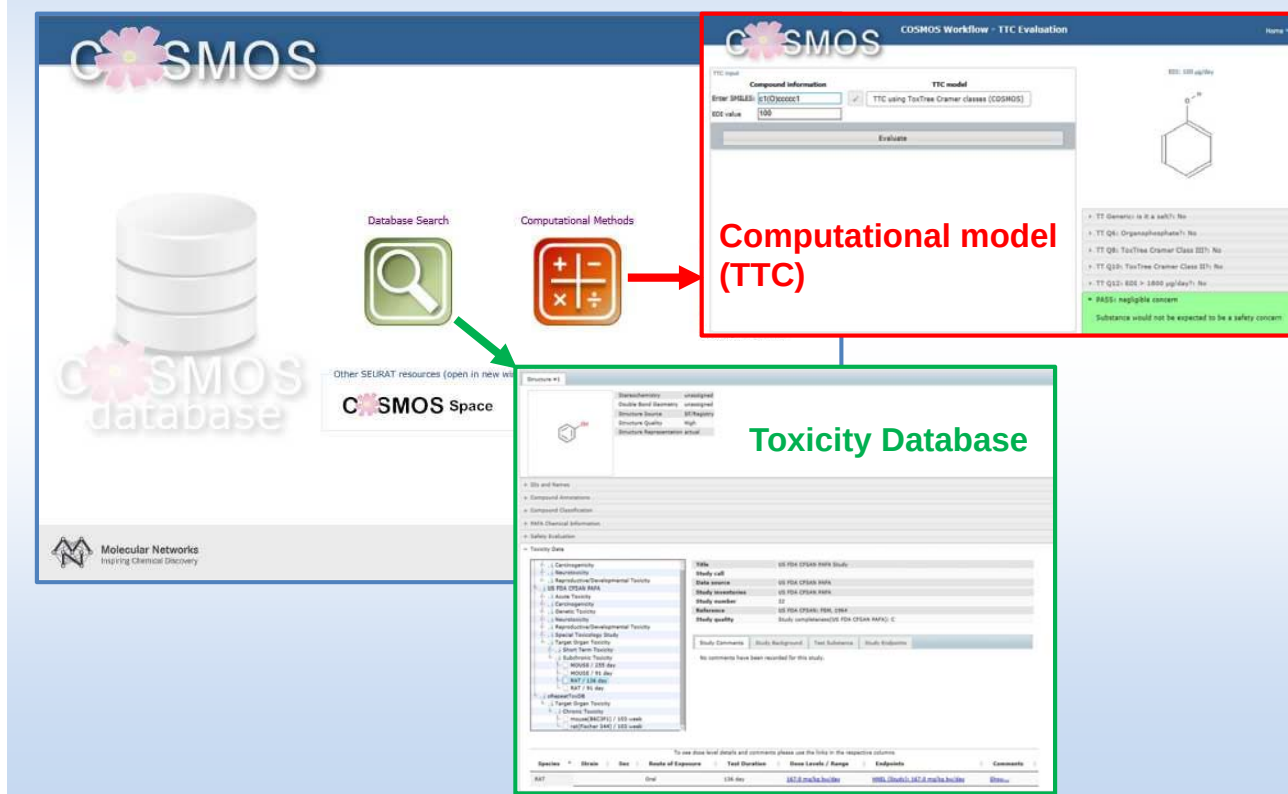
The project ran from January 2011 - December 2015.

Major results and links to the legacy tools are available from this website.



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COSMOS (cont.)



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electronic Toxicity: eTox



主要製薬企業、研究所等
コンソーシアムを形成

etox

Integrating bioinformatics and cheminformatics approaches
for the development of expert systems allowing the
in silico prediction of toxicities

Start January 2010 (5+2 years project)
18.7 M€ over 7 years



www.etoxproject.eu

efpia



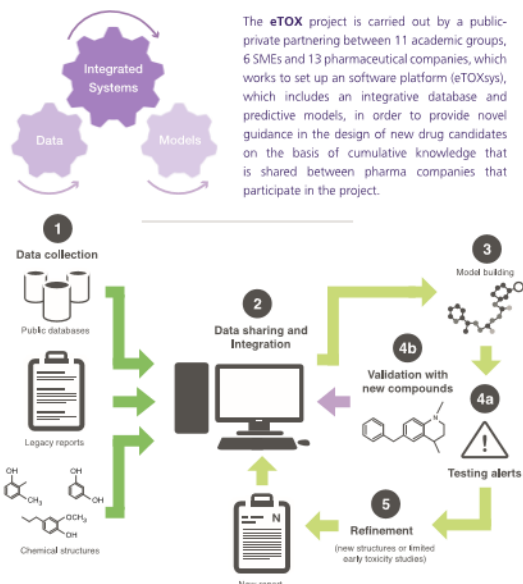
imi innovative medicines initiative

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eTox (cont.)

eTOX aims to develop innovative methodological strategies and novel software tools able to predict the in vivo toxicology of new molecular entities by **sharing and exploiting legacy toxicological information stored in the archives of the participating pharma companies**

データ共有(と保護)



Achievements up to date

Creation of a database of toxicology-related data combining public data and legacy reports from participating pharmaceutical companies. **Over 7,000 study records available in the eTOX Database corresponding to 4,749 reports and 2,176 compounds.**

Development of a common ontology to allow mapping of the terms used across the different companies and also in public literature to a single preferred term essential for cross-study data analyses. **Ontology of over 10 million verbatim terms.**

At present eTOXsys includes **74 in silico models** for diverse endpoints: 28 on ADME, 5 on transporters, 2 on physicochemical properties, 2 on carcinogenicity, 2 on genotoxicity, 16 on organ toxicities, and 19 on safety pharmacology.

eTOXsys incorporates a powerful, flexible and user-friendly engine for interrogating the database on the basis of any of their chemical, biological or toxicological properties, or combinations of them. **Version 2.0 released in May 2014.**



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innovative medicines initiative

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毒性データベース・毒性予測ツールの食品健康影響評価への活用

- リスク評価の優先順位付け
- 微量で多種類の物質を短期間で包括的に評価する必要がある場合
- 毒性試験が困難な場合(不純物、分解物、代謝物など、データがない場合の補完)
- リスク評価の高度化(メカニズム、人への外挿性)

試験報告書・文献・資料

毒性データベース

NO(A)EL

毒性プロファイル

毒性予測ツール

代謝シミュレータ

カテゴリー・予測モデル

MoA/AOP

国際的な動向を把握しつつ、
範囲を指定して活用経験を積む。

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ご清聴ありがとうございました。