EFSA ジャーナル(2005)282、1～31

遺伝毒性および発がん性を有する物質のリスク評価に対する調和のとれたアプローチに関する EFSA からの要請についての科学委員会の見解

(要請番号 EFSA-Q-2004-020)

(採択日 2005 年 10 月 18 日)

概要

科学委員会(Scientific Committee)は欧州食品安全機関(European Food Safety Authority)より、遺伝毒性および発がん性を有する物質のリスク評価について調和のとれたアプローチを提案するよう要請された。これらの物質は、生体細胞内の遺伝物質(DNA)と直接的に相互作用し、がんを引き起こす可能性がある。当該物質に関しては、特に日常的に消費される場合、少量であっても曝露に関連したリスクが生じる可能性があることから、いかなる曝露も好ましくないものであることが広く想定されている。影響は総曝露量に依存するが、本見解では食品からの曝露に焦点を当てる。

現在、遺伝毒性および発がん性を有する物質のリスク評価に最良のアプローチに関して国際的な科学的合意は得られておらず、世界各国で各種アプローチが使用されている。多くの国々において、特に欧州連合(European Union)内では、リスク評価者のリスク管理者に対する勧告は、当該物質への曝露を合理的に達成可能な限り低い(ALARA 原則として知られる)レベルまで低減するというものである。しかし、このような勧告はリスク管理者に対して、必要と思われる対策の緊急性または範囲のいずれに関しても措置の優先順位付けの基準を示していないことが認識される。

遺伝毒性および発がん性を有する物質のリスク評価に現在使用されているアプローチのいくつかは、各発がん物質ではその発がん性が異なること、すなわちこれらの物質は一定の投与量における腫瘍誘発の可能性が異なるという事実を考慮に入れている。発がん性に関する情報は、ヒトのデータがほとんど入手できないことから、大半は齧歯類での実験室試験から得られている。これらの試験では、検出可能かつ統計的に有意な腫瘍発生率が同定されるように動物を被験物質(1 つあるいは複数の物質)に高用量で生涯の大半に相当する期間曝露する。ヒトにおよぼし得る影響に関する勧告を提示するには、これらの動物での結果の有意性を、通常は実験室試験で使用される用量よりはるかに低いヒトでの曝露レベルの文脈において解釈しなければならない。

動物での試験における高用量をヒトでの低レベル曝露するに外挿する試みでは、単純な直線外挿法から極めて複雑な外挿法まで、広範なモデルが開発かつ使用されている。この結果、選択するモデルによって、同じ物質に関して異なる結論が引き出されている。さらに、特定の物質に関しては、選択したモデルが実際に基礎となる生物学的過程を反映しているか否かは明らかではない。したがって科学委員会は、曝露マージン(MOE)アプローチとして知られる別のアプローチの使用を勧告する。

MOE アプローチでは、しばしば動物での試験から得られ、かつ低レベルではあるが動物で測定可能な反応を誘発する用量に一致する基準点を使用する。次いでこの基準点を消費パターンの差を考慮して、ヒトにおける各種食事摂取量の推定値と比較する。

科学委員会は MOE の導出にはベンチマークドーズ(BMD)の使用を勧告する。ベンチマークドーズは実験データの観察範囲内で数学的モデリングにより動物データから得られる標準化した基準点である。これは、実験用量の範囲全体から得られる情報をすべて使用する。

参考資料 4

科学委員会は、げっ歯類において腫瘍発生率 10%に一致する BMD(ベンチマークドーズ)上の95%信頼区間の下限を示すBMDL10(ベンチマークドーズ信頼下限10%)の使用を勧告する。科学委員会は、ベンチマークドーズアプローチは、有効な場合、ヒトのデータにも適用可能であることを指摘する。

データがベンチマークドーズの算出には不適切な場合、腫瘍発生率 25%に対応する用量を表す T25 を使用することが勧告される。

科学委員会は、ヒトでの摂取量推定値の選択について、全集団や集団内の特定の群などに関して、考慮する物質と食事におけるその物質の分布に応じた異なる曝露シナリオを提示することを勧告する。すべての測定値はそれらに固有の不確実性とともに示すものとする。

科学委員会は、遺伝毒性および発がん性を有する物質が食品中に遺伝毒性および発がん性の残留物を残留させる場合、それらの物質の食品への意図的な添加あるいはフードチェーンの初期段階での使用を認可すべきではないとの見解である。曝露マージンアプローチは、遺伝毒性および発がん性を有する物質が、その起源に関わらず食品中に検出され、曝露している、または曝露した人々への潜在的リスクに関するガイダンスが必要な場合にのみ適用すべきである。

科学委員会は MOE の解釈方法についてのガイダンスを提示する。種間差(動物とヒトとの差)、種内差(ヒトの個体差)、発がん過程の特性、用量反応曲線上の基準点が考慮された。科学委員会は、動物での試験で得られた BMDL10 を基準にした場合、一般に 10,000 以上の MOE は、公衆衛生の観点からは問題は少なく、リスク管理措置の優先順位は低いものと見なされる可能性があるという見解である。しかし、このような判断は最終的にはリスク管理者が扱う問題である。さらに、この規模の MOE ではヒトでの曝露を低減させるリスク管理措置の適用を除外すべきではない。

キーワード

Risk assessment, carcinogenicity, genotoxicity, benchmark dose, margin of exposure

付託事項

科学委員会は欧州食品安全機関により遺伝毒性および発がん性を有する物質のリスク評価に対する調和のとれたアプローチに関する見解の提出を要請された。

背景

遺伝毒性および発がん性を有する物質のリスク評価に関して現在欧州連合内および世界規模でいくつかのアプローチが使用されている。適切なヒトの疫学的データはほぼ全ての事例において入手不可能であることから、高用量の動物バイオアッセイからのデータが使用されており、ヒトが通常曝露する低レベルへの外挿が必要である。しかし、各種外挿方法の背景にある科学には極めて多くの議論があり、多くの場合、遺伝毒性および発がん性を有する物質への曝露は合理的に達成可能な限り低い(ALARA)ことが勧告される(欧州委員会 [European Commission] SCF、2001、2002a、2002b、2002c による)。ALARA などの勧告はリスク管理者に対して、必要と思われる措置の緊急性または範囲のいずれに関しても行動の優先順位付けの適切な基準を提示しないことを認識すべきである。さらに、より低レベルの検出限界を得ることで分析法の感度および特異性が向上し、遺伝毒性および発がん性を有する物質を含む食品中の検出物質数が増加すると思われる。全体として、欧州食品安全機関の科学委員会および科学パネルがリスクを評価する場合、調和のとれた、科学的で透明性の高い、正当と認められるアプローチが必要なことは明白である。

1. はじめに

リスク評価過程はいかなる物質においても、ハザード関連情報整理、ハザードによる健康被害解析、曝露評価、リスク特性解析を含むいくつかの段階で構成される。リスク特性解析は、ハザードによる健康被害解析および曝露評価に関する入手可能データからの情報を意思決定での使用に適した勧告に統合するリスク評価の段階である(Renwick *et al.* 2003)。本見解は、リスク特性解析の部分に関する具体的なアプローチを扱うものであり、物質の遺伝毒性および発がん性の有無をエビデンスの重要性に基づいて決定するハザード関連情報整理およびハザードによる健康被害解析の段階は特に取り上げていない。ハザード関連情報整理およびハザードによる健康被害解析に関わる科学的知識は EU プロジェクト FOSIE(Food Safety in Europe、2002)の中で別の主題と共に詳細に述べられており、ここではさらなる検討は行わない。

食品安全性の最も困難な問題の一つは、発がん性および遺伝毒性を有する物質が食品中に存在し、それらを容易に除去あるいは回避できないことが認められる場合に、ヒトの健康に対する潜在的リスクについて勧告することである。

好ましくない物質は、食品中に発生する(たとえば、食用植物中に固有の天然成分として、あるいは環境中にある物質、真菌汚染、製造過程を介した汚染で生じる物質として)。ALARA(合理的に達成可能な限り低い)原則は、このような物質が発がん性および遺伝毒性のハザードであることが示された場合、それらの物質への曝露を最小にすることが一般に必要であることを提示している。科学委員会の本見解では、ALARA 原則を超えたアプローチを扱い、食品中に存在し、遺伝毒性および発がん性を有する特定の物質の毒性および発がん性レベル評価を可能にするものである。このようなアプローチは、これら物質すべてへの曝露を最小にするアプローチに代わるものではない。本アプローチでは、資源が限られている場合に、ヒトへのリスクが最大の物質を最優先することを確実にする。

科学委員会は、遺伝毒性および発がん性を有する物質が遺伝毒性および発がん性の残留物を食品中に残留させる場合、それらの物質が食品に意図的に添加されている、あるいはフードチェーンの初期段階で使用されているということを示唆するものではない。

遺伝毒性物質は通常、*in vitro* および *in vivo* の各種試験システムにおける陽性結果に基づいて特定される。直接的に、または代謝変換後に DNA と相互作用する遺伝毒性物質(直接作用性遺伝毒性化学物質)に関して、作用機序の閾値は存在しない、すなわち潜在的影響のない用量はないことが一般に仮定されている。一方、紡錘体機能および構造に影響をおよぼし異数性を誘導するもの(Parry *et al.* 1994)、トポイソメラーゼ阻害により染色体の完全性に影響をおよぼすもの(Lynch *et al.* 2003)、酸化ストレスなどにより間接的に DNA 損傷を引き起こすもの(Bolt *et al.* 2004)など、DNA に非反応性の遺伝毒性物質に関しては、閾

値に基づく機序が考えられる。本見解では、最後に述べた機序は詳述せず、DNA との直接的相互作用により遺伝毒性を示す発がん性物質のみを扱う。

in vitro 試験の結果のみが入手できる場合など、入手可能な遺伝毒性のデータが限られている場合、遺伝毒性のエビエンスの全般的重要性は、他の関連情報(化学反応性、代謝運命など)を考慮して個別に評価するものとする。

発がん物質が遺伝毒性を示さない場合、特定できる可能性がある非遺伝毒性機序を通して作用するものと見なされる。発がん性であるが発がん性の作用機序が特定されていない物質の場合、通常遺伝毒性が作用機序であると仮定する。これは他の情報が不足している場合の既定の立場であることを認識することが重要であり、遺伝毒性が実際に作用機序であると認めるものではもちろんない。

上述したように、本報告では、遺伝毒性作用を有すると見なされる物質によるがん誘発の側面を検討するにとどまる。食品科学委員会(Scientific Committee on Food [SCF])は 2002 年中に、これらの発がん物質のリスク評価の問題に関する議論を開始していたが、EFSA に引き継ぐ前にレビューを完成させることはできなかった(欧州委員会 SCF、2001、2002a、2002b、2002c)。この議論の目的は、リスク管理において、それまで採用されていた合理的に達成可能な限り低いレベル(ALARA)まで物質への曝露を軽減するという原則よりも有益な結論を提示することであった。

1.1 発がん過程に関する現時点での認識

正常細胞は、ゲノム安定性を失って遺伝的変化を獲得することにより、がん性細胞に成長するということが、現在一般的に認められている(Loeb and Loeb 2000、Gray and Collins 2000、Eyfjord and Bodvarsdottir 2005)。がん原遺伝子および腫瘍抑制遺伝子は主要な変異の標的として特定されている(Bishop 1991、Weinberg 1991)。発がん物質特異的変異パターンは、いくつかの動物腫瘍(Balmain and Brown 1988)およびヒトがん(Harris 1992、Semenza and Weasel 1997)におけるがん原遺伝子または腫瘍抑制遺伝子に観察されており、発がん物質曝露、遺伝的変化、がんの機序的関係が示唆される(Hussain and Harris 1999)。

ヌクレオチド除去修復に欠陥のある被験者における極めて高い腫瘍発生率から、がん発生過程における DNA 変化の重要な役割が裏づけられる(Stary and Sarasin 2002)。さらに、DNA 修復能における器官および細胞型特異的な差は各種実験状況下での腫瘍形成部位と相関することが示されている(Goth and Rajewski 1974、Kleihues and Margison 1974、Swenberg *et al.* 1984)。

遺伝毒性を除く大半の毒性過程には一般に曝露の閾値があり、この閾値を下回ると一般に生物学的に有意な影響は誘発されないと想定されている(Dybing *et al.* 2002)。閾値が実験的に立証または反証できないとしても、恒常性維持機構および細胞保護機構が存在し細胞標的が多数あることから、毒物学的に意味のある影響の誘発には、物質と重要部位との相互作用の程度またはそれらの部位の占有率が最低レベルに達していなければならない(Dybing *et al.* 2002)。この臨界(閾値)レベルを下回った場合、恒常性維持機構は、生体異物への曝露によって生じる撹乱に拮抗でき、構造または機能の変化は観察されないものと思われる。

しかし、電離放射線作用の「単一ヒット」モデルから類推して(Lea 1946)、直接作用性遺伝毒性化学物質と遺伝物質との相互作用がいかなる程度でも、ある程度の確率で反応を発生させることが想定されている(McMichael and Wooward 1999)。遺伝毒性および発がん性を有する物質の DNA との共有結合に関する研究では、低用量域での線形の用量反応関係が明らかであり、閾値は示されていない(Neumann 1980、Dunn 1983、Lutz 1987、Beland *et al.* 1988)。このことは、低用量では遺伝毒性の線形と、ひいてはがんリスクが線形に減少することを示唆すると考えられる可能性があり、遺伝毒性物質の単一分子への曝露であっても DNA 損傷を引き起こし、それによりある程度のリスクが生じる可能性のあることが示唆される。しかし、DNA 付加体はそれ自体では遺伝子に作用をおよぼさず、DNA 複製を通して変異が固定されなければならない。DNA 付加体が固定される確率は、用量の影響を受ける DNA 修復率および細胞増殖率によって異なるため、線形性から逸脱する可能性がある(Lutz 1990)。比較的高レベルの DNA 損傷は通常生理学的過程から生じることに注目すべきである(Beckman and Ames 1997)。これは、極めて低用量の遺伝毒性および発がん性を有する物質のバックグラウンドの損傷への寄与はごくわずかである可能性を示唆する。高用量での修復能の飽和または過負荷(Pegg and Dolan 1987)は、突然変異発生率および腫瘍量を増加させる可能性がある。さらに、細胞分裂速度を促進する、または細胞周期の遅延(DNA 修復に必要)を抑制する化学物質は、一次 DNA 損傷からの突然変異の固定を増強する。一例として、細胞増殖の促進は、2-アセチルアミノフルオレン投与マウスでは膀胱腫瘍(Cohen and Ellwein 1990)、N-ニトロソジエチルアミン投与ラットでは肝腫瘍(Peto *et al.* 1984)の誘発が非線形性であることに関与すると考えられている。通常、発がん性バイオアッセイに適用される高用量では、標的器官において再生性細胞増殖を示す重大な毒性が誘発されることから、実験データを低用量での影響に単純に直線外挿した場合、真の発生率をかなり過大評価する可能性がある。さらに、がんは多段階の過程であることが認められており、この過程は、正常細胞を悪性誘導物に進行性に転換させる方向にはたらく種々の遺伝変化からなる逐次的な段階である。(Hanahan and Weinberg 2000)。がんは複数かつ独立的な遺伝変化の結果であることから、発生率は理論的に、必要な独立事象数を反映する多項式関数に従い増加することが予測される。これは、変異性の増大により全過程の速度が上昇する場合にもあてはまる(Lengauer *et al.* 1998)。この結果、複数分子の相互作用から生じる別の毒性影響の場合と同様、遺伝毒性化学物質に誘発される発がん性の「実際的な」閾値が生じる可能性がある(Kirsch-Volders *et al.* 2000)。DNA 修復により低レベルの DNA 損傷の処理が成功裏に行われるという可能性は、遺伝毒性影響の生物学的閾値を推

定する機序として提唱されている(Purchase and Auton 1995)。

いくつかの毒性化学物質(遺伝毒性および発がん性を有する物質を含む)の用量反応曲線は、低用量では線形性から逸脱するという観察は、低レベルの毒性物質に誘発される刺激影響と定義されるホルミシス仮説の発展に弾みをつけている(Stebbing 1982)。しかし、この仮説とリスク評価との関連性は依然として特定されていない。

1.2 外挿モデル

ヒトでの特定化学物質の発がんリスクを評価するためには、動物での試験で有意な腫瘍発生率が検出され得る曝露とヒトが曝露するレベルとの差を埋める必要がある。典型的な齧歯類発がん性試験では、各投与量において雌雄のラットまたはマウス 50 匹の群を使用して、基本(対照)発生率がゼロの場合に 50 匹中約 5 匹の発生率を有意な増加と見なしている。このことから、動物データの数学的モデリングをヒトの発がんリスクの推定に使用することが示唆されている。

外挿に使用されるモデルの範囲は最近、Edler ら(2002)による、および英国の Committee on Carcinogenicity of chemicals in food, consumer products and the environment による化学発がん物質リスク評価の戦略に関するガイダンス(COC、2004)においてレビューされており、ここでは広範な検討は行わない。これらのモデルは、遺伝毒性化学物質の機序に閾値はないと仮定している。リスク評価のための最も単純な仮説機序は、さらなる作用の必要なく腫瘍形成に至る単一の変換事象、すなわち「ワンヒットモデル」の仮説であり、直線外挿を導く。より複雑なモデルでは、複数の事象およびその事象が相互作用して腫瘍形成に至る種々の経路に関して仮説を立てている。ワイブルモデルなどこれらのモデルのいくつかは工学プロセスから発展したもので、多成分系の破壊確率を表す。この概念は有用である可能性があるが、修復機構および競合する代謝経路が結果に大きな影響をおよぼし得る低曝露での発がん過程の生物学と関連付けようとする試みはほとんどない。有用性についての同様の議論は、開始点からの単純な直線外挿と、観察データに適合させるために微調整され得るがその範囲外の生物学には関連しない多項式モデルの両方に当てはまる。様々な方程式が観察範囲のデータに適合され得るが、残念ながら各方程式は、ヒトが曝露する可能性のある低用量に外挿された場合、極めて異なる結果を導き出す。図 1 は、各種モデルから得られる可能性がある予測範囲を示す。実際に遺伝毒性および発がん性を有する物質のリスク評価に使用されているのは、図 1 に示した数学的モデル中の主に 2 つであり、いくつの機関が使用するにとどまる。これは、線形化多段階モデルで、実験的用量反応曲線内の 1 つの点から低用量に直線外挿(ワンヒットモデル)したものである。後者のモデルは、データ要件が緩やかであること、多種多様のデータセットへの一般的な適用性、固有の保守的傾向のため、広範に使用されている。いずれのモデルも、低摂取量の生物学的過程では非線形性を示さないと思われる。

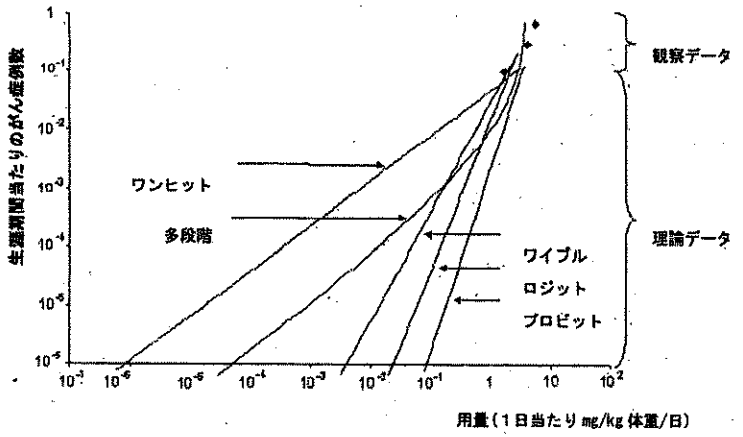

図 1

各種モデルを用いた動物発がん性データからの低用量外挿。図は、英国の Committee on Carcinogenicity of chemicals in food, consumer products and the environment の化学発がん物質リスク評価の戦略に関するガイダンス(COC、2004)からの複写、修正。

1.3 結論

科学委員会は、遺伝毒性および発がん性を有する物質の低曝露でのヒトへのリスクを推定するために、高用量での動物腫瘍データを数学的モデルを用いて外挿することには大きな懸念を示した。以下はその理由である。

- 特定の物質に関して、モデルが生物学的過程の基礎を実際に反映しているか否かはほとんど明らかではない。たとえば、低摂取量での毒物動態および作用機構に有意な非線形性が示される可能性がある一方、高用量での細胞毒性は動物での試験における用量反応関係の形に影響をおよぼす可能性がある。

- 得られるリスク予測値は使用するモデルによって大幅に異なり、実際のデータからはほとんど影響を受けない。この予測では、選択したモデルによって同じ物質のリスク予測値が桁違いとなる可能性がある。

したがって科学委員会は、リスク管理者への勧告を曝露マージンアプローチに基づいて行う可能性を検討した。

2. 曝露マージン

2.1 はじめに

食品中の物質によるリスクの性質および規模に関するリスク管理者への勧告は定量的、定性的に、種々の形態で行われる(Renwick *et al.* 2003)。曝露マージンアプローチが、食品中の物質に関するリスクについてのリスク管理者への勧告に使用されることは極めてまれであり、各種ハザードに関して確立され一般に認められた手法はない。

食品中の物質によるリスクに関するリスク管理者への勧告の作成は、ヒトの疫学的データに基づくことが理想であるが、そのようなデータはほとんど入手不可能であり、通常定量的評価には役立つものではない。

非遺伝毒性物質に関しては、動物での試験で得られた無毒性量(NOAEL)に通常 100 倍の不確実係数を適用して一日摂取許容量(ADI)などの健康基準限界値が得られる。100 倍の不確実係数は科学的判断に基づき、種差およびヒトの個体差を考慮している(WHO 1987、WHO 1994、WHO 1999)。遺伝毒性および発がん性を有する物質に関して、腫瘍形成の NOAEL を閾値に代わるものと見なすべきではなく、NOAEL は、試験では発生率の有意な増加が検出不可能な用量反応曲線上の基準点を明示するにすぎない。したがって、この NOAEL アプローチは遺伝毒性および発がん性の物質には適切ではない。

曝露マージン(MOE)は、有害影響の用量反応曲線上に定められた点とヒトの摂取量との比率であり、したがって、「安全な」摂取量についての絶対的な仮説を立てるものではない。したがって、科学委員会は、遺伝毒性および発がん性を有する物質に関してはこのアプローチがより適切であると見なす。

MOE アプローチを適用する場合、以下の段階を考慮する必要がある。

i. ヒトの摂取量との比較に用いる用量反応曲線上の適切な基準点の選択。
ii. ヒトの食事による曝露の推定。
iii. MOE の算出。

2.2 ヒトの摂取量との比較に用いる、用量反応曲線上の適切な基準点の選択

各種化学物質の発がん性を比較する数値的指標を確立するいくつかの方法が提唱されている。これらには、TD_{50}の同定(Peto *et al.* 1984b)、T25(Dybing et al. 1997)、ベンチマークドーズ法(米国環境保護庁［EPA］1996)が含まれる。

科学委員会は、原則としてこれらのアプローチはいずれも、ヒトの摂取量との比較に用いる基準点を決定する際に使用可能であると見なした。したがって、次項ではこれらの方法を簡単に説明する。

分析用のデータの選択時には、評価過程で通常用いられる試験のデザイン、実施、報告といった要素を考慮すべきである。これらについてはここでは検討しない。さらに、物質の動態と代謝に対する投与経路および投与方法の影響を、ヒトの摂食習慣との関連性について評価すべきである(例、強制経口投与と食事による投与では1日用量は類似していても代謝は異なる可能性があり、動態は確実に異なる)。

2.2.1 TD_{50}

Peto らは、動物での慢性曝露試験における化学物質の発がん性に関する数値的記述について、一般的な取り決めとしてTD_{50}の使用を提唱した(Peto *et al.* 1984b、Sawyer *et al.* 1984)。付属の文書では、Gold ら(1984)が Peto らが定義したTD_{50}を使用して発がん性データベース(Carcinogenic Potency Database)を構築した。その後、データベースは数回にわたり入手可能となった新規の試験で(Gold *et al.* 1986、1987、1989、1990、1991、1993、1995、1999)補完されており、全データベースがインターネット(http://potency.berkeley.edu/)上で利用可能である。TD_{50}はLD_{50}に類似する指標を導入するために選択され、当初は試験動物における50%腫瘍原性用量と定義された。言い換えると、TD_{50}は特定の標的部位(1つあるいは複数)に関して、標準の実験時間すなわち種の「標準的寿命」期間内に動物の半数に腫瘍を発生させる慢性用量(mg/kg 体重/日)である。しかし、対照動物における腫瘍発生および早期死亡を許容するため、いくつかの修正が導入されており、TD_{50}の定義は、「特定の性別、系統、種、一連の実験条件に関して、標準期間すなわちその種の「標準的寿命」の期間中慢性投与された場合に、その期間全体を通して腫瘍が発生しない確率を死亡率で補正した推定値を半減させる用量」(mg/kg 体重/日)と変更された(Peto *et al.* 1984b)。

TD_{50}は腫瘍性病変の特定のカテゴリ(悪性腫瘍のみ、肝腫瘍のみなど)または全腫瘍に関して算出可能である。Peto ら(1984b)は、試験するカテゴリは、「治療の影響を強く受ける腫瘍型」または「良性または悪性のすべての腫瘍型」のいずれかであることを提唱した。Peto らはまた、TD_{10}またはTD_{90}などの指数は、信頼できる方法で推定される場合TD_{50}と同様に有用であると見なし、TD_{50}の利点は多くの場合実験用量域に含まれることであり、このことからより正確な推定が行われる可能性があるとした。

2.2.2 T25

発がん性指数を評価する簡便な方法である T25 は、Dybing ら(1997)が提示した。T25 発がん性指数は「種の標準的寿命の期間中に特定の組織部位に発生する動物腫瘍の 25%(自然発生率で補正後)を生じさせる慢性投与量(mg/kg 体重/日)」と定義された。T25 は、高度な統計的方法およびコンピュータ処理を必要としない簡便な方法として、また、発がん性による順位付けが発がん物質の分類に必要であると考えられるような規制設定の場において特に使用されるものとして提唱された。T25 は現在、製剤のラベルに関連して、発がん物質の具体的な濃度限度を設定するために欧州連合内で使用されている(欧州委員会、1999)。

T25(1日当たり mg/kg 体重)の推定には、一般に、統計的に有意な反応を示す最低腫瘍発生率のデータが使用される。したがって、特定用量での最終的な発生率が 15%の場合、その用量に 25 あるいは 15 を乗じて T25 を推定すべきである。しかし、より高い腫瘍発生率によりより低用量の T25 が得られる場合は、低用量の T25 が推奨される。T25 の算出には、承認されたガイドラインに準じて実施された長期発がん性試験からのデータを使用することが望ましい。これができない場合、Dybing ら(1997)は、別の試験を T25 の推定に使用する場合に満たすべき一連の基準を提示している。

Dybing ら(1997)は、各種試験からの T25 算出方法に関して具体例をいくつか提示し、110 の物質に関する結果を Gold と同僚らが発がん性データベース用に算出した同腫瘍部位について対応する TD_{50}値と比較した。この結果から、発がん性の記述に関する T25 指数の使用は、TD_{50}と比較して容認可能なパラメータであることが示された。

2.2.3 ベンチマークドーズ

食品中の非遺伝毒性物質のリスク評価に動物データを使用する場合、最も感受性の高い(試験)種における物質の臨界影響に関して NOAEL および／または最小毒性量(LOAEL)をハザードによる健康被害解析の根拠として使用する。しかし、このアプローチは用量反応曲線の形を直接的に考慮しておらず、したがって利用可能な全情報が使用されるわけではない。また、NOAEL/LOAEL の値は用量の選択および実験的試験デザインにおける各用量間の間隔によって大きく異なる(例、用量設定間隔が大きい場合、真の無毒性量は実験データで示されるものよりも大幅に大きくなる可能性がある)。

ある物質に関する実験動物データの用量反応曲線を完全に利用した場合に、ヒト曝露レベルでの潜在的リスクの特性解析および定量化が改善される可能性があることは広く認められている(Edler *et al.* 2002)。これはベンチマークドーズ(BMD)を用いて実施可能である。BMD はまた、臨床試験および疫学的試験から得られるヒトのデータにも適用されている(Filipsson *et al.* 2003)。

Crump(1984)は BMD を非発がん性の健康影響に関する NOAEL および LOAEL の代替法として提唱したが、これは BMD が用量反応評価の第 1 段階においては NOAEL/LOAEL に比してより定量的な代替法であるためである。BMD モデリングでは、毒物学的な用量反応の特性に関して、より高用量では一般に反応における変化は抑制されないということ以外に特定の仮説を立てない。このような抑制が生じる可能性はあるが、この種の反応はリスク評価では考慮されない。BMD は、観察範囲の実験データに適合させた数学的モデルに基づき、低レベルだが測定可能な反応を誘発する用量(ベンチマークドーズ反応 BMR)を推定するが(米国 EPA)、これは通常対照より 5%または 10%高い発生率で選択される。BMD の下限値(BMDL)は、BMD に関する片側 95%信頼区間に対応する下限値を示す。下限値を使用する際には、特定の試験に固有の不確実性を考慮するため、(95%信頼区間により)選択した BMR を上回らないことが確実になる。図 2 は、BMD を用量反応曲線から算出する方法と、対照より 10%高い発生率が選択される場合に BMD10 および BMDL10 が取る値を図式的に示す。

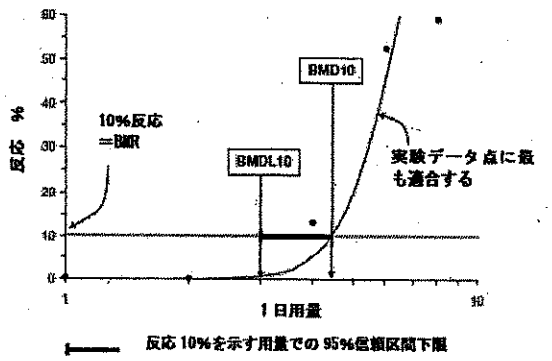

図 2

対照より 10%高い反応発生率に関する BMR、BMD、BMDL の概念を示す仮説的用量反応データ

T25 の算出と BMD の同定との基本的な相違は、T25 は用量反応曲線上の 1 つのデータ点から算出しているが、BMD は通常、用量反応曲線上で利用可能な全情報を考慮した用量反応モデリングによって得られることである。Van Landingham ら(2001)は、国家毒性プログラム(National Toxicology Program)が実施した 276 の慢性バイオアッセイを用いてこの 2 つの方法を比較した。2 年間のバイオアッセイ各々について、統計的有意性および生物学的有意性に基づき腫瘍型が選択されており、25%腫瘍発生率に関する T25 と BMD の算出値が決定されている。この評価の結果から、BMD では全投与群からのデータが含まれているため、より信頼性の高い推定値が得られることが示された。さらに、T25 を用いる方法では実験デザインの差に対してより高い感度が示された。

BMD アプローチは、腫瘍データのモデリングおよび発がん過程中の重要な前兆事象と考えられる他の(非発がん性)反応に関する米国 EPA の発がん物質リスク評価に関するガイドライン案において推奨されている(US EPA 1996)。用量反応評価の過程において BMD の使用を推進するために、米国 EPA(米国 EPA 2004)は、BMD ソフトウェアを開発しており、これはインターネット上で入手可能である(http://www.epa.gov/ncea/bmds.htm)。

2.2.4 結論

科学委員会はMOEアプローチにおける各種システムの妥当性を検討し、ヒトおよび実験動物データの評価に関して、BMD法を使用して用量反応曲線上の基準点を得ることを提唱するという結論に達した。科学委員会は現在、10%のBMRに関して算出されたBMDL(BMDL10)は、遺伝毒性および発がん性を有する物質に関し適切な基準点であるとの見解である。この値は、大半の試験で測定可能で統計的に有意に増加した発生率の下限であり、観察実験データ外への外挿は通常ほとんどあるいは全く必要ではないと思われる。全体的なBMDアプローチ自体に関しては、EFSA科学委員会によるさらなる取り組みが必要である。BMD10およびBMDL10の推定において用量反応データが不適切な場合、科学委員会は基準点としてT25の使用を推奨する。T25は適用が容易であり、欧州連合では既に使用されている。

2.3 ヒトの食事による曝露の推定

遺伝毒性および発がん性を有する物質に関する摂取量評価は他の毒物学的プロフィールを持つ物質での評価と相違ないことから、科学委員会は、本見解では摂取量の推定方法を詳細に示す必要はないと考える。しかし、高レベルでの急性曝露が生じる可能性はあるものの、遺伝毒性および発がん性を有する物質に関して主に懸念されるのは慢性曝露であることを理解するべきである。食事摂取量推定値は以下に関するものと考えられる。

- 全集団またはより望ましくは「消費者のみ」[1]
- 平均摂取量および摂取量中央値
- (食品の大量摂取または高度に汚染された食品の平均的な消費により)高曝露した個人の摂取量、集団の90、95、97.5、99パーセンタイルで示される。

関心の対象となっている物質が集団の大半が消費する食品中に発生する場合、推定は集団全体に基づいて行われ得る。集団の一部が消費する食品中に物質が発生する場合、集団全体で平均した摂取量では事実とは異なった低い曝露推定値になると思われる。このような場合、曝露は「消費者のみ」に関して推定しなければならない。摂取量推定値が平均から離れるにつれてその信頼性が低くなることから、集団での摂取量分布の90、95、97.5、99パーセンタイルに関する信頼区間を示すことがより一層重要である(Cullen and Frey 1999)。

[1] 消費者のみとは、食事調査期間中に1回以上、対象とする食品を消費した個人を示す。

提供された推定値範囲からの曝露シナリオの選択は、リスク管理者が行うべき決定であるが、これらのシナリオは各種推定値に関連する固有の不確実性についての記述と共にリスク評価者が提供すべきである。現在科学委員会では、曝露評価における不確実性に関する見解を準備中である。

2.4 曝露マージンの算出

MOEは、BMDL10またはT25などの基準点をヒトの摂取量推定値で除して算出する。

3. 算出した曝露マージンの解釈に関するガイダンス

許容範囲と見なされる特定の化学物質のMOEは社会的判断によるものであり、責任はリスク評価者ではなく、主としてリスク管理者に委ねられる。リスク評価者は、ハザードによる健康被害解析と摂取量データの質、使用データに固有の不確実性、MOEの規模についてリスク管理者に報告する義務がある。リスク評価者はさらにリスク管理者に対してMOEの規模の解釈について勧告するべきである。

科学委員会は、MOEの解釈に際して次の点を検討する必要があることを認識した。
a) 種間差および種内差(ヒトの個体差)
b) 発がん過程の特性
c) BMDL10またはT25など選択した基準点の種類

3.1 種間差および種内差の検討

非遺伝毒性物質に関する通常の初期係数100は、潜在的種間差とヒトの個体差をそれぞれ考慮した2つの10倍係数の積である(WHO 1987および1994)。これらの10倍係数は生理学的差および代謝的差を考慮したものであり、遺伝毒性および発がん性を有する物質にも有用だと思われる。これらの初期係数10は、たとえば国際化学物質安全性計画(IPCS)によって示されているように、適切な化学物質特異的データが使用可能な場合は増減する可能性がある(WHO/IPCS 2001およびIPCSウェブサイトhttp://www.who.int/ipcs/en)。

薬物代謝の多型が発がん感受性におよぼす影響は広範に調査されている。代謝経路における遺伝子多型は、体内物質の用量に10倍超の差をもたらし得るが、これはまれな情況であり、排出の主要経路における機能的多型である場合にのみ発生する(Dorne and Renwick 2005)。多くの実験室研究および疫学的症例対照研究の全体的な結論として、一般に低レベルの環境曝露に関連する個別の発がんリスクにおよぼす生体異物代謝酵素の遺伝的変化の影響は中等度であることが示されている(Hirvonen *et al.* 1999、Taningher *et al.* 1999、

Pavanello and Clonfero 2000)。これは、変異遺伝子型の各群に関して2未満のオッズ比が示された症例対照研究で得られた発がんリスク推定値のメタ分析によって裏づけられている(D'Errico *et al.* 1999)。

科学委員会は、同じ生理学的差および代謝的差が遺伝毒性および発がん性を有する物質にも当てはまると考えており、したがって、100以上という基準点とヒトの摂取量との間の差は、種間差および種内差を許容する十分な値であると思われる。

3.2 発がん過程に関する追加的検討

遺伝毒性および発がん性を有する物質の作用機序には、DNA損傷の恒久的かつ遺伝的変異への固定化など不可逆的段階が含まれる。不可逆的段階によりもたらされる結果は、単一の変異細胞のクローン増殖、遺伝的変化の蓄積、変異細胞のがんへの進行によって増強される。

遺伝因子は、環境曝露に関連した個別の発がんリスクを調節する(Shield and Harris 2000)。外因性または内因性の遺伝毒性物質への曝露後の重要標的における遺伝的変化の確率は、DNA損傷の修復および細胞周期調節の効率によって異なる可能性がある。DNA損傷に拮抗する変異の固定化によって個別の発がんリスクに影響をおよぼし得る候補遺伝子には、DNA修復遺伝子、免疫機能遺伝子、細胞周期とアポトーシスの調節遺伝子が含まれる(Brennan 2002)。

DNA修復と発がんリスクとの潜在的関連性は近年注目を集めている(Mohrenweiser and Jones 1998、Hu *et al.* 2002)。変異原感受性は二卵性双生児および同胞と比較して一卵性双生児間ではほとんど相違はなく、DNA損傷に対する個々の感受性における遺伝的根拠が示される(Cloos *et al.* 1999、Tedeschi *et al.* 2004)。ヒトのDNA修復の多様性に関する調査の大多数は、がん患者と非がん患者とを比較している。このような差はヒトのDNA修復に固有の差によるものと思われるが、腫瘍発生の結果として生じる可能性もある。保守的なアプローチとして、既報のDNA修復の個人差は非がん患者集団内で発生し得ることが仮定される。Mohrenweiser(2004)は最近、がん症例の被験者と健常対照被験者とのDNA修復能の測定値を比較した試験のレビューを行った。結論として、大多数の試験では、DNA修復能の20～35%の低下は発がんリスクの上昇と関連し、オッズ比は通常3～6であることが示された。

また分子疫学的研究からのデータは、DNA修復遺伝子の変異対立遺伝子と肺がん、乳がん、前立腺がんのリスク増加との間に認められる関連とも一致する(Goode *et al.* 2002)。

ゲノム不安定性を阻害する大部分の遺伝子および細胞増殖を調節する遺伝子は、ヒトでは多型であり、一般的な変異体は浸透度が低く発がん感受性に影響をおよぼす可能性がある。特に、TP53、p21、サイクリンD1の多型は乳がん(Powell *et al.* 2002)、膀胱がん(Wang *et al.* 2002)、肺がん(Qiuling *et al.* 2003)の感受性の増強／予後不良に関連づけられ、いずれもオッズ比は2～3である。

健常被験者の血液細胞を遺伝毒性物質により *in vitro* で処理した後、約1桁の範囲で反応の変化が報告されている(Gu *et al.* 1999)。低浸透度の多型の影響は理論上ほとんど検出不可能であるものの(Mohrenweiser *et al.* 2003)、DNA修復遺伝子の個々の変異対立遺伝子の寄与は2倍未満と中等度である。さらに、栄養因子および生活習慣因子が遺伝的多様性に付加されて、DNA損傷レベルを調節し、個々のDNA修復表現型に寄与する可能性がある(Collins 2003、Palli *et al.* 2003、Wei *et al.* 2003)。科学委員会は、これらの試験の大半が *in vitro* で実施されていること、また *in vivo* 下での関連性が依然として明らかではないことに留意した。

3.3 基準点の検討

上述したように、科学委員会はBMDL10を最適な基準点と見なした。動物の用量反応曲線上の本基準点は、弱いが測定可能な反応に関連するため、遺伝毒性および発がん性を有する物質に関する閾値の代替と見なすことはできない。さらに、基準点より下の用量影響関係、がん発生率が増加しないレベルを下回る用量レベルは不明であり、さらに不確実性が増す。

T25はBMDL10よりも保守的ではないことから、MOEを解釈する際にT25も考慮する必要があると思われる。

3.4 全体的な曝露マージンの検討

科学委員会は、がんの生態に対する現状の認識に基づき、遺伝毒性および発がん性を有する物質への曝露レベルに関し、そのレベルを下回るとがんの発生率が増加しないようなレベル(用量反応の生物学的閾値)があると結論づけるが、現時点ではこのような曝露レベルの数値は科学的根拠に基づいて同定することはできない。

科学委員会は、各種物質および摂取量のシナリオに関して算出されるMOEは大きく異なることがあり、小さいMOEはより大きいMOEよりも高いリスクを示すことから、リスク管理者は優先順位の設定のためにMOEの規模を使用できるとの見解である。類似のアプローチは、カナダ保健省(Health Canada)によりカナダ環境保護法(Canadian Environmental

Protection Act)の優先化学物質に関して(Health Canada 1994)、オーストラリアおよびニュージーランドの National Health and Medical Research Council により発がん性土壌汚染物質の毒性評価に関して(NHMRC 1999、Fitzgerald *et al.* 2004)、JECFA(Joint FAO/WHO Expert Committee on Food Additives)により汚染物質の評価に関して(JECFA 2005)使用されている。

科学委員会は、MOE の規模の解釈には各種不確実性 i、ii、iii の検討を含めるべきであるとの見解である。

i. 毒物動態学および毒物力学の基本的過程における種差およびヒトの個体差は、動物での試験からのデータをヒトのリスク評価に使用する際に内在するものである。非遺伝毒性物質のリスク評価においてこれらの不確実性を許容するため、通常 100 倍係数が使用されるが、類似の不確実性は遺伝毒性および発がん性を有する物質に適用可能であると思われる。
ii. 発がん過程に影響をおよぼす細胞周期調節と DNA 修復におけるヒト個体間の多様性のため、特に遺伝毒性および発がん性を有する物質に関しては、不確実性が増す。
iii. 基準点は NOAEL と同等ではなく、影響はより低用量で生じる可能性がある。基準点を下回る用量影響反応、そのレベルを下回るとがん発生率が増加しないような用量レベルは不明であり、さらに不確実性が増す。

要約すると、基準点とヒトの曝露間の 100 倍の差は、上記 i)に示した一般的な種差とヒトの個体差のみを許容すると思われる。100 倍の差を追加すれば、上記 ii)および iii)に示した追加的不確実性を許容すると思われる。

科学委員会は、MOE が動物試験で得られた BMDL10 に基づく場合、一般に 10,000 以上の MOE は公衆衛生の観点からは懸念が低く、リスク管理措置に対する優先順位は低いと見なすことは妥当であると思われるとの見解である。しかし、このような判断は最終的には、リスク管理者が扱う問題である。加えて、この規模の MOE でも、ヒトの曝露を低減させるためのリスク管理措置の適用を除外すべきではない。

10,000 以上の規模の MOE でも、たとえば T25 を使用して MOE を算出する場合、または基準点が不十分な動物データベースに基づいている場合など、より大きな不確実性が存在する状況下では健康への懸念は低いと見なされ得ないと思われる。

動物の用量反応データは、通常物質が試験中に毎日投与されるような試験から得られると思われる。したがって、原則として MOE の算出に使用されるヒトの曝露データは、長期平均摂取量であると思われる。ヒトの短期摂取量データは平均摂取量を過大評価する傾向がある。その結果、短期摂取量データは長期平均摂取量を使用した場合よりも低い MOE を示し、控えめな推定となると思われる。

動物データの使用に関連する不確実性は、ヒトがんの疫学的データを使用して基準点を得る場合には意味を持たないと思われる。不確実性の規模は、基準点の決定に使用される疫学的研究の集団の規模と性質によって異なると思われ、個別に検討すべきである。

その他の毒性影響は、BMDL または T25 のいずれかを得るために使用した用量と異なる用量、または多くの場合それより低用量で生じる可能性があり、全体的リスク評価において考慮するべきである。

4. 結論

1. 曝露マージンアプローチは遺伝毒性および発がん性を有する物質のリスク評価に提唱される。曝露マージンは、用量反応曲線上の基準点(通常、ヒトのデータがない場合は動物での実験に基づく)をヒトの推定摂取量で除したものと定義される。

2. 腫瘍発生率 10%に一致する BMD(ベンチマーク ドーズ)上の 95%信頼区間の下限を示す BMDL10(ベンチマーク ドーズ信頼下限 10%)の使用は、用量反応曲線上の基準点として推奨される。腫瘍発生率 25%に一致する(補正)用量を示す T25 は、データがベンチマーク ドーズ信頼下限の推定に不適切である場合に使用するべきである。

3. 各種曝露シナリオおよび集団内の群に関する一連のヒトの推定摂取量を、曝露マージンの算出に使用するべきである。

4. 各種物質および摂取シナリオに関して算出された曝露マージンは、大きく異なり得る。小さい曝露マージンはより大きい曝露マージンよりも高リスクであることを示す。したがって、リスク管理では優先順位付けにこの情報を使用できる。

5. 科学委員会では、一般に 10,000 以上の曝露マージンが、動物での試験で得られた BMDL10 に基づき、解釈の際に不確実性全体を考慮している場合、公衆衛生の観点からは懸念は低く、リスク管理措置に対する優先順位は低いと見なすことは妥当であるとの見解である。しかし、このような判断は、最終的にはリスク管理者が扱う問題である。さらに、この規模の曝露マージンでも、ヒトの曝露を低減するためのリスク管理措置を除外すべきではない。

6. 科学委員会は、曝露マージンアプローチは、遺伝毒性および発がん性を有する物質がその発生源に関わらず食品中に検出され、曝露している人、または曝露した人に対する潜在的リスクに関するガイダンスが必要である場合に適用可能であるとの見解である。

7. 科学委員会は、原則として遺伝毒性および発がん性を有する物質が、その活性をもった残留物として食品に残ってしまう場合には、食品に意図的に添加すべきではなく、また食糧生産の初期段階で使用すべきではないとの見解である。

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6. 略語

ADI:一日摂取許容量
ALARA:合理的に達成可能な限り低い
BMD:ベンチマークドーズ
BMDL:ベンチマークドーズ信頼下限
BMR:ベンチマークドーズ反応
LD50 :半数致死量

MOE:曝露マージン
NOAEL:無毒性量
LOAEL :最小毒性量
TD50:発がん性指数
T25: 簡易化発がん性指数

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謝辞

科学委員会は、本見解案の作成にあたった作業グループのメンバーに感謝の意を表す。

作業グループの長は Ada Knaap 氏が務め、構成メンバーは Christer Anderson 氏、Paul Brantom 氏、Jim Bridges 氏、Riccardo Crebelli 氏、Helmut Greim 氏、John Christian Larsen 氏、Douglas McGregor 氏、Andrew Renwick 氏、Josef Schlatter 氏であった。

The EFSA Journal (2005) 282, 1-31

Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic

(Request No EFSA-Q-2004-020)

(ADOPTED ON 18 OCTOBER 2005)

SUMMARY

The Scientific Committee has been asked by the European Food Safety Authority to propose a harmonised approach for the risk assessment of substances that have both genotoxic and carcinogenic properties. These are substances that have the potential to directly interact with the genetic material (DNA) in the cells of the body and to cause cancer. It is widely assumed for such substances that any exposure is undesirable since there may be a risk associated with exposure even to low amounts, especially if consumed on a regular basis. The effects depend on the total exposure, however, this opinion focuses on the exposure from food.

There is currently no international scientific consensus on what is the best approach for assessing the risk of substances that are both genotoxic and carcinogenic and different approaches are used around the world. In many countries and especially within the European Union, the advice given by the risk assessor to the risk manager has been to reduce the exposure to such substances to a level that is as low as reasonably achievable (known as the ALARA principle). However, it is recognised that such advice does not provide risk managers with a basis for setting priorities for action, either with regard to urgency or the extent of measures that may be necessary.

Several of the approaches currently used for risk assessment of substances that are both genotoxic and carcinogenic take into account the fact that carcinogens differ in their potency, that is, they differ in their likelihood of inducing a tumour at a given dose. Information about potency is mostly derived from laboratory studies on rodents, since human data are rarely available. In these studies, animals are exposed to the substance(s) of interest at high dose levels for the major part of their lifetime, so that any detectable and statistically significant tumour incidence can be identified. To provide advice on the possible consequences for humans, the significance of these animal results must be interpreted in the context of human exposure levels, which are usually much lower than the doses used in laboratory studies.

In an attempt to extrapolate from the high doses in animal studies to the lower levels to which humans are exposed, a wide range of models from simple linear extrapolation to very complex ones have been developed and used. This has resulted in differing conclusions for the same substance, depending on the model chosen. Moreover, for any particular substance, it is not known whether or not the model chosen actually reflects the underlying biological processes. The Scientific Committee therefore recommends using a different approach, known as the margin of exposure (MOE) approach.

The MOE approach uses a reference point, often taken from an animal study and corresponding to a dose that causes a low but measurable response in animals. This reference point is then compared with various dietary intake estimates in humans, taking into account differences in consumption patterns.

The Scientific Committee recommends the use of the benchmark dose (BMD) to obtain the MOE. The benchmark dose is a standardised reference point derived from the animal data by mathematical modelling within the observed range of experimental data. It uses all of

the information obtained over the range of doses from the experiment. The Scientific Committee recommends the use of the BMDL10 (benchmark dose lower confidence limit 10%) which is an estimate of the lowest dose which is 95% certain to cause no more that a 10% cancer incidence in rodents. The Scientific Committee notes that the benchmark dose approach can also be applied to human data when available.

In cases where the data would be unsuitable for deriving a benchmark dose, use of the T25, representing the dose corresponding to a 25% incidence of tumours, is recommended.

With respect to the selection of the human intake estimates, the Scientific Committee recommends that different exposure scenarios should be provided, e.g. for the whole population and for specific groups of the population, depending on the substance considered and its distribution in the diet. All estimates should be provided with their inherent uncertainties.

The Scientific Committee is of the opinion that substances which are both genotoxic and carcinogenic should not be approved for deliberate addition to foods or for use earlier in the food chain, if they leave residues with are both genotoxic and carcinogenic in food. The margin of exposure approach should only be applied in cases where substances that are both genotoxic and carcinogenic have been found in food, irrespective of their origin, where there is a need for guidance on the possible risks to those who are, or have been, exposed.

The Scientific Committee gives guidance on how to interpret the MOE. The following aspects were considered: inter-species differences (differences between animals and humans), intra-species differences (differences between human individuals), the nature of the carcinogenic process, and the reference point on the dose-response curve. The Scientific Committee is of the view that in general an MOE of 10,000 or higher, if it is based on the BMDL10 from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. However, such a judgment is ultimately a matter for the risk managers. Moreover an MOE of that magnitude should not preclude the application of risk management measures to reduce human exposure.

KEY WORDS

Risk assessment, carcinogenicity, genotoxicity, benchmark dose, margin of exposure.

TERMS OF REFERENCE

The Scientific Committee was requested by the European Food Safety Authority to prepare an opinion on a harmonized approach for the risk assessment of substances with both genotoxic and carcinogenic properties.

BACKGROUND

Several approaches are currently in use to assess the risk of substances with genotoxic and carcinogenic properties, within the European Union and at a global scale. Since in almost all cases no adequate human epidemiological data are available, data from high-dose animal bioassays are used, requiring extrapolation to the low levels to which humans are generally exposed. However, the science behind the different extrapolation methods is very much debated and often it is advised that the exposure to substances which are both genotoxic and carcinogenic should be as low as reasonably achievable (ALARA) (a.o., European Commission SCF, 2001, 2002a, 2002b, 2002c). It is to be realised that advice such as ALARA does not provide the risk manager with an adequate basis for setting priorities for action, either with regard to urgency or the extent of measures that may be necessary. Furthermore, improvements in analytical methods with respect to sensitivity and specificity leading to even lower detection limits will increase the number of substances detected in food including those that are both genotoxic and carcinogenic. Overall, there is an obvious need for a harmonised, scientific, transparent and justifiable approach when risks are assessed by the Scientific Committee and the Scientific Panels of the European Food Safety Authority.

ASSESSMENT

1. INTRODUCTION

The risk assessment process of any substance consists of several steps including hazard identification, hazard characterisation, exposure assessment and risk characterisation. Risk characterisation is the stage of risk assessment that integrates information from the available data on hazard characterisation and exposure assessment into advice suitable for use in decision-making (Renwick *et al.*, 2003). The present opinion addresses a specific approach for the risk characterisation part and does not address specifically the hazard identification and characterisation steps where the decision whether a substance has genotoxic and carcinogenic properties has been taken based on the weight of evidence. The science involved in hazard identification and hazard characterisation has been described among other topics in detail in the EU project FOSIE (Food Safety in Europe, 2002) and will not be considered further here.

One of the most difficult issues in food safety is to advise on potential risk to human health when it is found that substances which are both carcinogenic and genotoxic are present in food and their presence cannot be readily eliminated or avoided.

Undesirable substances occur in food (for example as an inherent natural constituent in the food plant or as contaminant through their presence in the environment, through fungal contamination or through preparation processes). The general need to minimise exposure to such substances, when they are demonstrated to present a carcinogenic and genotoxic hazard, is expressed in the ALARA (as low as reasonably achievable) principle. The opinion of the Scientific Committee addresses approaches beyond the ALARA principle allowing a level of potency assessment of specific substances which are present in food and which are both genotoxic and carcinogenic. Such an approach will not substitute for minimising exposure to all such substances. It will ensure that, where resources are limited, the highest priority is given first to those substances which present the greatest risk for humans.

It is not the Scientific Committee's intention to imply that substances which are both genotoxic and carcinogenic should be deliberately added to foods or used earlier in the food chain if they leave residues which are both genotoxic and carcinogenic in food.

Genotoxic substances are usually identified on the basis of positive results in different test systems *in vitro* and *in vivo*. For genotoxic substances which interact with DNA, directly or after metabolic transformation (direct-acting genotoxic chemicals), the absence of a threshold in their mechanism of action is generally assumed, i.e. there is no dose without a potential effect. On the other hand, threshold-based mechanisms are conceivable for genotoxic agents which do not react with DNA, such as those which affect spindle function and organization inducing aneuploidy (Parry *et al.*, 1994), or which affect chromosome

integrity through topoisomerase inhibition (Lynch *et al.*, 2003), or which indirectly cause DNA damage, e.g. through oxidative stress (Bolt *et al.*, 2004). The latter mechanisms are not considered further in the present opinion, which only concerns carcinogenic substances with genotoxic properties due to their direct interaction with DNA.

In cases where limited data on genotoxicity are available, e.g. only *in vitro* test results, the overall weight of evidence of genotoxicity is to be evaluated on a case-by-case basis taking into account other relevant information (e.g. chemical reactivity, metabolic fate).

If a carcinogen expresses no genotoxicity, it is considered to be acting through a non-genotoxic mechanism that may be identifiable. In the case of a substance that is carcinogenic, but its carcinogenic mode of action has not been identified, it will usually be assumed that genotoxicity is the mode of action. It is important to be aware that this is a default position based on a lack of other information, and is of course not an acknowledgement that genotoxicity is indeed the mode of action.

As mentioned above, this report only considers the aspect of cancer induction by substances considered to have a genotoxic mode of action. The Scientific Committee on Food (SCF) had already begun the debate on the problem of risk assessment for these carcinogens during 2002 but was unable to complete their review before the torch passed to EFSA (European Commission SCF, 2001, 2002a, 2002b, 2002c). The objective of those discussions was to provide a conclusion more helpful to risk management than the principle of reducing the exposure to such substances as low as reasonably achievable (ALARA) so far adopted.

1.1 Current understanding of the carcinogenic processes

It is now generally accepted that normal cells develop into cancerous cells by the loss of genomic stability and the sequential acquisition of genetic alterations (Loeb and Loeb, 2000; Gray and Collins, 2000; Eyfjord and Bodvarsdottir, 2005). Proto-oncogenes and tumour suppressor-genes have been identified as main mutational targets (Bishop, 1991; Weinberg, 1991). Carcinogen-specific mutational patterns have been observed in oncogenes or tumour suppressor genes in some animal tumours (Balmain and Brown, 1988) and in some human cancers (Harris, 1992; Semenza and Weasel, 1997), suggesting a mechanistic link between carcinogen exposure, genetic alterations, and cancer (Hussain and Harris, 1999).

The highly increased tumour incidence in subjects with defects in nucleotide excision repair supports the key role of DNA alterations in the process of cancer development (Stary and Sarasin, 2002). Moreover, organ and cell-type specific differences in DNA repair capacity have been demonstrated to correlate with site of tumour formation under a variety of experimental situations (Goth and Rajewski, 1974; Kleihues and Margison, 1974; Swenberg *et al.*, 1984).

For most toxic processes, excluding genotoxicity, it is generally assumed that there is a threshold of exposure below which no biologically significant effect will be induced (Dybing *et al.*, 2002). Even though the existence of a threshold cannot be proven or disproven experimentally, the presence of homeostatic and cytoprotective mechanisms, and the abundance of cellular targets, mean that a minimum degree of interaction of the substance with the critical sites or their occupancy must be reached in order to elicit a toxicologically relevant effect (Dybing *et al.*, 2002). Below this critical (threshold) level of interaction, homeostatic mechanisms would be able to counteract any perturbation produced by xenobiotic exposure, and no structural or functional changes would be observed.

However, by analogy with the "single hit" model of action of ionizing radiations (Lea, 1946), it has been assumed that any extent of interaction of direct-acting genotoxic chemicals with the genetic material poses a finite probability of generating a response (McMichael and Wooward, 1999). Studies on covalent binding of substances that are both genotoxic and carcinogenic to DNA show a linear dose-response relationship in the low-dose range, with no indication of a threshold (Neumann, 1980; Dunn, 1983; Lutz, 1987; Beland *et al.*, 1988). This might be thought to suggest a linear decrease of genotoxicity, and eventually of cancer risk, at low doses and implies that exposure to even a single molecule of a genotoxic substance could produce DNA damage and thereby some degree of risk. However, a DNA adduct does not in itself have genetic consequences, but it needs to be fixed into a mutation through DNA replication. The probability for a DNA adduct to be fixed is dependent on the rates of DNA repair and cell proliferation, which are influenced by dose; consequently, there is likely to be deviations from linearity (Lutz, 1990). It is to be noted that a relatively high level of DNA damage is normally produced by physiological processes (Beckman and Ames, 1997). This suggests that the contribution of very low doses of substances that are both genotoxic and carcinogenic to background damage may be negligible. The saturation or overload of repair capacity at high doses (Pegg and Dolan, 1987) may result in increased mutation incidences and tumour yields. Moreover, chemicals which stimulate the rate of cell division or reduce cell cycle delay (which is required for DNA repair) enhance the fixation of mutations from primary DNA lesions. As an example, the stimulation of cell proliferation is believed to be responsible for the non-linearity of bladder tumour induction in mice treated with 2-acetylaminofluorene (Cohen and Ellwein, 1990), and liver tumours in rats treated with *N*-nitrosodiethylamine (Peto *et al.*, 1984). As the high doses applied in carcinogenicity bioassays usually elicit significant toxicity with regenerative cell proliferation in target organs, simple linear extrapolation from experimental data to effects at low doses may lead to a considerable overestimation of true incidence. Moreover, cancer is acknowledged to be a multistep process, where sequential steps consist of different genetic alterations driving towards the progressive transformation of normal cells into malignant derivatives (Hanahan and Weinberg, 2000). Because cancer is the result of multiple, independent genetic alterations, the incidence is theoretically expected to rise as a polynomial function reflecting the number of independent events required. This also holds when the acquisition

of increased mutability speeds up the entire process (Lengauer *et al.*, 1998). This may result in a "practical" threshold for carcinogenesis induced by genotoxic chemicals, similarly to other toxic effects arising from multiple molecular interactions (Kirsch-Volders *et al.*, 2000). The possibility that DNA repair may cope successfully with low levels of DNA damage has been advocated as a putative mechanism for a biological threshold for genotoxic effects (Purchase and Auton, 1995).

The observation that the dose-response curve for some toxic chemical substances (including substances that are both genotoxic and carcinogenic) deviates from linearity at low doses, has triggered the development of the hypothesis of hormesis, which is defined as the stimulatory effects caused by low levels of toxic agents (Stebbing, 1982). However its relevance for risk assessment remains to be determined.

1.2 Extrapolation models

In order to assess the cancer risk of a particular chemical for the human population, there is a need to bridge the gap between the exposure at which a significant tumour incidence is detectable in animal studies and the levels to which the human population is exposed. A typical rodent carcinogenicity assay uses groups of 50 rats or mice of each sex at each treatment level and thus can only recognise an incidence rate of around 5 in 50 as significantly increased when the base (control) incidence is zero. This has led to the suggestion of using mathematical modelling of animal data to predict the human cancer risk.

The range of models used in extrapolation has been recently reviewed by Edler *et al.* (2002) and in the Guidance on a strategy for the risk assessment of chemical carcinogens of the UK Committee on Carcinogenicity of chemicals in food, consumer products and the environment (COC, 2004) and will not be extensively discussed here. The models assume that the mechanisms of genotoxic chemicals have no threshold. For risk assessment purposes the simplest assumption of mechanism is of a single transforming event that then leads on to tumour formation with no further action needed, the "one-hit" hypothesis, leading to linear extrapolation. More complex models make assumptions about multiple events and different ways in which their effects interact to result in tumours. Some of these models such as the Weibull model have evolved from engineering processes and represent a probability of failure of multi-component systems. The concept may be relevant but makes little attempt to connect with the biology of the process of carcinogenesis at low exposure where repair mechanisms and competing metabolic pathways may significantly affect the outcome. The same arguments, regarding relevance, apply to both simple linear extrapolation from a point of departure and to polynomial models that can be fine-tuned to fit the observed data but have no connection to any biology outside that range. A wide variety of equations can be made to fit the data in the observable range but unfortunately each will result in very different conclusions when extrapolated to low doses to which human population may be exposed. Figure 1 illustrates a range of predictions that may emerge from different possible models.

In practice, mainly 2 of the mathematical models shown in Figure 1 have been used for risk assessment of substances that are genotoxic and carcinogenic, and only by some agencies. These are the linearized multistage model and low dose linear extrapolation (One Hit) from a point within the experimental dose-response curve. The latter model has received wider use because of its lower data requirements, its general applicability to a wide variety of different datasets and its inherent conservatism. Neither model would reflect any non-linearities in biological processes at low intakes.

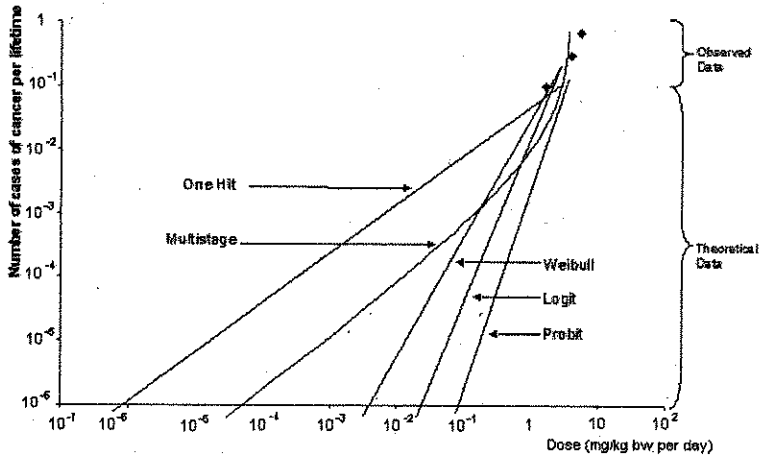

Figure 1
Low dose extrapolation from animal carcinogenicity data using various models. Figure reproduced and modified from the Guidance on a strategy for the risk assessment of chemical carcinogens of the UK Committee on Carcinogenicity of chemicals in food, consumer products and the environment (COC, 2004).

1.3 Conclusion

The Scientific Committee had serious reservations about extrapolating from animal tumour data at high doses using mathematical modelling in order to estimate risks to humans at low exposures from substances that are both genotoxic and carcinogenic because:

- It is rarely known, for a particular substance, whether a model actually reflects the underlying biological processes, for example there may be significant non-linearities in toxicokinetics and mode of action at low intakes, while cytotoxicity at high doses may influence the shape of the dose-response relationship in animal studies.

- The numerical estimate of risk obtained is critically dependent on which model is used and is very little influenced by the actual data; this can result in estimates of risk for the same substance varying by several orders of magnitude, depending on the model selected.

The Scientific Committee therefore explored the possibility of basing advice to risk managers on a margin of exposure approach.

2. Margin Of Exposure

2.1 Introduction

Advice to risk managers about the nature and the magnitude of risks from substances in food can take a variety of different forms, both quantitative and qualitative (Renwick *et al.*, 2003). A margin of exposure approach has been used only rarely in the advice to risk managers about the risks associated with substances in food, and there are no established or accepted methods for different types of hazard.

The formulation of advice to risk managers on the risk from substances in food would ideally be based on human epidemiological data but such data are rarely available and are usually not helpful for quantitative assessments.

For non genotoxic substances a 100-fold uncertainty factor is routinely applied to the No-Observed-Adverse-Effect-Level (NOAEL) from an animal study to derive a health based limit value, e.g. Acceptable Daily intake (ADI). The 100-fold uncertainty factor is based on scientific judgement and allows for species differences and human variability (WHO, 1987; WHO, 1994; WHO, 1999). For substances which are both genotoxic and carcinogenic, a NOAEL for tumour formation should not be regarded as a surrogate for a threshold; the NOAEL only defines the reference point on the dose-response curve where the study is unable to detect a significant increase in incidence. Consequently, the NOAEL approach is not appropriate for substances that are genotoxic and carcinogenic.

The margin of exposure (MOE) is the ratio between a defined point on the dose-response curve for the adverse effect and the human intake, and therefore it makes no implicit assumptions about a "safe" intake. Therefore, this approach is considered by the Scientific Committee as more appropriate for substances that are both genotoxic and carcinogenic.

When applying the MOE approach, the following steps need to be taken into account:

i. Selection of an appropriate reference point from the dose-response curve for comparison with human intake
ii. Estimation of human dietary exposure
iii. Calculation of an MOE

2.2 Selection of an appropriate reference point from the dose-response curve for comparison with human intake

Several procedures have been proposed to establish numerical indices for comparing carcinogenic potencies of different chemicals. These include the TD_{50} determination (Peto *et al.*, 1984b) as well as the T25 (Dybing *et al.*, 1997) and the Benchmark Dose procedure (US EPA, 1996).

The Scientific Committee considered that in principle any of these approaches could be used in deriving a reference point for comparison with the human intake. Therefore, the procedures listed above are briefly explained in the following sections.

It should be noted that, when selecting data for analysis, consideration should be given to elements of study design, conduct and reporting that are usual in the evaluation process. These will not be discussed here. In addition, the impact of dose route and dosing method on substance kinetics and metabolism should be assessed for their relevance to human dietary consumption habits (e.g. gavage versus dietary administration may result in similar daily dose rates, but metabolism may be different and kinetics will certainly be different).

2.2.1 TD_{50}

Peto *et al.* proposed to use the TD_{50} as a general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments (Peto *et al.* 1984b; Sawyer *et al.*, 1984). In an accompanying paper the TD_{50} as defined by Peto *et al.* was used by Gold *et al.* (1984) to establish their Carcinogenic Potency Database. Since then the database has been supplemented several times as new studies became available (Gold *et al.* 1986, 1987, 1989, 1990, 1991, 1993, 1995, 1999) and the entire database is available on the Internet (http://potency.berkeley.edu/). The TD_{50} was chosen in order to adopt a measure analogous to the LD_{50} and was initially defined as the tumourigenic dose rate for 50% of the test animals. In other words for a given target site(s), the TD_{50} is that chronic dose rate (in mg/kg bw per day) which would cause tumours in half of the animals within some standard experimental time – the "standard lifespan" for the species. However, several corrections have been introduced in order to allow for the tumour occurrence in control animals and premature deaths and the definition of the TD_{50} was changed as follows: "For any particular sex, strain, species and set of experimental conditions, the TD_{50} is the dose rate (in mg/kg bw per day) that, if administered chronically for a standard period – the "standard lifespan" of the species – will halve the mortality-corrected estimate of the probability of remaining tumourless throughout that period" (Peto *et al.*, 1984b).

A TD_{50} can be calculated either for a particular category of neoplastic lesion (e.g. malignant tumours only, liver tumours only) or for all tumours. Peto *et al.* (1984b) proposed that the category studied should be either "those tumour types that are strongly affected by treatment" or "all tumour types, benign or malignant". The same authors

considered that indices such as TD_{10} or TD_{90} might be as good as the TD_{50} provided that they can be reliably estimated, and stated that an advantage of the TD_{50} is that it will often be included in the experimental dose range, which may provide a more accurate estimation.

2.2.2 T25

A simplified method for assigning a carcinogenic potency index, the T25, has been presented by Dybing *et al.* (1997). The T25 potency index was defined as "the chronic dose rate in mg/kg bw per day, which will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard life time of that species". The T25 was proposed as a simplified method not requiring sophisticated statistical methods and computer power and to be specifically used in regulatory settings when a ranking according to carcinogenic potency is deemed necessary for classification of carcinogens. T25 is presently used within the European Union for setting specific concentration limits for carcinogens in relation to labelling of preparations (European Commission, 1999).

For estimating the T25 (in mg/kg bw per day) the lowest tumour incidence data showing a statistically significant response are generally used. Thus, in the case of a net incidence of 15% at a given dose rate, that dose should be multiplied by 25/15 to estimate the T25. However, if higher tumour incidence data give a lower T25 this latter value is recommended. The data used for calculating the T25 should preferentially be from long-term carcinogenicity studies conducted according to accepted guidelines. If this is not the case, Dybing *et al.* (1997) give a set of criteria to be met if other studies are to be used in T25 estimation.

Dybing *et al.* (1997) gave several specific examples of how to calculate the T25 from different studies and compared the results for 110 substances with the corresponding TD_{50} values for the same tumour sites calculated by Gold and co-workers for their Carcinogenic Potency Database. The result indicated to the authors that the use of the T25 index is an acceptable parameter as compared to the TD_{50} in describing carcinogenic potency.

2.2.3 Benchmark Dose

When animal data are used for risk assessment of non-genotoxic substances in food, the NOAEL and/or the Lowest-Observed-Adverse-Effect-level (LOAEL) for the critical effect of the substance in the most sensitive (test) species, is used as a basis for hazard characterisation. However, this approach does not take into account directly the shape of the dose-response curve, thus all available information is not used. The numerical NOAEL/LOAEL is also critically dependent on the choice of doses made and on the spacing between doses in the experimental study design (e.g. if there is wide dose spacing, the true no adverse effect level may be considerably higher than that indicated by the experimental data).

It is widely agreed that the characterisation and quantification of potential risks at human exposure levels can be improved if full use is made of the dose-response curve of the experimental animal data for that substance (Edler *et al.*, 2002). This can be done by using the Benchmark Dose (BMD). The BMD has also been applied to human data, derived from clinical and epidemiological studies (Filipsson *et al.*, 2003).

The BMD was put forward by Crump (1984) as an alternative to the NOAEL and LOAEL for non-cancer health effects because it provides a more quantitative alternative to the first step in the dose-response assessment than the NOAEL/LOAEL. BMD modelling makes no particular assumption about the nature of toxicological dose-responses, other than that the change in response generally does not decrease with higher doses. While such decreases may occur, this type of response is not taken into account in risk assessment. The BMD is based on a mathematical model being fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable response (the benchmark response BMR) typically chosen at a 5 or 10% incidence above the control (U.S. EPA 1995). The BMD lower limit (BMDL) refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD. Using the lower bound takes into account the uncertainty inherent in a given study, and assures (with 95% confidence) that the chosen BMR is not exceeded. Figure 2 illustrates schematically how the BMD is calculated from a dose response curve and where the BMD10 and BMDL10 would stand if a 10% incidence response above the control would be chosen.

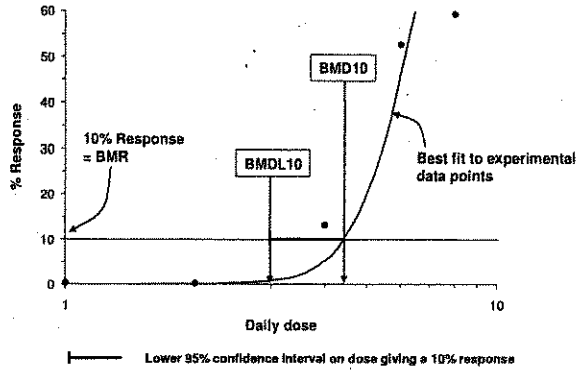

Figure 2
Hypothetical dose response data illustrating the concepts of BMR, BMD and BMDL for a 10% incidence response above the control.

The basic difference between the T25 calculation and the determination of the BMD is that the T25 is calculated from one data point on the dose-response curve , whereas the BMD is typically accomplished through dose-response modeling considering all available information on the dose response curve. Van Landingham *et al.* (2001) compared the two methods using 276 chronic bioassays conducted by the National Toxicology Program. In each of the 2 year bioassays a tumour type was selected based on statistical and biological significance and both the T25 and BMD calculated for a 25% tumours incidence have been determined. The results of this evaluation indicated that the BMD produces more reliable estimates because it includes data from all treatment groups. Additionally, the T25 method was shown to be more sensitive to experimental design differences.

The BMD approach is recommended in the US EPA's Proposed Guidelines for Carcinogen Risk Assessment (US EPA, 1996) regarding modelling tumour data and other (non-cancer) responses thought to be important precursor events in the carcinogenic process. In order to advance the use of the BMD in the dose-response assessment process, the US EPA (US EPA, 2004) has developed BMD software, which is available on the Internet (http://www.epa.gov/ncea/bmds.htm).

2.2.4 Conclusion

The Scientific Committee discussed the appropriateness of the different systems in its MOE approach and came to the following conclusion: for the evaluation of human and experimental animal data it proposes to use the BMD methodology to derive a reference point on the dose-response curve. The Scientific Committee is currently of the opinion that the use of the BMDL, calculated for a BMR of 10% (BMDL10), is an appropriate reference point for substances that are both genotoxic and carcinogenic. Such a value is the lowest statistically significant increased incidence that can be measured in most studies, and would normally require little or no extrapolation outside the observed experimental data. The whole BMD approach as such will be subject to further work by the EFSA Scientific Committee. In cases where the dose-response data are inadequate for deriving an estimate of the BMD10 and BMDL10, the Scientific Committee recommends the use of the T25 as the reference point; it can be easily applied and it is already in use in the European Union.

2.3 Estimation of human dietary exposure

The Scientific Committee considers that in the context of the present opinion there is no need for a detailed description on how to perform intake estimates because the intake assessment for a substance that is both genotoxic and carcinogenic is not different to that for substances with another type of toxicological profile. It is to be realised, however, that the main concern with regard to the presence of substances that are both genotoxic and carcinogenic is chronic exposure, although acute exposure to high levels may occur.
Dietary Intake estimates may relate to:

- the whole population or preferably for "consumers only"[1],
- the mean and median intakes,
- the intake by individuals highly exposed (due to high consumption of some foods or to average consumption of highly contaminated foods), as represented by the 90^{th}, 95^{th}, 97.5^{th} and 99^{th} percentiles of the population group.

If the substance of interest occurs in a food item consumed by almost all of the population, estimates could be based on the whole population. If the substance occurred in a food item consumed by a small part of the whole population, then an intake averaged over the whole population would produce a misleadingly low exposure estimate. In such cases exposure estimates should be performed for "consumers only". Since intake estimates become increasingly unreliable the further they are away from the mean, it is even more important that the confidence intervals should be provided for the 90^{th}, 95^{th}, 97.5^{th} and 99^{th} percentiles of the population intake distribution (Cullen and Frey, 1999).

[1] Consumers only are individuals who have consumed the food item under consideration at least once during the dietary survey period.

The choice of exposure scenarios from the range of estimates provided is a decision to be made by the risk managers, but these should be provided by the risk assessors with a description of the relevant inherent uncertainties related to the different estimates. An opinion on uncertainties in exposure assessment is currently in preparation by the Scientific Committee.

2.4 Calculation of the margin of exposure

MOEs are calculated by dividing the reference point, e.g. BMDL10 or T25, by the estimated human intakes.

3. GUIDANCE ON THE INTERPRETATION OF THE CALCULATED MARGIN OF EXPOSURE

An MOE for a particular chemical that would be considered acceptable is a societal judgement and primarily the responsibility of risk managers, rather than risk assessors. Risk assessors have the responsibility to inform risk managers about the quality of the hazard characterisation and intake data, the uncertainties inherent in the data used and the magnitude of the MOEs. The risk assessors should also advise the risk managers on the interpretation of the magnitude of the MOEs.

The Scientific Committee noted that the following aspects have to be taken into account for the interpretation of an MOE:
a) Inter-species differences and intra-species differences (human variability),
b) The nature of the carcinogenic process,
c) The type of reference point selected, e.g. BMDL10 or T25.

3.1 Consideration of inter- and intra-species differences

The usual default factor of 100 for non-genotoxic substances represents the product of two 10-fold factors, one to allow for possible inter-species differences, and one to allow for human variability (WHO, 1987 and 1994). These 10-fold factors allow for physiological and metabolic differences and these would also be relevant for substances which are both genotoxic and carcinogenic. These default factors of 10 could be reduced or increased when appropriate chemical specific data are available as described for instance by IPCS (WHO/IPCS, 2001 and IPCS website http://www.who.int/ipcs/en/)
The impact of polymorphisms of drug metabolism on cancer susceptibility has been widely investigated. Genetic polymorphism in a pathway of metabolism can lead to a more than 10-fold difference in the internal dose of the substance, but this is a rare situation and only occurs if it is a functional polymorphism in the major route of elimination (Dorne and

Renwick, 2005). The overall conclusion drawn from a number of laboratory and epidemiology case-control studies is that genetic variation in xenobiotic-metabolising enzymes has in general a modest effect on the individual cancer risk associated with low-level environmental exposure (Hirvonen *et al.*, 1999; Taningher *et al.*, 1999; Pavanello and Clonfero, 2000). This is substantiated by a meta-analysis of cancer risk estimates from case-control studies, which showed odds ratios lower than 2 for variant genotype population groups (D'Errico *et al.*, 1999).
The Scientific Committee considers that the same physiological and metabolic differences apply also for substances that are both genotoxic and carcinogenic, consequently a difference between the reference point and human intakes of at least 100 would be sufficient to allow for these inter- and intraspecies differences.

3.2 Additional considerations relating to the carcinogenic process

The mode of action for substances that are both genotoxic and carcinogenic includes irreversible steps, such as the fixation of DNA lesions into permanent and inheritable mutations. The consequences of irreversible steps are amplified by clonal expansion of a single mutated cell, accumulation of genetic changes and progression of the mutated cells into cancer.

Genetic factors modulate the individual risk of cancer associated with environmental exposures (Shield and Harris, 2000). The probability of genetic alterations at critical targets following exposure to exogenous or endogenous genotoxic substances may be dependent on the efficiency of repair of DNA damage and cell cycle control. Candidate genes which may influence individual cancer risk by counteracting fixation of DNA-lesions into mutations include DNA repair genes, immune function genes, and genes controlling cell-cycle and apoptosis (Brennan, 2002).

Attention has focused in recent years on the possible association between DNA repair and cancer risk (Mohrenweiser and Jones, 1998; Hu *et al.*, 2002). Mutagen sensitivity varies little between identical twins compared to dizygotic twins and siblings, indicating a genetic basis in the individual susceptibility to DNA damage (Cloos *et al.*, 1999; Tedeschi *et al.*, 2004). The majority of investigations on variations in DNA repair in humans involve a comparison between cancer patients with cancer free individuals. Such differences may be due to intrinsic differences in DNA repair within the human population but could also arise as a consequence of tumour development. As a conservative approach it is assumed that reported individual differences in DNA repair can occur within a cancer free population. Mohrenweiser (2004) recently reviewed studies that compared measures of DNA-repair capacity between cancer case subjects and healthy control subjects. The conclusion was that reductions of 20 to 35% in DNA-repair capacity were associated with elevations in cancer risk in the majority of studies, usually with odds ratios in the range of 3 to 6.

Data from molecular epidemiology studies also are consistent with an association between some variant alleles of DNA repair genes and increased risk of lung, breast and prostate cancers (Goode *et al.*, 2002).

Most genes preventing genome instability and the genes regulating cell proliferation are polymorphic in the human population, with common variants with low penetrance which may affect cancer susceptibility. In particular, polymorphisms of TP53, *p21* and cyclin D1 have been associated with increased susceptibility/poor prognosis of breast cancer (Powell *et al.*, 2002), cancer of the urinary bladder (Wang *et al.*, 2002) and lung cancer (Qiuling *et al.*, 2003), all with odds ratios of 2 to 3.

After *in vitro* treatment of blood cells from healthy subjects with genotoxic agents a variation in response in a range of around an order of magnitude has been reported (Gu *et al.*, 1999), but the contributions of individual variant alleles of DNA repair genes is modest, less than two-fold although the impact of low penetrance polymorphisms may theoretically be barely detectable (Mohrenweiser *et al.*, 2003). In addition, nutritional and lifestyle factors may be superimposed on the genetic diversity, modulating the level of DNA damage and contributing to the individual DNA repair phenotype (Collins, 2003; Palli *et al.*, 2003; Wei *et al.*, 2003). The Scientific Committee noted that most of these studies have been performed *in vitro*, and that their relevance to *in vivo* situations remains uncertain.

3.3 Consideration of reference point

As discussed above the Scientific Committee considered that a BMDL10 would be the most appropriate reference point. This reference point on the animal dose-response curve relates to a small but measurable response and so cannot be regarded as a surrogate for a threshold in the case of a substance that is both genotoxic and carcinogenic. In addition the dose effect relationship below the reference point, and the dose level below which cancer incidence is not increased are unknown, representing additional uncertainties.

Since the T25 is less conservative than the BMDL10, this would also need to be taken into account when interpreting the MOE.

3.4 Consideration of the overall margin of exposure

The Scientific Committee concludes that based on the current understanding of cancer biology there are levels of exposure to substances which are both genotoxic and carcinogenic below which cancer incidence is not increased (biological thresholds in dose-response), however, numerical values for such levels of exposure cannot be identified on scientific grounds at the present time.

The Scientific Committee is of the opinion that the magnitude of an MOE can be used by the risk managers for priority setting, since the MOEs, calculated for different substances

and intake scenarios, can vary broadly; a small MOE represents a higher risk than a larger MOE. Comparable approaches have been used by Health Canada for Priority Substances under the Canadian Environmental Protection Act (Health Canada, 1994), the National Health and Medical Research Council in Australia and New Zealand for the Toxicity Assessment for Carcinogenic Soil Contaminants (NHMRC, 1999; Fitzgerald *et al.*, 2004) and JECFA for the evaluation of contaminants (JECFA, 2005)

The Scientific Committee is of the opinion that the interpretation of the magnitude of an MOE should include consideration of the various uncertainties i, ii and iii

i. Species differences and human variability in the basic process of toxicokinetics and toxicodynamics are inherent in the use of data from studies in animals for human risk assessment. A factor of 100-fold is usually used to allow for these uncertainties in the risk assessment of non-genotoxic substances; similar uncertainties would be applicable to substances that are both genotoxic and carcinogenic.
ii. There are additional uncertainties specifically for substances that are both genotoxic and carcinogenic, because of the inter-individual human variability in cell cycle control and DNA repair, which influence the carcinogenic process.
iii. The reference point is not equivalent to a NOAEL and effects can occur at lower doses. The dose effect relationship below the reference point, and the dose level below which cancer incidence is not increased are unknown, representing additional uncertainties.

In summary, a 100-fold difference between the reference point and human exposures would allow only for general species differences and human variability described in i) above. An additional 100-fold difference would allow for the additional uncertainties covered under ii) and iii) above.

The Scientific Committee is of the view that in general an MOE of 10,000 or higher, if it is based on the BMDL10 from an animal study, would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions. However, such a judgment is ultimately a matter for the risk managers. Moreover an MOE of that magnitude should not preclude the application of risk management measures to reduce human exposure.

An MOE of an order of magnitude of 10,000 or higher would not be considered of low health concern under circumstances where there were greater uncertainties, for example if the MOE was calculated using a T25, or if the reference point were based on a poor animal database.

The animal dose-response data would normally be derived from studies in which the substance is administered daily throughout the study. Therefore, in principle the human exposure data used to calculate the MOE would be the long-term average intake. Short-

term human intake data tend to overestimate average intakes. In consequence short-term intake data would probably be conservative by giving a lower MOE than if long-term average intakes were used.

Uncertainties related to the use of animal data would not be relevant in cases where human cancer epidemiology data are used to derive a reference point. The magnitude of uncertainties would depend on the size and nature of the population in the epidemiology study used to define the reference point, and should be considered on a case-by-case basis.

Other toxic effects may occur at doses that are different from and often lower than those used to arrive at either a BMDL or T25 and are to be taken into account in the overall risk assessment.

4. CONCLUSIONS

1. The margin of exposure approach is proposed for the risk assessment of substances that have both genotoxic and carcinogenic properties. The margin of exposure is defined as the reference point on the dose-response curve (usually based on animal experiments in the absence of human data) divided by the estimated intake by humans.

2. The use of a BMDL10 (benchmark dose lower confidence limit 10%), representing the lower bound of a 95% confidence interval on a BMD (benchmark dose) corresponding to a 10% tumour incidence is recommended as a reference point on the dose-response curve. The T25, representing the (corrected) dose corresponding to a 25% tumour incidence, should be used if the data are inadequate for estimation of a benchmark dose lower confidence limit.

3. A range of human intake estimates relevant to different exposure scenarios and groups of the population should be used to calculate margins of exposure.

4. Margins of exposure, calculated for different substances and intake scenarios, can vary broadly. A small margin of exposure represents a higher risk than a larger margin of exposure. Consequently, risk management can use this information for priority setting.

5. The Scientific Committee is of the view that in general a margin of exposure of 10,000 or higher, if it is based on the BMDL10 from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view and might be reasonably considered as a low priority for risk management actions. However, such a judgment is ultimately a matter for the risk managers. Moreover a margin of exposure of that magnitude

should not preclude the application of risk management measures to reduce human exposure.

6. The Scientific Committee is of the opinion that the margin of exposure approach can be applied in cases where substances that are both genotoxic and carcinogenic have been found in food, irrespective of their origin, and where there is a need for guidance on the possible risks to those who are, or have been, exposed.

7. The Scientific Committee is of the opinion that in principle substances which are both genotoxic and carcinogenic should not be deliberately added to foods or used earlier in the food chain if they leave residues which are both genotoxic and carcinogenic in food.

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6. ACRONYMS

ADI: Acceptable Daily Intake
ALARA: As Low as Reasonably Achievable
BMD: Benchmark Dose
BMDL: Benchmark Dose Lower Confidence Limit
BMR: Benchmark Dose Response
LD_{50} : Lethal dose for 50% of the test animals

MOE: Margin of Exposure
NOAEL: No-Observed-Adverse-Effect-Level
LOAEL : Lowest- Observed-Adverse-Effect-Level
TD_{50}: Carcinogenic potency index
T25: a simplified Carcinogenic potency index

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ACKNOWLEDGMENT

The Scientific Committee wishes to thank the members of the Working Group for the preparation of the draft opinion.

The Working Group was chaired by Ada Knaap and composed of the following members: Christer Anderson, Paul Brantom, Jim Bridges, Riccardo Crebelli, Helmut Greim, John Christian Larsen, Douglas McGregor, Andrew Renwick and Josef Schlatter.