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.

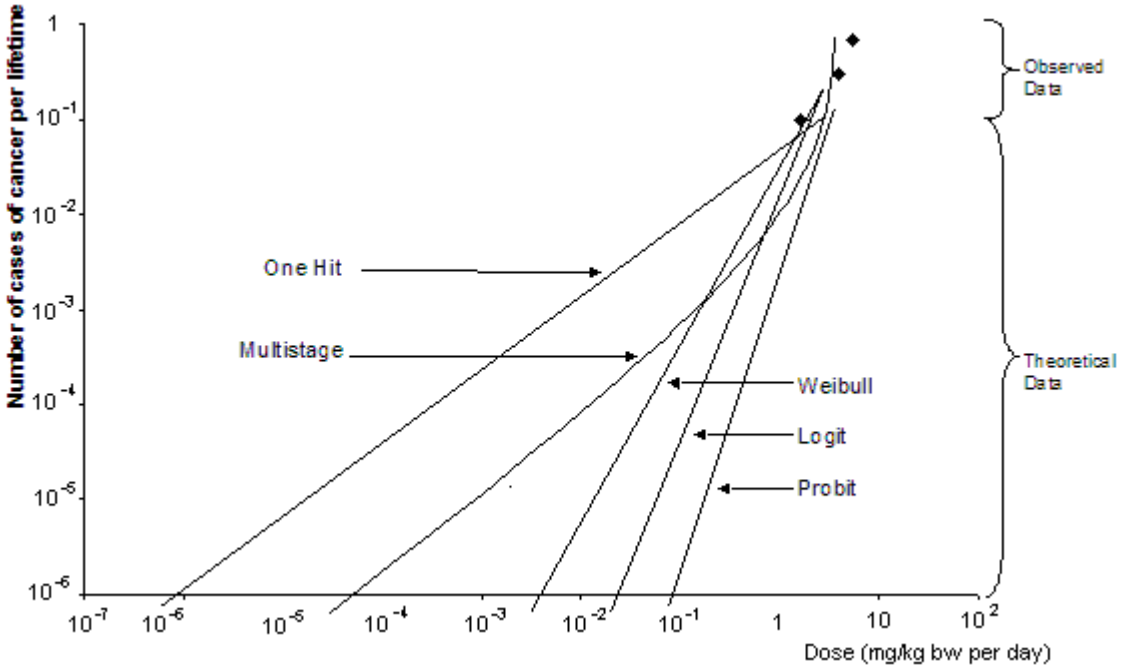

Figure 1
Low dose extrapolation from animal carcinogenicity data using various models. Figure reproduced and modified from the Guidance on a strategy for the risk assessment of chemical carcinogens of the UK Committee on Carcinogenicity of chemicals in food, consumer products and the environment (COC,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

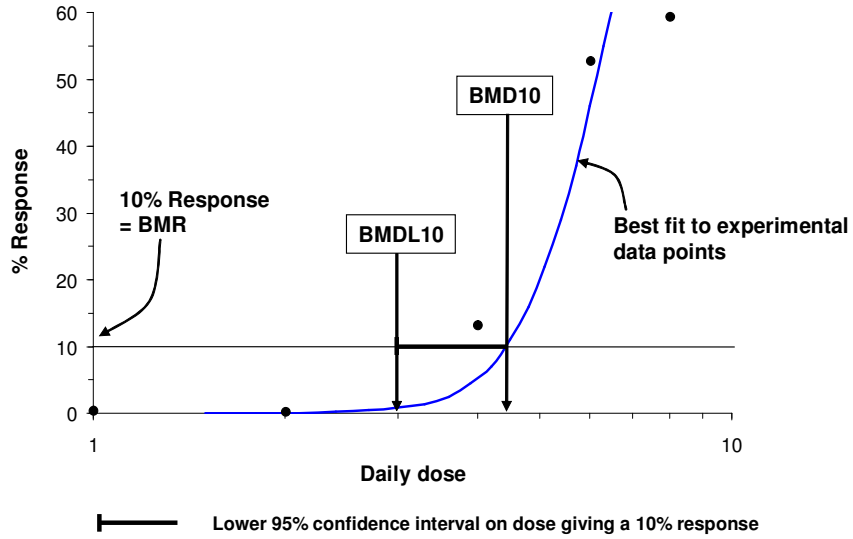

Figure 2
Hypothetical dose response data illustrating the concepts of BMR, BMD and BMDL for a 10% incidence response above the control.

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.

5. REFERENCES

Ames, B.N.; Magaw, R.; Gold, L.S. (1987) Ranking possible carcinogenic hazards. Science 236, 271-280.

Ames, B. and Gold, L.S. (1988) Carcinogenic risk estimation. Science 240, 1043-1047.

Au, W.W.; Salama S.A.; Sierra-Torres, C.H. (2003) Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ. Health Perspect. 111, 1843-50.

Balmain, A. and Brown, K. (1988) Oncogene activation in chemical carcinogenesis. Adv. Cancer Res. 51, 147-182.

Beckman, K.B. and Ames, N. (1997) Oxidative decay of DNA. J. Biol. Chem. 272 (32):19633-6.

Beland, F.A.; Fullerton, N.F.; Kinouchi, T.; Poirier, M.C. (1988) DNA adduct forming during continuous feeding of 2-acetylaminofluorene at multiple concentrations. In: Bartch, H., Hemminki, K. and O'Neill, I.K. eds., *Methods for Detection of DNA Damaging Agents in Humans: Application in Cancer Epidemiology and Prevention* (IARC Scientific Publication n. 89), Lyon, International Agency for Research on Cancer, pp. 175-180.

Berwick M. and Vineis, P. (2000) Markers of DNA repair and susceptibiity to cancer in humans: an epidemiological review. J. Natl. Cancer Instit. 92, 874-897.

Bishop, J.M. (1991) Molecular themes in oncogenesis. Cell 64, 235-248.

Bolt, H.M., Foth, H., Hengstler, J.G., Degen G.H. (2004) Carcinogenicity chategorization of chemicals – new aspects to be considered in a European perspective. Toxicol. Letters 151, 29-41.

Brennan, P. (2002) Gene-environment interactions and aethiology of cancer: what does it mean and how can we measure it? Carcinogenesis 23, 381-387.

Butterworth, B.E.; Bogdanffy, M.S. (1999) A comprehensive approach for integration of toxicity and cancer risk assessments. Regul. Toxicol. Pharmacol. 29, 23-36.

Committee on Carcinogenicity of chemicals in food, consumer products and the environment (COC) (2004) Guidance on a strategy for the risk assessment of chemical carcinogens. http://www.advisorybodies.doh.gov.uk/coc/guideline04.pdf

Cohen, S.M.; Ellwein, L.B. (1990) Cell proliferation in carcinogenesis. Science 249, 1007-1011.

Collins, A.R.; Harrington, V.; Drew, J.; Melvin, R. (2003) Nutritional modulation of DNA repair in a human intervention study. Carcinogenesis 24, 511-515.

Cloos, J.; Nierwenhuis, E.J.; Boomsma, D.I.; Kuik, D.J.;van der Sterre, M.L.; Arwert, F.; Snow, G.B.; Braakhuis, B.J. (1999) Inherited susceptibility to bleomycin-induced chromatid breaks in cultured peripheral blood lymphocytes. J. Natl. Cancer Instit. 91, 1125-1130.

Crump, K.S. (1984) An improved procedure for low-dose carcinogenic risk assessment from animal data. J Environm. Pathol. Toxicol. Oncol. 5, 339-348.

Cullen, A.C. and Frey, H.C. (1999) Probabilistic techniques in exposure assessment: a handbook for dealing with variability and uncertainty in models and inputs. Plenum Press, NY.

D'Errico, A.; Malats, N.; Vineis, P.; Boffetta, P. (1999) Review of studies of selected polymorphisms and cancer. In: W. Ryder (ed.) Metabolic polymorphisms and susceptibility to cancer. IARC Scientific Publication n. 148, Lyon, International Agency for Research on Cancer 1999, pp. 323-393.

Dorne, J.L.C.M., and Renwick, A.G. (2005) Refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol. Sci. 86, 20-26.

Duell, E.J.; Wiencke, J.K.; Cheng, T.J.; Varkonyi, A.; Zuo, Z.F.; Ashok, T.D.S.; Mark, E.J.; Wain, J.C.; Christiani, D.C.; Kelsey, K.T. (2000) Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 21, 965-971.

Dunn, B.N. (1983) Wide-range linear dose-response curve for DNA binding of orally administered benzo(a)pyrene in mice. Cancer Res. 43, 2654-2658.

Dybing, E.; Sanner, T.; Roelfzema, H.; Kroese, D.; Tennant, R.W. (1997). T25: A simplified carcinogenic potency index: Description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. Pharmacol. Toxicol. 80, 272-279.

Dybing, E.; Doe, J.; Groten, J.; Kleiner, J.; O'Brien, J.; Renwick, A.G.; Schlatter, J.; Steinberg, P.; Tritscher, A.; Walker, R.; Younes, M. (2002) Hazard characterisation of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food Chem. Toxicol. 40, 237-282.

Edler, L.; Poirier, K.; Dourson, M.; Kleiner, J., Mileson, B.; Nordmann, H.; Renwick, A., Slob, W.; Walton, K.; Würtzen, G. (2002). Mathematical modelling and quantitative methods. Food Chem. Toxicol. 30, 283-326.

European Commission (1999) Guidelines for setting specific concentration limits for carcinogens in Annex I of Directive 67/548/EEC - Inclusion of potency considerations. Commission Working Group on the Classification and Labelling of Dangerous Substances.

Luxembourg: Office for Official Publications of the European Communities, ISBN 92-828-7443-5

European Commission (2000), Scientific Steering Committee (SSC). First Report on the Harmonization of Risk Assessment Procedures. www.europa.eu.int/comm/food/fs/sc/ssc/out82_en.html

European Commission, Scientific Committee on Food (SCF) (2001). Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties (adopted on 12 December 2001). http://europa.eu.int/comm/food/fs/sc/scf/out116_en.pdf

European Commission, Scientific Committee on Food (SCF) (2002a). Minutes of the 132nd Plenary Meeting of the Scientific Committee on Food held on 15/16/17 April 2002 in Brussels. Agenda Item 4: Matters arising from previous plenary meeting. http://europa.eu.int/comm/food/fs/sc/scf/out132_en.pdf

European Commission, Scientific Committee on Food (SCF) (2002b). Opinion of the Scientific Committee on Food on new findings regarding the presence of acrylamide in food (expressed on 3 July 2002). http://europa.eu.int/comm/food/fs/sc/scf/out131_en.pdf

European Commission, Scientific Committee on Food (SCF) (2002c). Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food (expressed on 4 December 2002). http://europa.eu.int/comm/food/fs/sc/scf/out153_en.pdf

Eyfjord J.E., Bodvarsdottir, S.K. (2005) Genomic stability and cancer: Networks involved in response to DNA damage. Mutat. Res., in press.

Filipsson, A.F.; Sand, S.; Nilsson, J.; Viktorin, K. (2003) The benchmark dose method – Review of available models, and recommendations for application in health risk assessment. Critical Reviews in Toxicology, 33 (5), 505-542.

Fitzgerald, D. J.; Robinson, N.I.; and Pester, B.A. (2004) Application of Benzo(a)pyrene and Coal Tar Tumour Dose-Response Data to a Modified Benchmark Dose Method of Guideline Development. Environ. Health Perspect. 112, 1341-1346.

Food Safety in Europe (2002) Risk assessment of Chemicals in Food and Diet. Barlow, S.; Dybing, E.; Edler, L.; Eisenbrand, G.; Kroes, R.; van den Brandt, P. (Eds.) Food Chem. Toxicol. 40(2/3), 137-427.

Goth, R; Rajewski, M.F. (1974) Molecular and cellular mechanisms associated with pulse-carcinogenesis in the rat nervous system by ethylnitrosourea: ethylation of nucleic acids and elimination rates of ethylated bases from DNA of different tissues. Z. Krebs Forsch. 82, 37-64.

Gold, L.S.; Sawyer, C.B.; Magaw, R.; Backman, G.M.; de Veciana M.;, Levinson, R.; Hooper, N.K.; Havender, W.R.; Bernstein, L.; Peto, R.; Pike, M.C.; Ames, B.N. (1984) A carcinogenic potency database of the standardized results of animal bioassays. Environ. Health Perspect. 58, 9-319.

Gold, L.S.; de Veciana, M.; Backman, G.M.; Magaw, R.; Lopipero, P.; Smith, M.; Blumenthal, M.; Levinson, R.; Bernstein, L.; Ames, B.N. (1986) Chronological supplement to the carcinogenic potency database: standardized results of animal bioassays published through December 1982. Environ. Health Perspect. 67, 161-200.

Gold, L.S.; Slone, T.H.; Backman, G.; Magaw, R.; DaCosta, M.; Lopipero, P.; Blumenthal, M.; Ames, B.N. (1987) Second Chronological Supplement to the carcinogenic Potency Database: Standardized Results of Animal Bioassays Published through December 1984 and by the National Toxicology Program through May 1986. Environ. Health Perspect. 74, 237-329.

Gold, L.S.; Slone, T.H.; Bernstein, L. (1989) Summary of Carcinogenic Potency and Positivity for 492 Rodent Carcinogens in the Carcinogenic Potency Database. Environ. Health Perspect. 79, 259-272.

Gold, L.S.; Slone, T.H.; Backman, G.M.; Eisenberg, S.; DaCosta, M.; Wong, M.; Manley, N.B.; Rohrbach, L.; Ames, B.N. (1990) Third chronological Supplement to the Carcinogenic Potency Database: Standardized Results of Animal Bioassays Published through December 1986 and by the National Toxicology Program through June 1987. Environ. Health Perspect. 84, 215-285.

Gold, L.S.; Slone, T.H.; Manley, N.B.; Garfinkel, G.B.; Hudes, E.S.; Rohrbach, L.; Ames, B.N. (1991) The carcinogenic potency database: Analyses of 4000 chronic animal cancer experiments published in the general literature and by the US National Cancer Institute/National Toxicology Program. Environ. Health Perspect. 96, 11-15.

Gold, L.S.; Slone, T.H.; Stern, B.R.; Manley, N.B.; Ames, B.N. (1992) Rodent Carcinogens: Setting Priorities. Science 258, 261-265.

Gold, S.L.; Manley, N.B.; Slone, T.H.; Garfinkel, G.B.; Rohrbach, L.; Ames, B.N. (1993) The fifth plot of the carcinogenic potency database: results of animal bioassays published in the general literature through 1988 and by the national toxicology program through 1989. Environ. Health Perspect. 100, 65-135.

Gold, L.S.; Manley, N.B.; Slone, T.H.; Garfinkel, G.B.; Ames, B.N. Rohrbach, L.; Stern, B.R.; Chow, K. (1995) Sixth plot of the carcinogenic potency database: results of animal bioassays published in the general literature 1989 to 1990 and by the National Toxicology Program 1990 to 1993. Environ. Health Perspect. 103 (suppl. 8), 3-123.

Gold, L.S.; Manley, N.B.; Slone, T.H.; Rohrbach, L. (1999) Supplement to the carcinogenic potency database (CPDB): results of animal bioassays published in the general literature in 1993 to 1994 and by the National Toxicology Program in 1995 to 1996. Environ. Health Perspect. 107 (suppl. 4), 3-123.

Gray, J.W.; Collins, C. (2000) Genome changes and gene expression in human solid tumours. Carcinogenesis 21, 443-452.

Goode, E.L.; Ulrich, C.L.; Potter, J.D. (2002) Polymorphisms in DNA repair genes and association with cancer risk. Cancer Epidem Biomar 11, 1513-1530.

Gu, J.; Bondy, M. L.; Sigurdson, A., Spitz; M. R.; Hsu, T. C.; Wu, X. (1999) Three Measures of Mutagen Sensitivity in a Cancer-Free Population. Cancer Genet. Cytogenet. 110, 65-69.

Hanahan D. and Weinberg R.A. (2000) The hallmarks of cancer. Cell 100(1):57-70.

Hemminki, K.; Xu, G.; Angelini, S.; Snellman, E.; Jansen, C.T.; Lambert, B.; Hou, S.M. (2001) XPD exon 10 and 23 polymorphisms and DNA repair in human skin in situ. Carcinogenesis 22, 1185-88.

Harris, C.C. (1992) Tumour suppressor genes, multistage carcinogenesis and molecular epidemiology. In: Vainio, H., Magee, P., McGregor, D., and McMichael, A.J. eds., *Mechanisms of Carcinogenesis in Risk Identification* (IARC Scientific Publication n.116), Lyon, International Agency for Research on Cancer, pp. 67-85.

Health Canada (1994) Human Health Risk Assessment for Priority Substances (Priority Substances List assessment report). Health Canada. ISBN 0-662-22126-5. Canada Communication Group, Ottawa, Canada.

Hirvonen, A. (1999) Polymorphisms of xenobiotic-metabolizing enzymes and susceptibility to cancer. Environ. Health Perspect. 107 (suppl. 1), 37-47.

Hou, S.-M.; Falt, S.; Angelini, S.; Yang, K.; Nyberg, F.; Lambert, B.; Hemminki, K. (2002) The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. Carcinogenesis 23, 599-603.

Hu, J.J.; Mohrenweiser, H.W.; Bell, D.A.; Leadon, S.A.; Miller, S. (2002) Symposium overview: genetic polymorphisms in DNA repair and cancer risk. Toxicol. Appl. Pharmacol. 185, 64-73

Hussain, S.P. and Harris, C.C. (1999) p53 mutation spectrum and load: the generation of hypothesis linking the exposure of endogenous or exogenous carcinogens to human cancer. Mutat. Res. 428, 23-32.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), sixty-fourth meeting, 8-17 February 2005 http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

Kirsch-Volders, M.; Aardema, M., Elhajouji, A. (2000) Concepts of threshold in mutagenesis and carcinogenesis. Mutat. Res. 464(1):3-11.

Kleihues, P; Margison, G.P. (1974) Carcinogenicity of N-methylnitrosourea: possible role of repair of *O*6-methylguanine from DNA. J. Natl. Cancer Inst. 53, 1839-1841.

Kroes, R.; Muller, D.; Lambe, J.; Lowik, M.R.; van Klaveren, J.; Kleiner, J.; Massey, R.; Mayer, S.; Urieta, I.; Verger, P.; Visconti, A. (2002) Assessment of intake from the diet. Food Chem. Toxicol. 40, 327-385.

Lea, D.E. (1946) Actions of radiations on living cells. University Press, Cambridge.

Lengauer, C.; Kinzler, K.W.; Vogelstein, B. (1998) Genetic instabilities in human cancers. Nature 396, 643-649.

Loeb, K.R.; Loeb, L.A. (2000) Significance of multiple mutations in cancer. Carcinogenesis 21, 379-385.

Lutz, W.K. (1987) Quantitative evaluation of DNA-binding data *in vivo* for low-dose extrapolation. Arch. Toxicol. Suppl. 11, 66-74.

Lutz, W.K. (1990) Dose-response relationship and low dose extrapolation in chemical carcinogenesis. Carcinogenesis 11, 1243-1247.

Lynch, A.; Harvey, J.; Aylott, M.; Nicholas, E.; Burman, M.; Siddiqui, A.; Walker, S.; Rees, R. (2003). Investigation into the concept of a threshold for topoisomerase inhibitor-induced clastogenicity. Mutagenesis 18, 345-353.

McMichael, A.J.; Wooward, A. (1999) Quantitative estimation and prediction of human cancer risk: Its history and role in cancer prevention. In: Moolgavkar, S., Krewski, D., Zeise, L., Cardis, E., Møller, H. eds. *Quantitative estimation and prediction of human cancer risk.* IARC Scientific Publication n.131, International Agency for Research on Cancer, Lyon, pp. 1-10.

Mohrenweiser, H.W. and Jones, I.M. (1998) Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the premise and perils of individual and population risk estimation? Mutat. Res. 400, 15-24.

Mohrenweiser, H.W.; Wilson III, D.M.; Jones, I.M. (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. Mutat. Res. 526, 93-125.

Mohrenweiser, H.W. (2004) Genetic variation and exposure related risk estimation: will toxicology enter a new era? DNA repair and cancer as a paradigm. Toxicol. Pathol. 32, 136-145

Neumann, H.G. (1980) Dose-response relationship in the primary lesion of strong electrophilic carcinogens. Arch. Toxicol. Suppl. 3, 69-77.

NHMRC (1999) Toxicity assessment for carcinogenic soil contaminants. NHMRC Technical Working Party on Carcinogenic Risk Assessment for Soil Contaminants. National Health and Medical Research Council of Australia.

Palli, D.; Masala, G.; Vineis, P.; Garte, S.; Saieva, C.; Krogh, V.; Panico, S.; Tumino, R.; Munnia, A.; Riboli, E.; Peluso, M. (2003) Biomarkers of dietary intake of micronutrients modulate DNA adduct levels in healthy adults. Carcinogenesis 24, 739-746.

Parry, J.M.; Fielder, R.J.; McDonald, A. (1994) Thresholds for aneuploidy-inducing chemicals. Mutagenesis 9, 503-504.

Pavanello S. and Clonfero, E. (2000) Biological indicators of genotoxic risk and metabolic polymorphisms. Mutat. Res. 463(3):285-308.

Pegg, A.E.; Dolan, M.E. (1987) Properties and assay of mammalian *O*6-alkylguanine-DNA-alkylguanine-alkyltransferase. Pharmacol. Ther. 34, 167-179.

Peto, R.; Gray, R.; Brantom, P.; Grasso, P. (1984a) Nitrosamine carcinogenesis in 5120 rodents: chronic administration of sixteen different concentrations of NDEA, NDMA, NPYR and NPIP in the water of 4440 inbred rats, with parallel studies on NDEA alone of the effect of age of starting (3, 6 or 20 weeks) and of species (rats, mice or hamsters). IARC Sci. Publ. 57, 627-665.

Peto, A.E.; Pike, M.C.; Bernstein, L.; Gold, L.S.; Ames, B.N.(1984b) The TD_{50}: a proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic- exposure animal experiments. Environ. Health Perspect. 58, 1-8.

Powell, B.L.; Iris, L.; van Staveren, I.L.; Rooske, P.; Grieu, F.; Berns, E.M.J.J.; Iacopetta, B. (2002) Associations between common polymorphisms in TP53 and p21WAF1/Cip1 and phenotypic features of breast cancer. Carcinogenesis 23, 311-315.

Purchase, I.F.H. and Auton, T.R. (1995) Thresholds in chemical carcinogenesis. Regul. Toxicol. Pharmacol. 22, 199-205.

Qiuling, S.; Yuxin, Z.; Suhua, Z.; Cheng, X.; Shuguang, L.; Fengsheng, H. (2003) Cyclin D1 gene polymorphism and susceptibility to lung cancer in a Chinese population. Carcinogenesis 24, 1499-1503.

Renwick, A.G.; Barlow, S.M.; Hertz-Picciotto, I.; Boobis, A.R.; Dybing, E.; Edler, L.; Eisenbrand, G.; Greig, J.B.; Kleiner, J.; Lambe, J.; Müller, D.J.G.; Smith, M.R.; Tritscher, A.; Tuijtelaars, S.; van den Brandt, P.A.; Walker, R.; and Kroes, R. (2003) Risk characterization of chemicals in food and diet. Food Chem. Toxicol. 41, 1211-1271.

Sawyer, C.; Peto, R.; Bernstein, L.; Pike, M.C. (1984) Calculation of carcinogenic potency from long-term animal carcinogenesis experiments. Biometrics 40, 27-40.

Semenza J.C., Weasel, L.H. (1997) Molecular Epidemiology in environmental health: the potential of tumor supressor gene p53 as a biomarker. Environ. Health Perspect. 105 (suppl.1), 155-63.

Shen, M.R.; Jones, I.M.; Mohrenweiser, H. (1998) Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res. 58, 604-608.

Shield, P.G. and Harris, C.C. (2000) Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J. Clinical Oncol. 18, 2309-2315.

Stary, A. and Sarasin, A. (2002) The genetics of the hereditary xeroderma pigmentosum syndrome. Biochimie 84, 49-60.

Stebbing, A.R.D. (1982) Hormesis – the stimulation of growth by low levels of inhibitors. Sci. Total Environ. 22, 213-234.

Swenberg, J.A.; Dyroff, M.C.; Bedell, M.A.; Popp, J.A.; Huh, N.; Kirstein, A.; Rajewski, M.E. (1984) O4-Ethyldeoxythymidine, but not O6-ethyldeoxyguanosine accumulates in DNA of hepatocytes of rats exposed continuously to diethylnitrosamine. Proc. Natl. Acad. Sci. USA 81, 1692-1695

Taningher, M.; Malacarne, D.; Izzotti, A.; Ugolini, D; Parodi, S. (1999) Drug metabolism polymorphisms as modulators of cancer susceptibility. Mutat. Res. 436(3); 27-61.

Tedeschi, B.; Cicchetti, R.; Argentin, G.; Caporossi, D.; Pittaluga, M.; Parisi, P.; Vernole, P. (2004) Aphidicolin and bleomycin induced chromosome damage as biomarker of mutagen sensitivity: a twin study. Mutat. Res. 546(1-2); 55-64.

US EPA (US Environmental Protection Agency) (1995). The use of the benchmark dose approach in health risk assessment. EPA/630/R-94/007. Risk Assessment Forum, Washington DC.

US EPA (US Environmental Protection Agency) (1996) Proposed guidelines for carcinogenic risk assessment. Federal Register, 17960-18011.

US EPA (US Environmental Protection Agency) (2004) Benchmark Dose Software (BMDS) Version 1.3.2. National Center for Environmental Assessment. U.S. Environmental Protection Agency. http://www.epa.gov/ncea/bmds.htm.

Van Landingham C.B.; Allen B.C.; Shipp A.M.; Crump K.S. (2001) Comparison of the EU T25 single point estimate method with benchmark dose response modeling for estimating potency of carcinogens. Risk Analysis 21, 641-656.

Wang, L.; Habuchi, T.; Takahashi, T.; Mitsumori, K.; Kamoto, T.; Kakehi, Y.; Kakinuma, H.; Sato, K.; Nakamura, A.; Ogawa, O.; Kato, T. (2002) Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 23, 257-264.

Wei, Q.; Shen H.; Wang, L-E.; Duphorne, C.M.; Pillow, P.C.; Guo, Z.; Qiao, Y.; Spitz, M.R. (2003) Association between low dietary folate intake and suboptimal cellular DNA repair capacity. Cancer Epidemiology, Biomarkers and Prevention 12, 963-969.

Weinberg, R.A. (1991) Tumour suppressor genes. Science 254, 1138-1146.

WHO (1987) Principles for the assessment of risk to human health from exposure to chemicals. Environmental Health Criteria, 70, World Health Organization, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc70.htm

WHO (1994) International Programme on Chemical Safety (IPCS): Assessing human health risks of chemicals: Derivation of Guidance values for health-based exposure limits. Environmental Health Criteria, 170, 73 pp., World Health Organisation, Geneva.

http://www.inchem.org/documents/ehc/ehc/ehc170.htm

WHO (1999) International Programme on Chemical Safety (IPCS): Assessing human health risks of chemicals: Principles for the assessment of risk to human health from exposure to chemicals. Environmental Health Criteria 210, World Health Organisation, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc210.htm

WHO (2001) International Programme on Chemical Safety (IPCS): Draft guidance document for the use of data in development of chemical-specific adjustment factors (CSAFs) for interspecies differences and human variability in dose/concentration-response assessment. WHO/PCS/01.4, World Health Organisation, Geneva.
http://www.who.int/ipcs/publications/methods/harmonization/en/csafs_guidance_doc.pdf

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

SCIENTIFIC COMMITTEE MEMBERS

Sue Barlow, Andrew Chesson, John Collins, Tito Fernandes, Albert Flynn, Tony Hardy, Bo Jansson, Ada Knaap, Harry Kuiper, Pierre Le Neindre, Josef Schlatter, Vittorio Silano, Philippe Vannier, and Josep Vives-Rego.

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.