

Annex 1

FSA Project: T01051

INTERPRETATION OF MARGINS OF EXPOSURE FOR GENOTOXIC CARCINOGENS

Final Report for Objective 1

Critical Review of Existing Proposals for a Level of Concern and Alternative Approaches for Interpreting Margins of Exposure for Genotoxic Carcinogens

20 October 2010

Introduction

For many years the advice provided to risk managers tasked with the responsibility of controlling the risks posed by substances that are both genotoxic and carcinogenic has traditionally been to reduce exposure as far as possible, following the “as low as reasonably achievable” (ALARA) principle. In this context, the term genotoxic generally describes a substance or its active metabolite that reacts directly with DNA (Barlow et al., 2006). Historically, the assumption reserved for such compounds is that they do not exhibit a threshold in the dose-response relationship and any level of exposure could cause an effect, hence the application of a precautionary approach to the management of the potential risks posed by such chemicals.

While the ALARA approach offers a pragmatic means of dealing with such chemicals, there are circumstances when it is of limited value, as it does not enable the risk manager to discriminate between potent and weak carcinogens, where some prioritisation is often required. This approach is also unhelpful for the purposes of risk communication because even in cases where the exposure can be reduced to as low as reasonably achievable, it is difficult for risk managers to provide guidance about the magnitude of the risk associated with that particular low exposure level (Benford et al., 2010, O'Brien et al., 2006)

Alternative approaches to ALARA for the characterisation of genotoxic carcinogens involve the use of more quantitative methods, which combine data from the dose response relationship with estimates of the exposure. The former include linear extrapolation to risk at low doses and the threshold of toxicological concern (TTC).

The linear extrapolation approach involves the mathematical modelling of high dose carcinogenicity data obtained from animal experiments, to produce quantitative estimates of the risks of the cancer effects for humans who are normally exposed at doses several orders of magnitude lower than the lowest experimental dose administered in an animal study (Barlow et al., 2006; Pratt et al., 2009). Extrapolation from the high doses observed in animals to the low doses to which humans are exposed can be carried out through the selection of reference points (RPs) or so called “points of departure” (PODs) on the modelled dose-response curve. The RPs/PoDs

normally selected for this approach include the benchmark dose (BMD10) or the statistical lower bound estimate of the 95% confidence interval (BMDL10) for a 10% response, the lowest observed adverse effect level (LOAEL) or carcinogenic potency estimates such as TD50 or T25 (simplistically, the effect dose for a 50% and 25% response, respectively, of the exposed population) (Dybing et al., 1997, O'Brien et al., 2006, Peto et al., 1984, Pratt et al., 2009).

Linear extrapolation provides an estimation of the level of exposure that is associated with an upper bound, lifetime risk of cancer of 1 in a million (1×10^{-6}) or 1 in a hundred thousand (1×10^{-5}), assuming linearity in the dose-response curve. However, one of the major limitations of this approach is that the models are highly conservative. A major criticism of the application linear extrapolation is that such methods do not take into consideration the complexity of events that occur between the exposure to the chemical carcinogen and the occurrence of cancer (e.g. cell repair capabilities). In addition some of the assumptions inherent in the models such as the induction time for cancer in humans being proportional to the expected lifetime in the test species (i.e. 70 years in humans proportional to 2 years in rodents) may be incorrect (COC, 2004).

The TTC approach has been applied for the risk characterisation of substances where biological data are lacking but the chemical structure of the substance being considered is known and there are also good exposure data for the substance (Kroes et al., 2004). If the substance of concern is known to be genotoxic or has a structural alert for genotoxicity (after exclusion of a small number of very potent structures), the distribution of doses estimated to be associated with a 1 in 10^{-6} risk of cancer (using linear extrapolation) for a large number of carcinogens is used to derive the exposure value below which there is considered to be a very low probability of an appreciable risk. The exposure value associated with the 5th percentile of the distribution has been termed a threshold of toxicological concern (TTC) and corresponds to an exposure level of 0.15µg/person/day which is intended to indicate the level of negligible risk or a low priority for risk managers (Kroes et al., 2004). For non-genotoxic compounds, higher TTC values, based on broad structural considerations, are used (Kroes et al., 2004). The application of the TTC approach has proven of value for the risk assessment of a number of data-poor chemicals such as flavouring substances in food. However, its use depends upon the availability of robust exposure data or explicit exposure scenarios to improve the confidence placed on the interpreted outcome (Barlow et al., 2006, Pratt et al., 2009).

More recently, international efforts to harmonise approaches to dealing with the risks associated with compounds that are both genotoxic and carcinogenic led to the proposal of the margin of exposure (MOE) approach by the European Food Safety Authority (EFSA, 2005) and by the Joint FAO/WHO Expert Committee on Food Additives – JECFA (JECFA, 2005). The MOE used for characterising the risks associated with genotoxic carcinogens is defined as the ratio between the dose leading to a specified tumour response in experimental animals¹ and the human intake. The MOE approach aims to inform risk managers on the relative risks associated with exposure from different genotoxic carcinogens and also helps to prioritise risk management action for dealing with such compounds. Delegates at an international conference organised by the WHO and EFSA, with the support of ILSI, to discuss the

¹ There are a number of ways in which such a dose can be expressed. These are discussed later in the report.

risk assessment of substances that are both genotoxic and carcinogenic considered the MOE approach to be a credible scientific approach to risk characterisation and the formulation of advice provided to risk managers for genotoxic carcinogens. This approach was also considered to offer some advantages over the risk characterisation methods discussed above (Barlow et al., 2006, EFSA, 2005, Pratt et al., 2009).

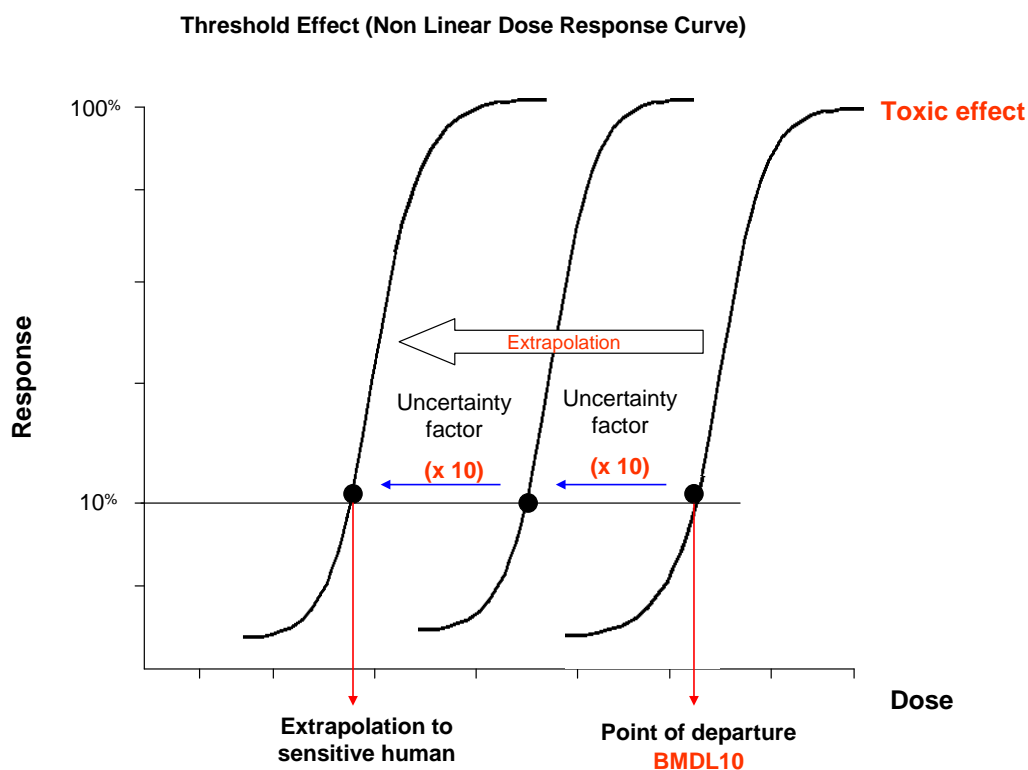
Interpretation of the Dose Response Curve and considerations for the interpretation of a level of concern in risk characterisation

An important consideration when dealing with genotoxic carcinogens is the shape of the dose response curve and the effect on the interpretation of the level of concern in the application of the MOE approach to characterising the effects of genotoxic carcinogens.

The method of extrapolation from the dose response curve depends on whether or not a threshold exists (or is assumed to exist) for the effect under consideration. The threshold is considered to be the exposure level below which no toxic effects are expected to occur in the organism.

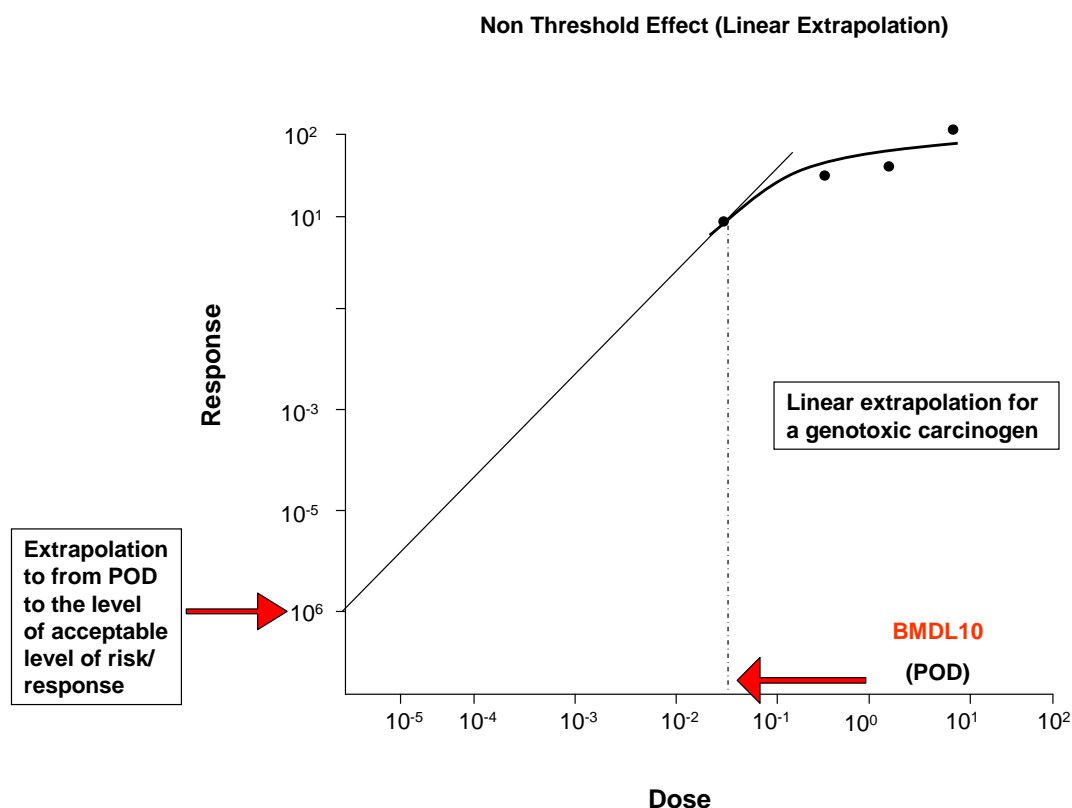
If a threshold is assumed on a dose response curve, extrapolation is carried out from the POD (e.g. NOAEL, representing the dose without observable risk) to the exposure level for sensitive humans, through the application of conventional uncertainty factors (10 X 10) to account for the toxicodynamic and toxicokinetic differences between test animals and humans (Figure 1). In this type of dose response assessment, referred to as “nonlinear”, the POD from experimental data is divided by an uncertainty (or safety) factor to obtain a health based guidance value such as the Acceptable Daily Intake (ADI) or the Tolerable Daily Intake (TDI) which negates the need to consider the level of concern associated with the level of exposure, when it is below this value (USEPA, 2010).

Figure 1: Extrapolation for a Threshold Effect



In cases where no threshold can be assumed, based on biological considerations, then an approach favoured by the U.S. Environmental Protection Agency (USEPA) is linear, low dose extrapolation, to extrapolate from the high doses used in test animals to lower doses that correspond to a predefined, 'acceptable' level of risk in humans (Figure 2). For the latter purpose, a risk level of 1 in a million (1×10^{-6}) was adopted as "*essentially zero*" or *de minimus* for the regulation and management of environmental chemicals (Kelly, 1991). The origins of this arbitrarily derived level of *de minimus* risk (Kelly, 1991) can be found in a notice of the Federal Register of the U.S Food and Drug Administration (FDA, 1973) and indicates the level below which the risks of cancer for humans are not considered to be of regulatory concern (Kelly, 1999). The 10^{-6} risk level has been strongly endorsed by the USEPA, although a range of cancer incidence risks between 1 in 10,000 (1×10^{-4}) and 1 in 1,000,000 (1×10^{-6}) depending on the situation and the circumstances of exposure, are in use in low dose extrapolation (Health-Canada, 2004, Kelly, 1991, USEPA, 2005).

Figure 2: Extrapolation for a Non-threshold Effect



It is important to distinguish between the two metrics, that obtained by linear low dose extrapolation and the MOE; one being the level of exposure associated with an “acceptable level” of population incidence of cancer and the other being the ratio between the exposure level that leads to an observed effect in animals and the actual human exposure. There is no direct relationship between the two metrics and any effort to convert one into the other would require that specific assumptions are made about the dose response curve and a thorough consideration of the nature of this relationship. Indeed an international conference (Barlow et al., 2006) organised to discuss the use of the MOE approach (among other approaches) for use in the risk assessment of genotoxic carcinogens highlighted that a MOE value that is considered a potential level of concern (LOC) should not be seen as an automatic threshold for triggering risk management action (similar to the risk assessment/regulatory use of a defined level of acceptable risk, such as 1 in 10^5 or 1 in 10^6 , as the level to trigger regulatory action). The 64th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006) also alluded to this in their General Considerations by stating that the linear extrapolation approach offered no advantage over the MOE, since the numerical estimates obtained were open to misinterpretation as a quantification of the actual risks. Therefore attempts to associate or compare a MOE with a theoretical incidence of cancer would have the same limitations as those cited for linear extrapolation (COC, 2004).

The first step in calculating a MOE is the determination of a BMDL, which requires no assumption about whether or not a threshold exists in the dose response curve as one obtains the BMDL on the basis of a defined level of response within the

observable range. It is the interpretation of the MOE subsequently calculated from this value that depends on the assumptions made about the existence of a threshold. If a threshold is assumed, then the same considerations used to derive a health based guidance value would be applicable. Hence, just as normally the POD is divided by an uncertainty factor of (usually) 100 (10 X 10) to establish a TDI or ADI, so the acceptable MOE of such an effect would generally be 100 or greater. If no threshold can be assumed then, as already stated above, this would require consideration of the associated level of concern.

Linear dose response assessment is often assumed to provide a reasonable worst case estimate of risk and is used as such for risk assessment by some authorities including the USA and the Netherlands. Other authorities including the UK Committees on Carcinogenicity and of Mutagenicity of Chemicals in Food, Consumer products and the Environment (COC/COM) do not support the use of mathematical models in carcinogenic risk assessment and prefer rather to apply the ALARA approach for the assessment of genotoxic carcinogens. It is the COC's view that the modelling approach does not take into account the complexity of events that occur between the exposure to a chemical carcinogen and the development of a neoplasm (COC, 2004). However, COC/COM has agreed that the MOE approach does have merit for prioritisation and risk communication of carcinogens. One of the main limitations of the modelling approaches highlighted by the COC is the assumption inherent in the linear model that the dose response is without threshold at a low dose. This assumption does not take into account the different repair capabilities and in cases where the true effect is sub-linear, such an assumption would result in an overestimation of the risk extrapolation. This issue has also been highlighted by EFSA (2005), who point out that mathematical models that are used to model the observed carcinogenicity data may not reflect the underlying biological processes and significant nonlinearities in toxicokinetics and MOA may occur at low exposure levels. Cytotoxic effects at high doses may also influence the shape of the dose response curve. If one considers the probability of a mutated gene resulting in initiation and the subsequent survival of a transformed cell, such an effect would result in the "flattening" out of the dose response curve.

However, there is also some level of agreement that there are some situations where the observed response is in fact linear or close to linear. This has been illustrated by both ethylnitrosourea (ENU) and methylnitrosourea (MNU) which have been observed to exhibit a linear dose response relationship (Doak et al., 2007). In such cases, the assumption of linearity would be a reasonable conservative approach.

Current proposals and alternative approaches for interpreting margins of exposures for genotoxic carcinogens.

The application of the MOE approach is still very much a developing area of chemical risk assessment, although it is gaining momentum within the international community. Efforts have been made by international experts to provide guidance on the application of the MOE approach and the advantages of its use in the risk characterisation of compounds that are both genotoxic and carcinogenic have been discussed (Barlow et al., 2006). An area of particular attention has been the attempt to

gain international consensus on how the LOC associated with a calculated MOE should be interpreted.

It is generally agreed that the magnitude of the MOE provides an indication of the LOC, where generally speaking, a higher MOE indicates a lower associated risk from exposure to the compound under consideration and vice versa. This paper aims to critically review existing literature on MOE and its use for genotoxic carcinogens, focusing specifically on the identification of existing proposals and alternative approaches for the interpretation of a LOC associated with a MOE.

Current advice from various advisory bodies on how to interpret a LOC associated with a MOE falls into 2 main categories discussed below.

1. Defined values of MOE to indicate one or more levels of concern for public health. Some authorities have proposed a single value to indicate the LOC, while others have proposed using bands based on a series of defined MOE values, representing differing levels of concern. Both these approaches could be used as a basis for prioritising chemicals for risk management consideration.
2. Case by case interpretation of the LOC associated with a MOE to take into account the various levels of uncertainty.

1. *Defined values of MOE representing one or more levels of concern*

The opinion of the EFSA scientific committee (2005) provided a comprehensive guide on different aspects that should be considered when interpreting the LOC from a public health point of view, for a calculated MOE. Although the EFSA opinion acknowledged that the determination of the MOE that would be considered socially acceptable from a public health point of view was ultimately a matter for risk managers, they nevertheless considered it their responsibility as risk assessors to advise risk managers on how best to interpret the magnitude of a calculated MOE. As such the committee adopted a defined magnitude of MOE above which, the LOC could be interpreted as being of low concern to human health, in cases where the MOE had been calculated under specific criteria.

The EFSA opinion considered that a MOE above 10,000, calculated as they proposed, would indicate a low priority for risk management action if this magnitude of MOE was not associated with an unreasonable degree of uncertainty. One criterion for the application of this approach was that the MOE should be calculated using the benchmark dose (BMD10) approach, where the lower 95% confidence interval on the benchmark dose (BMDL10), corresponding to a 10% response above background in studies in experimental animals, was selected as the point of departure (POD, also known as the reference point, RP) for the MOE calculation.

Initial justification for 10,000 as the determinant of the LOC

The uncertainties associated with physiological and metabolic differences for non-genotoxic substances were considered to apply also to substances that are both genotoxic and carcinogenic. The default factor of 100 made up from the product of 10

to account for inter-species differences and 10 for human variability, normally reserved for substances that are non genotoxic, was also considered relevant for substances that are both genotoxic and carcinogenic for these components of uncertainty. The committee considered it appropriate to apply chemical specific adjustment factors (IPCS, 2001) where available and the application of the latter would render the uncertainty factor applied to be more or less than the normal default of 100, for these components of uncertainty.

The EFSA committee considered that in addition to uncertainties associated with species differences and human variability, the nature of the carcinogenic process and type of reference point selected in the calculation of the MOE should be taken into account when interpreting the MOE. Owing to the nature of the effect, the committee considered it appropriate to add an additional factor of 10 to take into account some of the additional uncertainties associated with compounds that are both genotoxic and carcinogenic e.g. inter-individual human variability in cell cycle control and DNA repair and 10 for the use of a reference point rather than a NOAEL in acknowledgment of the uncertainties associated with not knowing the true dose below which cancer incidence is not increased. The product of the safety factors considered by EFSA led to the derivation of the MOE value of 10,000, below which the LOC was considered to be such that there would be priority for risk management action.

The EFSA committee considered the BMDL10 as the most appropriate reference point for deriving a MOE when using data from studies in experimental animals. In the absence of this reference point, the T25 was recommended for use although it was considered to be less conservative than the BMDL10. The committee noted that the interpretation of the LOC should take into account the reference point applied.

The committee added that a MOE above 10,000 should be interpreted as being of low concern for risk management action if the value was based on a BMDL10 from an animal study. Where greater uncertainties were encountered from the use of the T25 as the reference point or in cases where the BMDL10 was derived from the use of poor animal data, the interpretation of the MOE should reflect this, by appropriately taking into account the increased uncertainty.

The EFSA committee recommended that interpretation of the LOC from the value of 10,000 should take into consideration the overall uncertainties. They were cautious in their conclusion, adding that a MOE >10,000 should not be interpreted to mean that risk management action was unnecessary to reduce human exposure. In other words, a MOE should be used in addition to the ALARA principle not instead of it. They added that the final interpretation of the potential human risk associated with the MOE, regardless of the magnitude and after the consideration of all associated uncertainties, should ultimately be the judgement of the risk managers.

Although not stated explicitly in the EFSA opinion, it is worth noting that the value of 10,000 is equivalent to calculating a risk-specific dose for an excess lifetime cancer risk of 1 in 100,000 (10^{-5}) from the BMDL10 using low dose linear extrapolation, without any allowance for uncertainties including inter- and intra-species differences.

The above rationale put forward for the derivation of the value of 10,000 as the determinant of the LOC associated with a MOE value was discussed at an international conference organised by EFSA and the World Health Organization

(WHO) with the support of the International Life Sciences Institute (ILSI). Concerns were raised by delegates about the rationale put forward by EFSA for the derivation of the value of 10,000 (using 2 x 100-fold uncertainty factors (UFs)). Barlow et al (2006) reported that there was “some agreement” that the use of the first 100-fold UF (for inter- and intra-species variability) is scientifically justifiable, but that this “might actually” also cover inter-individual human variability in cell cycle control and DNA repair, and that scientific justification for a factor of 10 for use of the BMDL10 as a reference point rather than a NOEL was lacking.

The 64th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) also considered the formulation of advice for dealing with compounds that are both genotoxic and carcinogenic within the General Considerations of the report of their meeting.

Similar to the conclusion reached by the EFSA scientific committee, the JECFA reached the conclusion that the MOE approach was the most pragmatic option currently available to provide advice on compounds that are both genotoxic and carcinogenic. The report was not explicit in its recommendations on how best to interpret a MOE based on a BMDL10. Rather, the committee simply stated that advice should be given to risk managers on how to interpret the MOE along with information that relates to the strengths and weaknesses inherent in the data used to calculate the MOE.

A summary of the MOEs calculated by the EFSA and JECFA committees between 2005 and 2010 is provided in tables 1 and 2 respectively. The MOE was calculated for different exposure intakes and the interpretations of these findings related to the indication of the level of “concern” for human health. It was interesting to note that in practice, similar to the interpretation by EFSA, a MOE of 10,000 (and above) was considered by JECFA to be of “low concern for human health”.

Table 1: Summary of the MOE evaluations by EFSA from 2005 to 2010

Compound evaluated	Year of evaluation	POD	End point	MOE	EFSA Interpretation
Aflatoxin	2007	BMDL10	Hepatocellular carcinomas	69 - 173 from animal data 355 - 888 from human data (based on calculations using UB and LB exposure estimates)	EFSA concluded that exposure to aflatoxins from all sources should be as low as reasonably achievable.
Ethyl carbamate	2007	BMDL10	Alveolar and bronchiolar neoplasms	18,000 - for exposure to EC in food excluding alcohol 5,000 – food consumed with a variety of alcoholic beverages 6,000 – high consumers of fruit brandy	EFSA concluded that the EC content in stoned fruit brandy indicated a health concern.
PAHs - (B[a]P, PAH2, PAH4, PAH8)	2008	BMDL10	Carcinogenicity	Mean estimates of exposure: 15900 -17900 Median of 97.5 th percentile exposure: 9500 -10800	EFSA concluded that the mean MOEs for the different PAHs were of low concern for consumer health. However concluded that MOEs close to or less than 10,000 indicated a potential concern for human health and a possible need for risk management action.

Table 2: Summary of the MOE evaluations by JECFA from 2005 to 2010.

Compound evaluated	Year of evaluation	POD	End point	MOE at		JECFA Interpretation
				Mean dietary exposure	High dietary exposure	
Acrylamide	2005	BMDL10	Mammary tumours in rat	300	75	MOEs were considered to be low and may indicate a human health concern.
Ethyl carbamate	2005	BMDL10	Alveolar and bronchiolar neoplasms	20,000 (Intake from food only)	3,800 (Intake from both food and alcohol)	Intake from foods excluding alcohol would be of low concern.
						Intake from foods and alcohol combined is of potential concern.
Poly aromatic hydrocarbons (PAHs)	2005	BMDL10	Carcinogenicity	25,000	10,000	Intakes of PAHs were of low concern for human health.
1,3-dichloro-2-propanol	2006	BMDL10	Carcinogenicity	65,000	24,000	Low concern for human health.
Acrylamide	2010	NOAEL	Morphological changes in nerves in rats ²	200	50	JECFA concluded that although these effects were unlikely at the estimated exposure levels, morphological changes in nerves could not be excluded in individuals with high dietary exposure to acrylamide
		BMDL10	Mammary tumours in rats	310	78	These MOEs were considered to indicate a potential health concern.
			Harderian gland tumours in mice	180	45	
Furan	2010	BMDL10	Hepatocellular adenomas and carcinomas in female mice	960	480	The MOE were considered to indicate a potential health concern.

² Non-cancer effect, so interpretation of MOE differs from that for a genotoxic carcinogen.

Justification for banding approach

Between 2005 and 2007, the UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) and its sister committee on Mutagenicity (COM) considered the MOE approach within the context of risk communication.

In 2006 and 2007 the COC considered ways in which the communication of advice to the general public about the potential risks of carcinogenic and mutagenic chemicals could be improved (COT/COM/COC, 2007). At the same time, at the request of the Food Standards Agency (FSA), the COC considered the usefulness of the MOE approach proposed by the EFSA, WHO and ILSI Europe (COT/COM/COC, 2007) as a way of prioritising the risks associated with exposure to genotoxic carcinogens in food. The COC concluded that the MOE approach was consistent with their 2004 guidelines that related to the Minimal Risk Level (MRL) approach. The MRL is defined as “*an estimate of daily human exposure to a chemical identified by expert judgement that is likely to be associated with a negligible risk of carcinogenic effect over a specified duration of exposure (usually lifetime)*” (COC, 2004). It was felt that the MOE approach would help the prioritisation of genotoxic chemicals and would aid the communication of the associated risks with a wider audience.

The COC and COM considered a banding approach to expand on proposals put forward by JECFA and EFSA. The idea of a banding approach was also discussed by Barlow et al. (2006). The banding approach was intended to aid the communication of relative risk for genotoxic carcinogens. COC extended the MOE banding to include the value of 1 million as it was considered that this would improve risk communication with the public (COC, 2006). Although it was noted that the banding approach was arbitrary, the consensus from the committee was that the banding approach would improve the communication of advice on genotoxic carcinogens to a lay audience (COC, 2006).

Table 3: Proposed banding of MOE values for risk communication.

MOE Band	Interpretation
<10,000	May be a concern
10,000 – 1,000,000	Unlikely to be a concern
>1,000,000	Highly unlikely to be a concern

As part of an investigative exercise for the application of the MOE approach for the evaluation of genotoxic carcinogens, the COC considered 3 case study examples of genotoxic soil contaminants, namely; hexavalent chromium, benzo(a)pyrene (B[a]P) and 1,2-dichloroethane (COC, 2007). The MOEs were calculated for different exposure scenarios using data obtained from exposures to the compounds in air, water and food. The lowest BMDL10 value obtained from each cancer dataset and the upper and lower bound estimates of human exposure were used to calculate MOE ranges.

Benzo[a]pyrene

MOE values calculated ranged from 130,000 - 7,000,000, 700,000-34,000,000 and 700,000-80,000,000 for the exposure of B(a)P in the diet, air and water respectively. Based on the COC's suggestion for defining MOE bands, the B[a]P values would be interpreted as being "unlikely or highly unlikely to be a concern".

Chromium

The MOE values calculated for chromium ranged from 9,100-90,000³, 11,000,000-1,000,000,000 and 11,000,000-1,000,000,000 for exposure in the diet, air and water respectively. Based on the COC banding, MOE values calculated for exposure to Cr from water and air would be interpreted to be "unlikely or highly unlikely to be a concern".

1,2-dichloroethane

MOE values calculated for 2-dichloroethane ranged from 4,000,000-192,000,000 and 71,000-64,000,000 for exposures in the air and water, respectively. Exposure from the diet was considered to be negligible. Based on the COC banding, these MOE values would be interpreted as being "unlikely to highly unlikely to be a concern".

Case by case interpretation of the Level of Concern associated with a MOE through thorough evaluation of the related levels of uncertainty

An international conference organised by the EFSA and the WHO with the support of the ILSI Europe (November 2005) provided a forum for discussion and review of proposed approaches for evaluating the potential risks from substances that are both genotoxic and carcinogenic.

Delegates at the conference concluded that the MOE approach was the preferred means of providing advice to risk managers for substances that are both genotoxic and carcinogenic, but a consensus was not reached on how best to interpret the MOE in terms of risk to human health, particularly with regards to the magnitude of the MOE calculated.

To gain a better understanding of the application and interpretation of MOE, ILSI Europe established an expert group, which evaluated 12 case studies of genotoxic and carcinogenic compounds to identify critical issues to aid in the interpretation of MOE. Provisional results of the expert group were reviewed at a workshop with additional invited experts (ILSI, 2008).

Questions were raised about the scientific rationale initially proposed by the EFSA for the LOC associated with a MOE of 10,000 as an indication of a low LOC for public

³ It is important to note that the COC applied the BMDL for Cr_{VI} which is genotoxic and carcinogenic but the data from the dietary exposure assessment was for total Cr which is virtually all Cr_{III} which is not carcinogenic. The evaluation of Cr here exemplifies the necessity to look at the correct speciation of a chemical when carrying out such analysis.

health. Concerns have been expressed about the potential to misuse or interpret such a value to wrongly justify the exclusion of risk management actions that could include the application of ALARP. The application of the factor of 10 to account for interindividual human variability in cell cycle control was also questioned by ILSI (Barlow et al., 2006, ILSI, 2008).

The system of banding MOE values to aid interpretation of the LOC associated with MOE values was also considered by participants at the ILSI Europe workshop where the use of logarithmic intervals such as: 1-100, 1000-10,000, 10,000-100,000 was discussed. However, the consensus at the conference was that such an approach was immature at the time of discussion as more work was needed to improve the understanding of the uncertainties surrounding the derivation of a MOE.

The work of the ILSI Europe expert group was published in 12 case study examples to exemplify the use of the MOE approach to substances in food that are both genotoxic and carcinogenic, together with an overview paper published in a special issue of Food and Chemical Toxicology (Benford et al., 2010). The evaluation of the different case studies highlighted specific areas that needed to be addressed when attempting to interpret the MOE.

The experience of the evaluation of the 12 case study compounds by the ILSI Europe expert group was that interpretation of a MOE value required thorough assessment and consideration of the many uncertainties that were inherent in the estimates of dietary exposure and the selected POD on the dose response curve for carcinogenicity. Thus a case by case interpretation of individual MOE values is their preferred approach. A review of the outcome of the work of the ILSI Europe expert group and details of the recommended areas for consideration when interpreting a MOE are provided by Benford et al (2010) and briefly discussed below.

The exposure data for the MOE calculations were obtained from estimates of human dietary intakes. Such data have been recommended for use in the estimation of different exposure scenarios and estimations of the exposures encountered by specific population groups (EFSA, 2005, ILSI, 2008). Benford et al (2010) highlight the variability and uncertainties associated with exposure data. Owing to the fact that dietary intake data is country specific, uncertainties arise from both differences in estimates of consumption patterns as well as differences in methodologies employed to carry out such estimations. In the absence of population-specific data on occurrence of the chemicals of interest in foods, and on consumption of those foods, the dietary exposure assessments are usually carried out using highly conservative estimations. This is particularly the case for substances where intermittent exposure is known to occur (e.g. from incidents of contamination or adulteration).

It was noted that the choice of carcinogenicity data and the mathematical modelling methods employed mean that it is possible to obtain very different PoDs/RPs and MOE values for the same compound. The expert group highlighted the importance of transparency in the selection and treatment of the data used to calculate the MOE. It was therefore considered necessary to provide a narrative to describe and justify the selection and treatment of the data to aid the interpretation of the calculated MOE.

A comprehensive assessment of factors that affect the uncertainties in the dose-response modelling was carried out and considered the following:

- biological relevance of observed tumour type for humans,
- quality of the data used to derive POD/RP,
- comparison of model averaging and benchmark analysis,
- choice of doses for modelling,
- suitability of model,
- selection of BMR and
- choice of POD/RP (T25/ BMDL10).

The reader is referred to the review by Benford et al (2010) for further information on the various aspects indicated.

Upon consideration of the different factors that affect the level of uncertainty associated with the dose response and exposure assessment, the ILSI Europe expert group reached the conclusion that MOE values are often not directly comparable, particularly when the type of exposure is taken into account (i.e. intermittent or continuous exposure). Nevertheless, the ILSI Europe expert group concluded that the magnitude of the MOE would be helpful in the prioritisation of risk management actions. For the 12 case study compounds evaluated, a number of compounds had MOE values in the range 200 to 4000, which were interpreted as being of higher priority compared to the other compounds, which had MOEs that ranged from 20,000 to 100,000,000.

ILSI Europe emphasised the importance of considering the mode of action for the tumour as well as the human relevance of the observed tumourigenic response when interpreting the MOE. It was also suggested that the human health implications of relatively low MOEs could be better understood through improvement of the exposure assessment. By reducing the number of conservative assumptions in the exposure assessment, the level of confidence in the calculated MOE may be improved. It was noted that interpretation of different MOEs should consider the fact that the MOEs are estimates, with varying levels of uncertainty, which all need to be taken into account when interpreting the level of priority for risk management action.

Table 5: Summary of Publications Identified To Have Applied the MOE Approach in Their Risk Assessment

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Exposure data and exposure assessment	Interpretation of MOE
Lachenmeier et al 2009a	Acetaldehyde (in mouth wash)	BMDL - 56mg/kg/d	Mean MOE = 217,604 Median: 238,021 90 th percentile: 121,172 95 th percentile: 108,699 99 th percentile: 82,581	Carcinogenicity data was obtained from a study by Soffritti et al. (2002) – using the same BMDL calculated from the previous study (Lachenmeier et al., 2009a).	The study investigators examined the level of acetaldehyde in alcohol-containing mouthwashes (n=13) using healthy non smoking volunteers (n=4). Different exposure scenarios and corresponding MOEs were calculated.	EFSA- authors stated that as their MOEs were significantly higher than the threshold of 10,000, exposure to acetaldehyde from mouth wash was of low risk after being systematically distributed through the body. Barton-2005: the authors suggested that an additional safety factor of 3 may be required to protected children between 2-15yrs of age (therefore their MOE threshold was 30,000). This value was also not exceeded and so considered not to be a high risk for children aged 2-15.

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Exposure data and exposure assessment	Interpretation of MOE
Lachenmeier et al 2009b	Ethyl carbamate in alcoholic beverages sold in Mexico and Guatemala.	BMDL10 0.3mg/kg bw/day	MOE for 1 drink at different scenarios: Mean: 14,400 Median: 18,000 90 th percentile: 7,200 95 th percentile: 5,143 99 th percentile: 3,273 MOE for 2 drinks or more at the different exposure scenarios ranged from 7,200 to 655 for mean to high consumers at 99 th percentile.	Carcinogenicity data was not reviewed by the authors. The authors applied the BMDL value for ethyl carbamate obtained by the JECFA. Chose the BMDL for the incidence of alveolar and bronchiolar neoplasms which was the most sensitive end points observed.	Levels of ethyl carbamate were estimated from the sampling of 110 Mexican agave spirits collected over a 3 year period from major production sites in 4 different Mexican states. The data from the sampling in Mexico were used to estimate whole population exposure scenarios. The authors also provided exposure scenarios for individual drinkers, providing the estimated exposures for different numbers of drinks per day and for different concentrations of ethyl carbamate.	The authors concluded that the levels of EC were of low priority for risk management action as the MOEs for the whole population risk assessment of EC in Mexico for the annual per capita consumption of 1 litre of pure alcohol in the form of agave spirit (for a 60kg person). This was due to the fact that the MOEs for all scenarios for the different concentrations were above 10,000. The authors found that MOEs were below 10,000 for individuals that drank more than 2 drinks a day. However evaluation of the survey showed that the proportion of Mexicans consuming more than 20g pure alcohol per day (about 2 drinks) per capita was about 20.5% overall (36.4% men & 5.8% women)

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Exposure data and exposure assessment	Interpretation of MOE
Lachenmeier et al 2009c	Acetaldehyde in alcoholic beverages (outside ethanol metabolism)	BMDL10	For mean concentrations of alcoholic consumption the MOEs were: Mean MOE: 498 Median: 634 90 th percentile: 249 95 th percentile: 187 97.5 th percentile: 140 99 th percentile: 100	3 different studies were considered for the dose-response modelling. A lifetime study of carcinogenicity by Soffritti et al (2002) was selected which administered acetaldehyde (0, 50, 250, 500, 1500 or 2500 mg/l) to rats in drinking water. Although carcinogenic effects were observed in various organs and tissues, the data for increase in total malignant tumours was used to in the modelling. Best fitting model was obtained from male tumour bearing rats.	Data on the levels of acetaldehyde content in alcoholic drinks. EU data on alcohol consumption from 15 different countries was combined with data available for data available for acetaldehyde concentrations in alcoholic beverages. Exposure to acetaldehyde from alcoholic beverages was calculated for different exposure scenarios for different acetaldehyde concentrations in the beverages as well as exposure scenarios for different amounts of alcoholic beverage consumption in Europe (mean, 90th, 95th, 97.5th and 99th percentiles).	EFSA recommendations for the use the types of data to be used for D-R modelling, exposure assessment scenarios and MOE calculation for different exposure scenarios were all considered. The authors stated that the calculated MOEs were below the 10,000 threshold, which demonstrated that the levels of acetaldehyde in alcoholic beverages were of a public health concern.

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Exposure data and exposure assessment	Interpretation of MOE
Lachenmeier et al 2009d	Furan (in home cooked foods for infants and young children)	T25	<p>MOEs calculated for babies aged 6 months: Mean MOE: 2692 Median: 2991 90th percentile: 1224 95th percentile: 997</p> <p>Overall the MOE calculated for babies aged 3 to 12 months ranged from 6060 to 930 for mean to high exposure at 99th percentile respectively.</p>	The carcinogenic data were not examined in this paper nor was the dose-response carried out as it was beyond the scope of the study. The T25 identified by Sanner et al. (2001) was selected for the preliminary risk assessment.	The authors carried out a survey of the levels of furan in commercial ready to eat baby foods. No information relating to the nature of the dietary exposure of the animal study, from which the T25 was obtained, was provided.	Risk assessment was conducted according to recommendations by EFSA 2005. Calculated MOE was below 10,000 and was interpreted as a potential health concern at the levels of exposures observed. Using recommendations from Barton et al., 2005; an extra safety factor of 10 was applied to account for the fact that the exposure was in children aged 0-2yrs.
Akpambang V.O.E 2009	PAHs - BaP & PAH8	BMDL10 calculated by EFSA CONTA M panel for BaP & PAH8	MOE calculated on the basis of 100g fish or meat consumed daily. The MOEs for B[a]P ranged from 1346 to 17,722 and from 1816 to 8437 for PAH8.	The carcinogenicity data were not considered by the authors, as the focus of the study was on quantifying the levels of PAHs observed in commonly eaten Nigerian fish.	The levels of PAHs in different types of commercially smoked fish and meat sold in 4 different markets in Akure, Nigeria were determined. The authors also estimated the levels of PAHs using laboratory grilled/ smoked techniques. Daily human exposure levels were calculated on the basis of 100g of fish/ meat consumed daily based on a body weight of 60kg.	MOE values were compared to the EFSA value of 10000. The authors concluded that the low MOE values observed indicated a potential concern for consumer health. The authors also highlighted the need for risk management action.

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Exposure data and exposure assessment	Interpretation of MOE
Wang J et al 2009 (abstract only - article in Chinese)	Aflatoxin	Not stated	204 to 12,305	Details of the animals studies used to calculate the MOEs were not provided in the abstract.	Data collected from health investigations carried out among Chinese residents. Mean and high level (97 percentile) exposure scenarios were calculated for the whole country, urban and rural areas.	The authors cited the use of data applied by the JECFA for the mathematical modelling of the available data. The risks interpreted from average level exposure to aflatoxins were said to be "middle" (assumed to mean moderate) while those people whose dietary exposure levels was high were considered to be at a higher risk.
Schuetze et al 2008	Veterinary drug malachite green (in eels) [Note that JECFA vet also determined MOE for this compound in 2008/9]	LOEL obtained from the studies of the US National Toxicology program (2005).	1.8 million to 49 million for acute and chronic consumption of eels.	Not reviewed. Data from the US NTP was used. However the LOEL observed in the study was used to calculate MOE.	Maximum residues were calculated from sampling carried out in fishing areas. Highest residue found in a study reviewed by The Committee For Medicinal Products For Veterinary Use was used in the calculation. MOE was calculated for acute and chronic exposure scenarios for both adults and children. the worst case scenario consumption data was used to calculate the MOE	Interpretation was based on EFSA threshold of 10,000. The risk was therefore interpreted to be low for both single and casual consumption of eels containing the maximum residues detected in the study. The study investigators still made reference to the application of the precautionary principle. Adding that the occurrence of genotoxic / carcinogenic agents such as MG and LMG in foods for human consumption should not be tolerable even at low concentrations.

First Author	Substance	POD selected	MOE	Quality of carcinogenicity data and dose-response modelling	Quality of exposure data and exposure assessment	Reference for interpretation of MOE
Zielmaker et al 2006	N-nitrosodimethylamine (NDMA)	BMDL10	7,000 for children aged 1yr old & 72,500 for adults (>25yrs).	The authors reviewed an extensive range of chronic carcinogenicity data for NDMA.	Food consumption survey data was used which enabled different exposure consumption scenarios to be calculated. The 95 percentile of long-term exposure distribution for children of 1yr of age and adults was calculated.	The authors stated that the calculated MOE values for children and adults should be interpreted as margins of uncertainty in the (single) MOE due to the fact that exposure is a function of age, which as a whole contributes to the cancer risk.
Tardiff et al 2009	Acrylamide (AA) & glycidamide (GA) in cooked foods	BMDL10 for average & high consumer	200 and 1200 for AA and GA respectively (average exposure) and 50 (AA) & 300 (GA) (high exposure consumers)	Data used by JECFA was used in the determination of the MOE	Physiologically based toxicokinetics (PBTK/ PBPK) was used to characterize the range of intakes for humans.	A non linear approach (see text) was used to calculate the MOE for the cancer endpoint (based on the evidence for the MOA reviewed by the authors). The authors concluded that the MOEs for average exposures to AA in cooked foods provided an adequate margin of safety for tumour formation. This is an example of the use of MOA information to help in the interpretation of the MOE.

Identification of Publications on the Application of the MOE Approach for the Risk Assessment of Genotoxic Carcinogens

A search strategy was designed to identify peer reviewed publications and grey literature on the use of MOEs when dealing with genotoxic carcinogens. The search for peer reviewed publications was limited to PubMed and the search engine Google was used to identify grey literature in the subject area. Full information on the search strategy including the search terms and the criteria applied to limit the results to relevant publications is provided as supplementary information.

Thirty two publications were identified from PubMed, Google and citation searching. This included the 12 different case studies published by the ILSI, Europe expert group as part of their assessment of the MOE approach for evaluating genotoxic and carcinogenic substances, 9 papers that had applied the MOE approach in the evaluation or risk assessment of carcinogenic compounds and 11 other publications which fell under the category of review papers and grey literature.

The review papers identified provided the background to this MOE project. The published papers provided extensive reviews on the current state of science for the risk assessment of genotoxic carcinogens (Barlow et al., 2006, Dybing et al., 1997, O'Brien et al., 2006, Schlatter et al., 2010). The search also identified the report of the International Conference organised by EFSA and WHO with the support of ILSI, Europe (Barlow et al., 2006) and also the report of the work of the ILSI Europe expert group set up to discuss specifically the application of the MOE approach to a series of case studies for genotoxic carcinogens (Benford et al., 2010). The 12 case studies identified by this search strategy were the supporting information for the review carried out by Benford et al (2010). The MOE approach was also discussed, although in less detail in the remaining 3 review papers. Crebelli (2006) discussed the current state of science for the risk assessment of genotoxic carcinogens in the European Union and discussed some of the limitations of the use of the ALARA approach. The MOE approach was applied and briefly discussed in a review by Hernandez et al (2009) as part of their consideration of different mechanisms for non-genotoxic carcinogens, however the authors did not provide details of the MOE calculations. Similarly, Pratt et al (2009) highlighted the MOE approach as part of a more general review of the influence of thresholds in the risk assessment of genotoxic carcinogens.

The 3 main publications from EFSA, JECFA and ILSI Europe on MOE were identified within the grey literature. The *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* (2005) provided an overview of the current state of risk assessment for genotoxic carcinogens as well as guidelines for the recommended use of the MOE approach. The report of the 64th meeting of the JECFA (2005) provided details on the MOE approach within the *General Considerations* which highlighted the committee's preference for the use of this approach for the risk assessment of genotoxic carcinogens. A summary report of a workshop organised by ILSI Europe: *Application of the Margin of Exposure Approach to Compounds in Food which are both Genotoxic and Carcinogenic* (2008) detailed the work of the ILSI Europe expert group on the development and use of the MOE approach. In addition to these key papers, opinions of the Federal Institute for Risk Assessment were also identified within the grey literature (BfR, 2005b, BfR, 2005a) Crucially, the opinions from BfR included general comments on the published

EFSA draft opinion of 2005. The Annual Report of the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (2007) was also identified from the search. Aside from the relevant publications identified using the search strategy and discussed briefly above, efforts were made to obtain further relevant publications on an ad hoc basis.

Application of the MOE approach in a risk assessment setting

Nine papers published between 2006 and 2009 were identified by the search strategy. A summary of the different publications is provided in table 4. A review of the studies identified focused on identifying the methodology applied to interpreting the LOC associated with the calculated MOE. The papers are discussed in more detail below.

Acetaldehyde in alcoholic beverages and mouth washes (Lachenmeier et al., 2009a, Lachenmeier et al., 2009c)

The MOE approach was applied by Lachenmeier *et al.* in their risk assessment of acetaldehyde (ALDH) in mouth wash and alcoholic beverages. The authors carried out dose-response analysis applying the bench mark dose approach and also exposure assessment using publicly available consumption data (alcohol) and estimated levels of acetaldehyde in saliva (after mouthwash usage).

The authors carried out the risk assessment according to the recommendations provided by EFSA. Different studies were reviewed for the dose-response analysis and a life time carcinogenicity oral study was selected on the basis that best reflected the exposure route for humans. The ALDH concentrations in different amounts of alcohol at varying exposure scenarios were calculated. MOEs of 498, 634, 249, 187, 140 and 100 for the mean, median, 90th percentile, 95th percentile, 97.5th percentile and 99th percentile respectively were calculated. The limitations of the risk assessment were identified by the authors (Lachenmeier et al., 2009c).

For exposure to ALDH in mouth wash (Lachenmeier et al., 2009a), the authors calculated MOEs of 217604, 238021, 121172, 108699 and 82581 for the mean, median, 90th percentile, 95th percentile and 99th percentile respectively. The authors interpreted the magnitude of the calculated MOE values on the basis of the potential health concerns or the level of priority for risk management action. This was determined by comparing the magnitude of their calculated MOE value with the magnitude of 10,000 put forward by EFSA.

With respect to risk assessment carried out for ALDH in mouth washes, the authors felt it was necessary to add an additional safety factor of 3 as recommended by Barton et al (2005) when considering exposure of children between the ages of 2-15 years. The authors interpreted the MOE of 217,604 for exposure to ALDH in alcohol containing mouth washes to be of low risk as it exceeded the amended MOE threshold of 30,000.

The authors considered the different limitations of the risk assessment which included the choice of the study, the exposure assessment and the possibility of the effects of

genetic polymorphisms. It was concluded that the calculated MOE did not overestimate the risks even after taking into consideration the various limitations. In addition, the authors applied an additional safety factor of 10 (producing a MOE threshold of 100,000) in their assessment of ALDH in alcohol to reflect the risks posed to ALDH dehydrogenase-deficient humans. The mean MOE of 498 for exposure to ALDH in alcoholic beverages was therefore interpreted as being of a potential health concern and a priority for risk management action.

Ethyl carbamate in alcoholic beverages (Lachenmeier et al., 2009b)

Lachenmeier et al (2009b) carried out a risk assessment of ethyl carbamate in alcoholic beverages in an effort to determine whether exposure to this compound was a contributing factor in the reported levels of liver cirrhosis observed in Mexico. The authors carried out a sampling survey to estimate the levels of ethyl carbamate in spirits purchased within selected states in Mexico. This information was combined with data on consumption obtained from the Global Information System on Alcohol and Health for the year 2004 to calculate the MOE.

The authors did not carry out the dose-response modelling themselves, rather they chose to use the BMDL10 calculated by JECFA (and also used by EFSA) for the most sensitive end point (incidence of alveolar and bronchiolar neoplasms) in their MOE calculations.

The authors noted that their estimations of the levels of ethyl carbamate in spirits were comparable to those estimated by EFSA for European spirits. For a consumption of 1 drink per day, MOEs of 14400, 18000, 7200, 5143, 3273 were calculated for the mean, median, 90th percentile, 95th percentile and 99th percentile respectively.

The authors highlighted their sampling strategy (restricted to only a small sub-group of Mexican states) as a limitation in their exposure assessment but concluded that the levels estimated were unlikely to have been different in other Mexican states. Although the authors did not obtain a BMDL10 for a liver cancer endpoint, they nevertheless concluded that as their calculated average MOE of 52,560 for ethyl carbamate (calculated using data obtained from lung cancer) was unlikely to play a role in the high rates of liver cirrhosis reported in Mexico.

Calculation of MOEs for different intake scenarios (1 to 5 drinks/ day for a 60kg person) showed that the MOE was below 10,000 for mean and median levels of exposure. However lower levels of MOE were calculated for estimated consumption of 2 or more drinks/ day (7,200 – 2,880). The authors however focused their final conclusions and interpretations on the mean MOEs calculated from the scenarios based on the ethyl carbamate concentrations, highlighting that available survey and per capita information showed that the proportion of Mexicans consuming more than 2 drinks per day was estimated to be 20.5% overall.

Furan in commercially produced baby foods (Lachenmeier et al., 2009d)

Lachenmeier et al also carried out a risk assessment of furan in commercially jarred baby foods using the MOE approach. The authors sampled 230 commercial ready to eat baby foods over a 3 year period to determine the levels of furan they contained.

The exposure for the intake scenarios for 4 different groups (3, 6, 9 and 12 months) was calculated. The authors did not carry out dose-response modelling of carcinogenicity data themselves, but applied the T25 value obtained from a study by Sanner et al (2001) to calculate their MOE.

The MOEs for the different exposure scenarios calculated with the T25⁴ were compared to the 10,000 threshold value put forward by EFSA. The MOEs for babies aged 3 to 12 months were calculated at different exposure scenarios. The MOEs for babies aged 6 months were estimated to be 2692, 1224, 997 and 792 for mean, 90th percentile, 95th percentile and 99th percentile exposure scenarios respectively. The MOEs calculated for all exposure scenarios were all interpreted to be a potential health concern for this contaminant. In addition the authors felt that it may be necessary to add an additional safety factor of 10 as recommended by Barton et al (2005) as the threshold of 10,000 was derived for adults. The authors acknowledged the uncertainties in using the T25 in their MOE calculation but concluded nevertheless that their preliminary MOE value justified the need to find ways of reducing the levels of furan in baby foods.

PAHs in smoked/ grilled fish (Akpambang et al., 2009)

Akpambang et al carried out a sampling survey of the levels of PAHs present in commercially and laboratory smoked meat and fish. The authors sought to investigate the PAH content of smoked/ grilled fish and meat commonly consumed in Nigeria. The authors applied a MOE approach to determine the LOC associated with the calculated levels of PAHs. No exposure assessment or dose-response modelling was carried out in this study, rather; the authors used the BMDL10 calculated for B[a]P and PAH8 by EFSA (2008) and assumed a daily consumption of 100g of meat/ fish per person per day based on a body weight of 60kg based on calculations carried out by Yeh et al (1996).

The authors interpreted the MOE calculated for a single exposure scenario (consumption of 100g/day) and did not consider different exposure scenarios to take into account low and high consumers. MOEs for B[a]P were 1346, 17722, 8008, 2218, 5015, 6652 for commercially smoked mudfish, jackfish, mackerel, croaker, suya and antelope respectively. The authors concluded that legal limits for PAHs in traditionally smoked foodstuffs in Nigeria were necessary and there was a need for risk management action for those products for which the MOE values were calculated to be less than 10,000.

⁴It should be noted that the EFSA committee do not recommend the use of the 10,000 to interpret the LOC in cases where the T25 rather than the BMDL10 has been applied.

Aflatoxins (Wang et al., 2009)

Wang et al applied the MOE approach to their evaluation of the risks attributed to dietary aflatoxins. Only an abstract published in English was available for review as the article was published in Chinese. The authors cited the use of the data used in the mathematical model used by JECFA⁵ and carried out the exposure assessment using nutritional data and data obtained from food inspection for food contaminants in China. The authors calculated MOEs for high and average exposure scenarios for the whole country, and also separately for urban and rural areas and obtained MOE values of 9017, 12304, 8006 respectively for average exposure and 242, 345 and 204 respectively for high exposures. The authors interpreted the LOC for the MOEs for average users to be “middle” and for the MOEs from high exposures to be “high”.

Level of malachite green in eels (Schuetze et al., 2008)

Schuetze et al carried out a survey of the levels of malachite green (MG) in wild eels caught from different lakes, a river and canal in Berlin, Germany. The residue levels of MG were established from the tissues of the eels. The authors calculated the MOE based on “worst case” scenario consumption data estimated for lifetime exposure and the LOEL for a slightly raised incidence of neoplasms in an NTP study in rats. An MOE of 1.8 million was calculated for children and 3.4 million for adults. Although the authors accepted that their calculated MOEs were significantly higher than the EFSA value of 10,000 considered to be of low concern for genotoxic carcinogens, they felt that the precautionary principle should be applied to avoid any exposure to a genotoxic carcinogen in foods.

N-nitrosodimethylamine (Zielmaker et al., 2010)

Zielmaker et al carried out risk assessment for the compound nitrosodimethylamine (NDMA) and modelled acute and chronic carcinogenicity data for children (1year old) and adults (>25 years) using the BMD approach. The authors used consumption data obtained from food consumption surveys. The authors considered the factors affecting the variability of the results obtained for the exposure assessment and the impact of the associated uncertainties on the exposure estimates. The MOE for children aged 1 was calculated to be 7,000 and for adults aged 25 years and above it was 72,000.

The authors were keen to note that care should be taken in the interpretation of the LOC associated with the calculated MOE values for chronic exposure in children (7000) and adults (72,500). It was their opinion that the lower MOE in children should not be interpreted as an indication that children were at a higher risk than adults, adding that it is the lifetime risk that is of concern and therefore the MOE in children, based on their higher exposure, does not reflect the lifetime risk of exposure to NDMA in humans.

⁵ It should be noted however that JECFA did not carry out an evaluation of aflatoxin so it is unclear which JECFA data the paper is referring to.

In acknowledging the fact that that MOE values are sometimes difficult to interpret, the authors considered the magnitude of the levels of exposure between the doses examined in the animal study compared to human exposure. They concluded that the relative differences in exposure in young and adult rats mirrored the scenarios in humans. The authors argued that the higher exposures (as a result of the higher dose per body weight) in children had also been observed in the young rats; therefore the effects of age-related change in exposure had already been accounted for in the animal study. The factor differences between the exposures in both humans and animals were calculated as well as the level of exposure over an entire lifetime at different exposure scenarios. These values were then compared to the dose calculated to be associated with 10^{-6} cancer risk (this value was considered to be the worst case estimate of the cancer risk in humans as it was calculated by linear extrapolation) in humans.

The authors then argued that the risks posed to those that exceeded the exposure limit might be assessed by considering the distribution of risks associated with the variability in human exposure for the upper 95th percentile of the population. The comparison of the level of exposure associated with 10^{-6} cancer risk showed that 5% of children and 50% of adults exceeded the exposure limit, corresponding to a risk estimate of 6×10^{-6} and 8×10^{-7} for children and adults respectively. The authors concluded that similar to the interpretation of the LOC for MOE levels in adults and children, the estimated risk lay somewhere between 6×10^{-6} and 8×10^{-7} for 95th percentile of the population.

The authors considered the relevance of their estimated risks in terms of life expectancy by evaluating time-to-tumour data. This evaluation therefore considered the relationship between the cancer risk and the decrease in time-to-tumour at the same dose. Applying the most conservative model, the authors concluded that even the higher estimate of cancer risk in children which corresponded to high but relatively short exposures, resulted in an estimated loss of healthy lifetime of less than 9 days in a large fraction of the population.

The authors considered the carcinogenicity data from the rat to be an appropriate model for humans and so therefore felt that a low assessment factor for interspecies differences in carcinogenicity would be appropriate. Although chemical specific information was lacking, the authors felt that the outcome of the probabilistic evaluation in their study was an appropriate surrogate for the usual default assessment factor of 10. The authors concluded that after consideration of the limitations of their risk assessment and the conservative nature of their risk estimates and exposure assessment, the exposure to NDMA as a result of the consumption of vegetable meals appeared to result in only marginal increases in human cancer risk.

Acrylamide (Tardiff et al., 2009)

Tardiff et al reviewed evidence from mutagenic and epidemiological studies for acrylamide (AA) and concluded there was sufficient evidence of nonlinearity for the cancer MOA of AA. The authors focused on the evidence of nonlinearity for AA acting as a weak dopamine agonist and suggested that the effects of AA on dopamine receptors may serve as the initial step for tumour formation in rats. The authors concluded that the involvement of several endocrine sensitive tissues (thyroid, testes, mammary gland and CNS) were indicative of the involvement of the endocrine system for AA-mediated carcinogenesis. Data were therefore pooled together for the four endpoints which were subsequently modelled using mathematical models that provided the best overall fit for the data.

The authors calculated the geometric mean for the PODs for the four tumour endpoints obtained from the dose response curves and divided this value by a net uncertainty factor (UF) of 75 to obtain nonlinear cancer TDI values for AA. The authors applied the same UF of 7.5 (2.5 (inter-species dynamics) X 3 (intra-species dynamics) X 1 (inter-species kinetics) X 1 (intra-species kinetics)) as that which would be applied for the calculation for the TDI for a non cancer endpoint but applied an additional UF of 10 to account for the severity of effect. In addition two cancer TDIs were calculated based upon information obtained from the dose-response, using the AUC as an estimate of internal exposure, for AA (where AA was considered to be responsible for the adverse effect) and for GA (where GA was considered to be responsible for the adverse effect).

In the scenario where AA was considered to be responsible for the effect, Tardiff et al. applied a human PBTK model and calculated the human equivalent doses of AA to the external TDI values of 4 and 3µg/kg/d for AA and GA respectively. Where GA was considered to be responsible for the effect the external TDI values were calculated to be 25 and 15µg/kg/d for AA and GA respectively.

As an alternative approach to characterising the risks from the cancer endpoints evaluated, the authors also calculated the MOE for the tumour endpoints. The JECFA (2005) dietary exposures estimated for average and high consumers were used for the MOE calculations and reference was made to the ILSI Europe recommendations for the use of MOE in risk characterization (Barlow et al., 2006). The geometric mean BMDL10 values were divided by the average and high daily intakes of food borne AA to obtain the MOE. The JECFA estimate of 1 µg/kg-day for an average consumer was used to obtain an MOE value of 200 for AA and 1200 for its metabolite GA. Using the JECFA exposure level for a high consumer (4µg/kg-day) gave MOE values for AA and GA of 50 and 300, respectively.

As a hypothetical exercise, the authors also calculated risk estimates based on low-dose linearity. Estimated daily intakes corresponding to the maximum likelihood estimate using a 1×10^{-5} risk level was calculated to be 2µg and 14µg per person for AA and GA respectively (assuming a default 75kg body weight). The dietary risk estimates corresponding to 1×10^{-5} upper bound risk level was estimated to be 1µg and 9µg per person for high and average consumers respectively. It was noted

however that for both cases, the lower-bound of the risk estimate was zero. The authors concluded that the calculated MOE for average exposure to AA in cooked foods provided an “adequate margin of safety... to preclude tumour formation”. They also noted that they had a high level of certainty in their conclusions based on their use of their human internal dosimetry model and a reasonable understanding of the MOA for AA.

Although not stated explicitly in their paper, it is clear that Tardiff et al have used the argument of non-linearity to suggest that the level of concern should be less than 10,000 hence their conclusion that the MOE values of 200 and 50 for average and high consumers of AA would provide an adequate margin of safety to preclude tumour formation. In doing so it is presumed that they have made the assumption that there is a direct relationship between the interpretation of the LOC for MOE and the dose response assessment.

Discussion

The aim of this review was to undertake a comprehensive and critical review of current advice and practice on the interpretation of the LOC associated with a particular magnitude of MOE. The search strategy identified relevant literature relating to the use of the MOE approach for the evaluation of genotoxic carcinogens. In addition, 9 studies met the criteria which sought to identify those that had applied the MOE approach to the risk characterisation of substances that were both genotoxic and carcinogenic.

Discussions of the MOE approach by international experts have resulted in concern being expressed about the feasibility of directly comparing MOE values between different substances and also about the rationale put forward for the derivation of the 10,000 threshold by EFSA. An expert working group organised by ILSI Europe calculated MOEs for 12 case study compounds to further explore these issues. Based on these evaluations, the working group made recommendations for the consideration of factors that have the potential to significantly affect the magnitude of the MOE calculated and ultimately affect how the MOE should be interpreted and communicated. The application of the MOE approach to the evaluation of the 12 different case study compounds was reviewed by Benford et al (2010). The publication of the work carried out by the ILSI Europe expert group represents the most recent effort by the international community to harmonise the application of the MOE approach to dealing with compounds that are both genotoxic and carcinogenic. Benford et al. concluded that the direct comparison of MOEs obtained for different compounds was difficult as a case by case assessment of the uncertainties associated with the individual MOE had to be undertaken. The latter included the consideration of uncertainties relating to the dose response modelling of the carcinogenicity data, the exposure assessment and consideration of the type of exposure likely to be experienced by humans.

Since the initial proposal for the use of the MOE approach for risk assessment of chemicals that are both genotoxic and carcinogenic by the EFSA Scientific Committee in 2005, only 9 studies published between 2006 and 2009 were found to have met the criteria of having applied the MOE approach to the risk assessment evaluations of a genotoxic and carcinogenic compound. Of the 9 papers identified to

have applied the MOE in their evaluation of compounds that were both genotoxic and carcinogenic, 7 had applied the EFSA threshold approach to interpret the findings of their MOE calculations. Only 2 of the studies (Tardiff et al., 2009, Zeilmaker et al., 2010) identified applied a different approach from that recommended by EFSA on the basis that the MOE values calculated were interpreted after considering the different uncertainties that were inherent in the exposure assessment calculations and mathematical treatment of the carcinogenicity data.

Only 3 of the publications carried out their own dose response modelling (Lachenmeier et al., 2009c, Tardiff et al., 2009, Zeilmaker et al., 2010). The 3 studies reviewed different carcinogenicity data and provided justification for the choice of study. In addition all 3 authors considered the different uncertainties associated with the exposure assessments and the dose response modelling. Tardiff et al (2009) and Zeilmaker et al., (2006) however provided the most comprehensive assessment of the varying uncertainties associated with their MOE calculation in contrast to Lachenmeier et al, who while they considered the limitations inherent in their study, focused mostly on the comparison of their MOE value with the EFSA value of 10,000.

The remaining studies focused mainly on carrying out exposure assessments, through the consideration of consumption data, and estimations of dietary intakes at different exposure scenarios for different compounds in either food stuffs or consumer related products. To calculate the MOE, the authors obtained the relevant reference points (mostly BMDL10) from evaluations carried out by JECFA, EFSA or the US NTP. The levels of concern were then interpreted at different exposure scenarios by comparison of the values with the EFSA value of 10,000.

The main differences that arose between the studies in their interpretation of the levels of concern either through comparison of their calculated MOE with the value of 10,000 or through their own interpretation upon consideration of the uncertainties inherent in their calculation of MOE, related to the interpretation of MOEs calculated for children and the interpretation of MOEs for genotoxic compounds that exhibit thresholds for certain end points. Both issues are discussed in further detail below.

Interpretation of the MOE values calculated for children

Although the risk assessments carried out by Lachenmeier et al (2009a and 2009d) applied the MOE level of 10,000 as their bench mark from which to interpret the LOC, additional safety factors were applied by the authors on the basis of recommendations by Barton et al (2005) to establish more conservative values for the comparison of the MOEs calculated for children. Factors of 10 and 3 were applied for the risk assessments carried out for the exposure of children aged 0 to 1 year to furan (in commercial baby foods) and children aged 2 to 15 years to acetaldehyde (in alcohol containing mouthwashes), respectively. In their risk assessment of furan, Lachenmeier et al (2009d) applied the T25 for the calculation of the MOE. The comparison of the calculated MOEs with the value of 10,000 for the interpretation of the LOC was not consistent with the recommendations put forward by EFSA. The EFSA are of the view that a MOE would not be considered to be of low concern if the MOE was calculated using a T25 the resulting MOE would be associated with greater uncertainties.

In contrast to the approach applied by Lachenmeier et al (2009a and 2009d), Zielmaker et al (2006), who calculated the MOE for exposure of children to NDMA as 7,000 and for adults as 72,000, respectively, concluded that the MOE values calculated for children and adults should be interpreted as margins of uncertainty around a single MOE without the distinction between ages. They considered exposure to be a function of age, which contributed to the cancer risk, but the level of this contribution was unknown, although in part it was taken into account in the animal cancer studies.

It was their opinion that the lower MOE in children should not be interpreted as an indication that children were at a higher risk than adults, adding that it is the lifetime risk that is of concern and therefore the MOE in children, based on their higher exposure, does not reflect the lifetime risk of exposure to NDMA. The authors raised the point that the interpretation of the level of risk associated with an MOE for a child versus an adult should consider whether the cancer risk is driven by exposures in early or later in life. In cases where the former was true, the MOE in children would clearly be deemed more relevant. However, in acknowledgment that in reality, exposure in both early and later life were probably relevant, the authors considered the “real” MOE to lie somewhere between the two calculated values. The authors noted that since exposure was a function of age, which as a whole contributed to the cancer risk, the MOEs calculated for both children and adults should be considered as the risks associated with chronic exposure without a distinction between age groups.

To aid the interpretation of the LOC from exposure to NDMA, in their risk characterisation Zielmaker et al not only calculated the MOE levels but also estimated the levels of exposure associated with 10^{-6} extra cancer risk. After consideration of the uncertainties associated with the different approaches to characterising the risk from NDMA, the authors’ final interpretation of the LOC was provided by reference to the level of associated cancer risk, which for NDMA was considered to lead to only marginal increases of human cancer risk.

Interpretation of the MOE for genotoxic compounds that exhibit a threshold.

The estimation of the safe dietary intake levels of acrylamide (AA) in humans was considered by Tardiff et al (2010). The authors concluded that there was sufficient evidence of nonlinearity for the cancer MOA of AA and therefore calculated a TDI for AA and its metabolite. As an alternative approach to characterising the risks from the cancer endpoints considered, the authors also calculated the MOE for the grouped cancer end points (mammary gland, CNS, thyroid and testes) and established MOE values of 200 for average intakes and 50 for high intakes. and concluded that the MOE for average users provided an “adequate margin of safety to preclude tumour formation”. However, it should be noted that the latter figure (50) is smaller than the uncertainty factor that is normally applied for inter- and intra-species variation (100). In acknowledgment that some may prefer to apply a default approach to the dose response assessment the authors also estimated the dietary risk estimate corresponding to a 1×10^{-5} upper bound risk level as part of a hypothetical exercise. It was noted however that for both cases, the lower-bound of the risk estimate was zero.

Acrylamide was also evaluated as one of the case study compounds reviewed by the ILSI Europe expert group. Bolger et al (2010) applied data reviewed by JECFA (2005) in their calculation of the MOE and concluded that AA is carcinogenic by a genotoxic mode of action (MOA) involving GA. The authors calculated BMDLs at 1, 5, 10% response for the calculation of the corresponding MOEs. The MOEs for high and average exposure levels were calculated for 2 tumour end points, peritesticular mesothelioma and mammary tumours. The MOE of 200 (based on calculation with BMDL10) calculated for the occurrence of mammary tumours upon average exposure was interpreted by the ILSI Europe expert group as indicating a high priority for risk management (Benford et al., 2010).

The UK Committee on Mutagenicity (COM) also conducted an extensive review on the mutagenic effects of AA (COM, 2009). The COM considered the multiple modes of action (MOAs) for AA and its metabolite and acknowledged that some but not all of the MOAs had been shown to exhibit a threshold. It was the opinion of the COM that the different MOAs could contribute to the genotoxicity exhibited but they were not mutually exclusive. The COM concluded that AA was an *in vivo* mutagen and that until there is conclusive evidence of a threshold with supportive mechanistic data in each of the potential genotoxic MOAs, it was necessary to adopt a default assumption that there is no level of exposure that is without risk. This view was supported by a more recent evaluation carried out by JECFA (2010) which estimated MOE values of 180 and 45 for mean and high dietary exposure levels. The 2010 JECFA concluded that as the compound was both genotoxic and carcinogenic, the derived MOE values indicated a health concern.

Although not stated explicitly in their paper, it is clear that Tardiff et al (2009) used the argument of non-linearity to suggest that the level of concern should be less than 10,000 hence their conclusion that the MOE values of 200 and 50 for average and high consumers of AA would provide an adequate margin of exposure to preclude tumour formation, a very different conclusion from those of Benford et al. (2010), COM (2009) and JECFA (2010). In doing so it is presumed that they have made the assumption that there is a direct relationship between the interpretation of the LOC for MOE and the dose response relationship. These assumptions are discussed in more detail below.

Assumption 1: a nonlinear dose response relationship is used to derive the value of 10,000 for the interpretation of the level of concern.

By assuming that the LOC should be less than the proposed value of 10,000, Tardiff et al (2009) are effectively suggesting that the value for the interpretation of the LOC associated with a MOE should be derived using a nonlinear dose response assessment (as demonstrated by their application of UFs to derive TDI values). Using this rationale, the MOE (assuming that 10,000 was considered to be the “acceptable” level of risk) would result in a lower risk than that already considered “tolerable” and the MOE associated with this new “tolerable” risk would be less than 10,000. Using the values applied by Tardiff et al. in their calculation of the TDI, this assumption would result in the reduced MOE value of 133 rather than 10,000 (obtained by dividing 10,000 by the UF of 75) for the interpretation of the LOC. Comparison of the MOE of 200 for average users to the latter value would therefore explain their conclusion that the MOE would provide an adequate level of safety. However, it is noteworthy that

the MOE obtained by Tardiff et al. for high consumers (50) is lower than the uncertainty factor normally applied for inter- and intraspecies variation.

Assumption 2: if a default linear approach for characterising the carcinogenic risk for AA is preferred, then the MOE of 10,000 is equivalent to the “acceptable” cancer risk level of 1 in 10^{-5} .

Tardiff et al. applied assumption 2 as part of a hypothetical exercise because they considered that some may prefer to apply a linear default to the dose response relationship for AA carcinogenesis. This was demonstrated by their estimation of the human dietary intake that corresponds to a 1 in 10^{-5} “acceptable” cancer risk which they then compared with the estimated values for the daily intakes for AA and GA at this level cancer incidence. By basing their conclusions about the LOC for the MOE on the results obtained from this assumption, it is clear that they have equated the MOE value of 10,000 with a level of exposure that would result in a cancer incidence of 1 in 10^{-5} .

The assumptions made by Tardiff et al imply that the LOC associated with a MOE of 10,000 is effectively the same as carrying out a linear dose response assessment. If one were to accept this link between the LOC and the potential risk value then it is clear that this would require a more thorough consideration of the nature of this association, and of whether any additional allowance should be made for other uncertainties (including inter- and intra-species variation). As discussed in earlier sections of this document, there is currently international agreement that the MOE value of 10,000 should not be seen as some kind of threshold for automatically triggering concern or risk management action (Barlow et al, 2006). Both EFSA and JMPR are cautious about making this association in an effort to preserve the advantages of the MOE.

General considerations

One of the issues raised by Tardiff et al (2009) in their evaluation of AA is the growing body of evidence to support a nonlinear dose response relationship for some genotoxic carcinogens. Known genotoxic and carcinogenic alkylating agents, methylmethane sulfonate (MMS) and ethylmethane sulfonate (EMS), were observed by Doak et al (2007) to exhibit clear thresholds in experiments carried out in cells. It should be noted however that in the same experiment, Doak et al (2007) observed that two other chemicals (MNU and ENU) exhibited a linear dose response relationship. The authors observed no increases in DNA damage above background levels at some low dose exposures and were able to identify a LOEL for the observed mutagenic effects. It was suggested that the observed nonlinearity of the dose response relationship was likely to be the result of cellular homeostatic maintenance by DNA repair. Similar results were observed in experiments carried out *in vivo* in the rat and mouse. A NOAEL of 20mg/kg/day for organ toxicity was observed in a 4 week study in wistar rats (Pfister and Eichinger-Chapelon, 2009). Genotoxicity tests carried out in the mouse showed that for doses up to 25mg/kg/d and 80mg/kg/day for lacZ mutant frequency and micronucleus induction respectively, no deviations from background measures in mouse bone marrow were observed (Gocke et al., 2009). While the issue

of potential thresholds for genotoxic carcinogens is an important one, it is beyond the scope of this paper and so will not be discussed further here.

An important issue raised by the Tardiff et al (2009) paper and indeed by the review by Benford et al (2010) is the consideration of factors (e.g. mode of action and associated uncertainties) that affect the interpretation of the LOC associated with a MOE. While the ILSI Europe Expert group highlighted the difficulties in comparing MOEs for different compounds on a like for like basis, of more concern is the potential for some within the scientific community to associate the value of 10,000 proposed by EFSA with a defined level of acceptable risk.

It is easy to see how this link is being made when one considers that the justifications put forward for this value are underpinned by application of two 100-fold UFs that are intended to account for inter and intra species variability and other factors deemed appropriate for consideration in the evaluation of genotoxic carcinogens (e.g. DNA repair activity, cell cycle control and the application of BMDL rather than NOAEL for the calculation of the MOE). Although EFSA was not explicit in making the link between the value of 10,000 and excess lifetime cancer risk of 1 in 10^{-5} , other advisory bodies like the Toxicology Advisory Group (New Zealand) have been more explicit about making this direct association between MOE and methods applied in dose response assessment. The Toxicology Advisory Group reached a consensus for a default factor of 10,000, stating that this value which corresponds to the risk level of 1 in 100,000 was appropriate for the derivation of toxicological intake values for non-threshold contaminants (we note again that this does not include any explicit allowance for other uncertainties that are normally considered relevant, such as inter- and intra-species variation). The Toxicology Advisory Group recommended that this default of 10,000 be applied to the preferential selection of carcinogenic potency estimates (Ministry for the Environment, 2010). This approach for the consideration of carcinogenic potency estimates is very similar to the intended use of the MOE approach to aid the prioritisation of compounds for risk management action.

As mentioned earlier, EFSA (2005) and the EFSA/WHO/ILSI conference (Barlow et al., 2006) do not favour quantification of cancer risk and prefer to interpret MOE in terms of levels of concern. Barlow et al. (2006) report some agreement that the use of a factor of 100 for inter- and intra-species variation is scientifically justifiable, but that inter-individual human variability in cell cycle control and DNA repair might actually be covered by the first 100 and that scientific justification for a factor of, say, 10 for further uncertainties was lacking.

These differing perspectives suggest there is a need for more clarification and transparency about the justifications for values that are to be used as indicators of a low LOC associated with a MOE value.

Conclusions and recommendations

This review consolidates current advice available for the interpretation of the LOC associated with a MOE for a genotoxic carcinogen. The review of studies identified within the public domain which applied the MOE approach as part of their risk assessment indicates that while the proposed MOE approach provides a way of harmonising approaches in risk characterisation for genotoxic carcinogens, much work is still needed in this area, particularly with regards to harmonising approaches on the interpretation of the LOC.

While the work carried out by the ILSI Europe expert group (Benford et al., 2010a) on the application of the MOE approach advocates a case by case interpretation of the LOC through the consideration and description of the different uncertainties encountered in the calculation of the MOE, the majority of the studies reviewed here cited the recommendations provided by EFSA and compared their MOE values to the value of 10,000 proposed to indicate the LOC. However, the use of the proposed value of 10,000 remains controversial, not least because consensus is yet to be reached within the international community about the scientific justification behind the uncertainty underlying this proposed value.

We conclude that there is a need for further guidance in this area with regards to the communication of the LOC associated with the MOE value. Perhaps it is not enough to simply state that “in general terms the higher the MOE, the lower the degree of concern” (Barlow et al., 2006). It begs the question of how the value of 10,000 can be scientifically justified as indicating the LOC? If a value of 10,000 is not considered appropriate on the basis of lack of scientific justification, then what other figure should this be replaced with, if at all?

Establishing a scientifically justifiable value to indicate a LOC associated with a MOE will require a thorough consideration of the issues relating to the dose response curve for a genotoxic carcinogen. As illustrated by the paper by Tardiff et al (2009) reviewed here, there is need for further clarity in this area particularly with regards to the issues (such as human variability, inter-species variability in response and the levels of uncertainty considered to be “acceptable” when estimating the risk) that are normally considered as part of the dose response assessment. It is imperative that guidance be provided on how these issues may also impact on the interpretation of the LOC associated with a MOE value.

The application of the EFSA approach for interpreting MOEs in the different studies reviewed here indicates that it is potentially a useful tool for the interpretation and communication of the LOC. Both in the case studies evaluated by the ILSI Europe expert group and the published studies identified, the MOE approach has been illustrated as a potentially useful tool for ranking chemicals in terms of priorities for risk management action.

There clearly is a need for risk managers to be presented with all of the scientific justification behind a calculated MOE and to take into account the various uncertainties to aid in the interpretation and prioritisation of risk management actions for different compounds. On the other hand, it seems that there may also be a need for a more simplified approach to communicating the interpretation of the LOC associated with the MOE through the clarification of the magnitudes of MOE that

indicate a LOC after taking all uncertainties and affecting factors (such as the MOA for the compound of interest) into account.

This review also raises the issue of how to interpret the LOC for MOEs calculated for children. The difference in approaches to dealing with this issue indicates there is a need for further guidance on this matter.

In summary, many challenges remain to be addressed to enable harmonised use of the MOE approach in dealing with genotoxic carcinogens and the interpreted LOC. The findings of this review suggest that it may be useful to consider a dual role for the interpretation of the LOC associated with a MOE; one that focuses on the interpreted LOC for the prioritisation of risk management action and the other for a more simplified communication of the interpreted LOC.

List of Acronyms and Abbreviations

ALARA	As low as reasonably achievable
AA	Acrylamide
ADLH	Acetaldehyde
BMD	Benchmark dose
BMDL	95% lower confidence limit on the benchmark dose
BMDL10	95% lower confidence limit on the benchmark dose corresponding to a 10% response above background.
BfR	Federal Institute for Risk Assessment
EFSA	European Food Safety Authority
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
LOC	Level of concern
MOE	Margin of exposure
MRL	Minimal risk level
NOAEL	No observed adverse effect level
NDMA	Nitrosodimethylamine
POD	Point of departure
RP	Reference point
T25	Chronic dose rate in mg/kg bw/day which will give 25% of the animal's tumours at a specific tissue.
TD50	The dose rate in mg/kg bw/day which, if administered chronically for the life-span of the species, will half the probability of remaining tumourless throughout that period.
TTC	Threshold of toxicological concern
WHO	World Health Organization

Supplementary Information

Search Strategy to Identify Papers on Margin of Exposure

A search using the terms “margin of exposure” was carried out in PubMed to identify any publications on margins of exposure. All papers that had “margin of exposure” or “MOE” in the abstract were initially considered. This first stage identified 57 potentially relevant papers. To ensure the search terms applied here were not too restrictive a number of searches were carried out to combine the terms “margin of exposure” with either “genotoxic” or “carcinogen*” or the combination of both (Table 1). The searches with the new combined terms did not pick up any potentially relevant papers that were different from those already identified by using the search terms “margin of exposure”.

Table 1: Summary of search terms carried out in PubMed

Search terms	Number of papers identified in PubMed	Number of papers considered to be Relevant
“Margin Of Exposure”	98	57
“Margin of Exposure” AND “Genotoxic”	35	30
“Margin of Exposure” AND “Carcinogen”	15	7
“Margin of Exposure” AND “Carcinogenic”	40	30
“Margin of Exposure” AND “Genotoxic Carcinogen”	5	5
“MOE” AND “Genotoxic Carcinogen”	4	4
“MOE” AND “Genotoxic”	33	27
“MOE” AND “Carcinogenic”	35	27

The abstracts of the 57 papers identified from the search using the search terms “margin of exposure” were examined to exclude all publications that applied the MOE approach to evaluate non cancer end points. The latter resulted in the exclusion of 28 papers leaving 29 relevant papers. Figure 1 summarises the search strategy applied to identify relevant papers on margin of exposure.

A search of the references from the 29 papers was carried out to identify grey literature and any relevant publications that may have not have been picked up by the search in PubMed. In addition, key terms from the papers were used to produce a more refined search terminology. Key terms applied by the majority of the publications cited included: risk assessment, carcinogen, genotoxic, MOE and margin of exposure. The citation searching identified 2 new relevant publications that had not been identified by the original search in PubMed.

Further searches were carried out in PubMed using a combination of the key terms identified 2 new studies that the original search did not pick up, however they were the same publications identified from the citation search. In total 31 relevant publications that met the criteria for the search were identified from PubMed.

A separate search was carried out to identify publications relating to the interpretation of MOE. Search terms included:

“Levels of concern” AND “Genotoxic carcinogens”

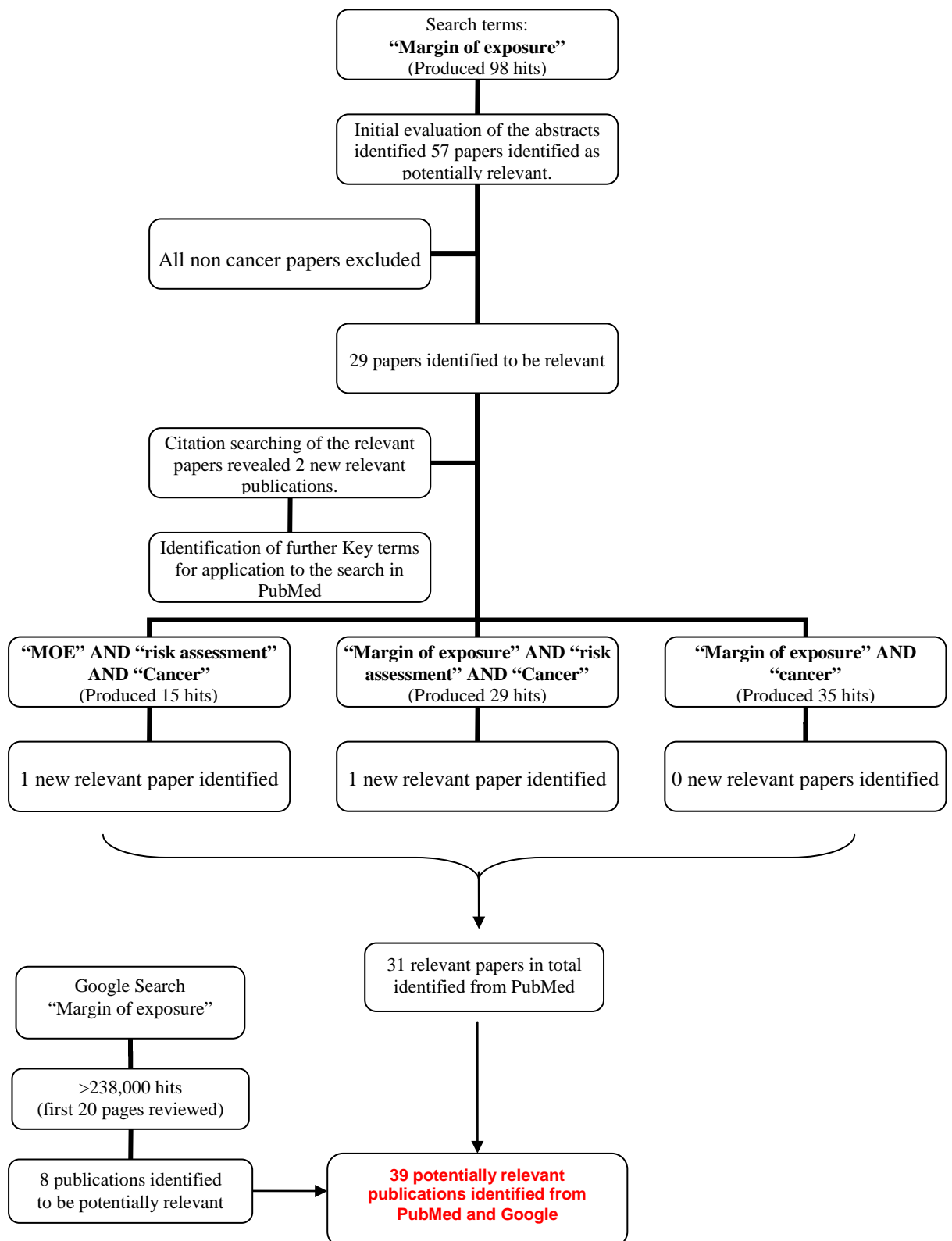
“prioritisation of carcinogens” AND “risk assessment”

“risk assessment” AND “Genotoxic Carcinogens”

These search terms produced no results.

A less thorough search was also carried out using the search engine Google, again using the search terms “margin of exposure”. More than 238,000 hits were identified. Examination of the results was limited to the first 20 pages. Eight potentially relevant publications from the grey literature were identified.

Figure 1: Search strategy to identify relevant papers on margin of exposure⁶



⁶ The bold terms within the quotation marks depict the actual search terms applied in PubMed and show how the key terms were meshed together to carry out the searches.

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