

# APPLICATION OF THE MARGIN OF EXPOSURE APPROACH TO COMPOUNDS IN FOOD WHICH ARE BOTH GENOTOXIC AND CARCINOGENIC



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SUMMARY REPORT OF A WORKSHOP HELD IN OCTOBER 2008

Organised by the ILSI Europe Risk Assessment of Genotoxic  
Carcinogens in Food Task Force

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*By Anne Constable and Susan Barlow*

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## SUMMARY

**T**he margin of exposure (MOE) approach for advising risk managers on potential health concerns arising from the presence of genotoxic carcinogens in foods has been discussed by several international expert groups. The ILSI Europe Task Force on Risk Assessment of Genotoxic Carcinogens in Food set up an expert group to work through the elaboration of MOEs for different chemicals detected in foods, covering ranges of carcinogenic potencies and using data on carcinogenicity and exposure that was of variable quantity and quality. The aim was to develop a better understanding of the MOE approach and how MOEs might be interpreted. A workshop was organised, attended by 40 delegates from Europe, USA and Japan, from academia, national and EU regulatory/advisory authorities and from the food industry, to discuss the outcomes of this work. Members of the expert group presented their results and the critical issues identified during the analysis of 12 case studies. In three parallel working groups and in plenary sessions, participants discussed various technical issues with specific reference to the choice of cancer data, the mechanism of action, mathematical modelling and the selection of reference points, and exposure considerations. The value, interpretation and communication of the MOE approach was also discussed during plenary sessions.

It was agreed that the choice of cancer site(s) to be modelled and the human relevance of the tumours require careful consideration. Possibilities for combining data within and across studies and for taking account of different dosing durations were discussed. For derivation of a suitable reference point (the  $BMDL_{10}$ ), a model-averaging approach is recommended once the software is better validated and made more user-friendly. The validity of the output of the dose-response modelling methods depends on the strength of the experimental data used. Depending on which databases and datasets are used for exposure modelling, different MOEs can be calculated for a specific chemical. Consistency in choice of upper end exposures is required when using MOEs to compare levels of concern for different chemicals.

Appropriately derived MOEs, accompanied by a suitable narrative, can be used as a prioritisation tool to compare different chemicals, to inform risk managers on the level of potential health concerns and to identify knowledge gaps and uncertainties. The MOE approach might not be suitable when there are too many uncertainties or weaknesses in the available data. Outstanding issues and further guidance requirements were also identified and recommendations made.

The work of the expert group was agreed to be a valuable contribution to the development and use of the MOE approach. The manuscript elaborated by the ILSI Europe expert group will be revised before being published, taking into account the discussions at the workshop.

## BACKGROUND

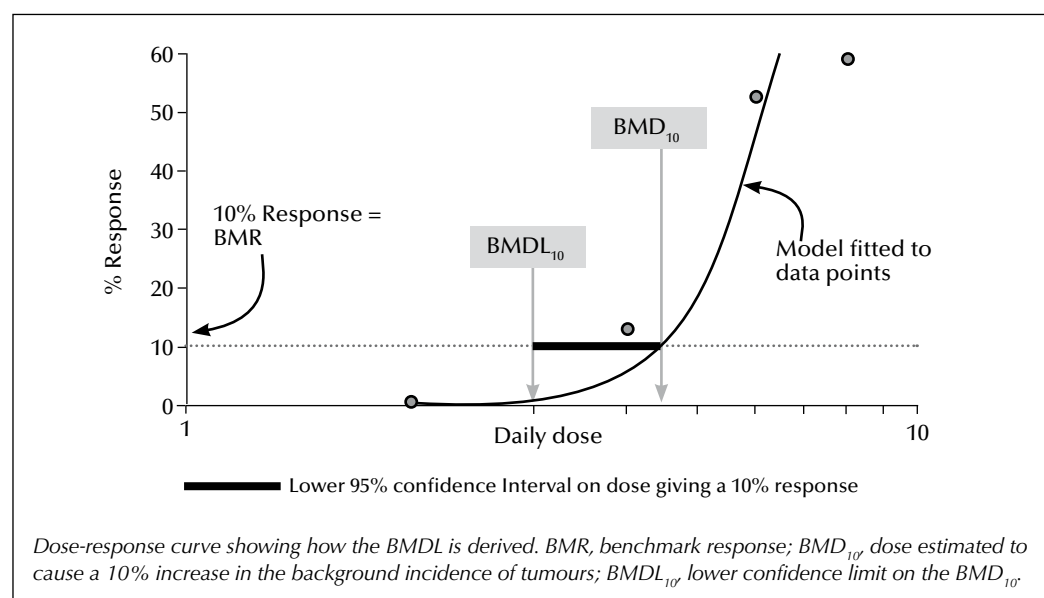
One of the most difficult issues in food safety is to advise on potential risks to human health when it is found that substances which are both genotoxic and carcinogenic are present in food and that their presence cannot be readily eliminated or avoided. Currently, various approaches are used to assess the risks from substances that are genotoxic and carcinogenic (hereafter referred to as genotoxic carcinogens).

Several of the approaches that are used take into account the fact that genotoxic carcinogens differ in their potency, that is, they differ in their likelihood of inducing a tumour at a given dose or exposure. Information about carcinogenic potency is mostly derived from laboratory studies on rodents. One of the approaches that can be used is to estimate possible human risk by extrapolation from the high doses used in animal studies to the generally much lower levels to which humans are exposed. For this purpose, a wide range of mathematical and statistical models, from simple linear extrapolation to very complex models, have been developed and used. This has resulted in differing conclusions for the same substance, depending on the model chosen.

In order to avoid the potentially large uncertainties in the use of such models in risk assessment, a margin of exposure (MOE) approach has been considered by various international groups and advisory bodies. In 2002, the ILSI Europe Task Force on Risk Assessment of Genotoxic Carcinogens in Food convened an expert group to propose a structured approach for the evaluation of genotoxic carcinogens in food, following a critical review of the approaches then available, including the MOE approach (O'Brien *et al.*, 2006). The MOE approach was also considered around the same time by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and the European Food Safety Authority (EFSA, 2005). These bodies independently recommended that the MOE approach was preferable to other approaches that could be used to develop advice on the risks of exposure to genotoxic carcinogens in food. Both JECFA and EFSA have used the MOE approach in their assessments of several genotoxic carcinogens present as contaminants in food (JECFA, 2005, 2007; EFSA 2007a,b).

The MOE approach compares the margin between a dose or an exposure causing cancer in animals or humans with the estimated human exposure to that substance. It uses a reference point, usually taken from an animal cancer bioassay in which the substance has been administered for most of the animal's life span. The reference point corresponds to a daily dose causing a low but measurable increase in the incidence of tumours. This reference point (also called a point of departure) is then divided by the estimate of human dietary exposure to the substance to give a dimensionless ratio that is the MOE. Several MOEs can be calculated for an individual substance if estimates of exposure vary within the human population.

An important parallel development was the work of the International Programme on Chemical Safety (IPCS) on the mathematical and statistical modelling of the dose–response curve within the observable range (WHO/IPCS, 2004; WHO/IPCS, 2009). This built on the earlier work of others and discusses in detail the derivation of a benchmark dose (BMD). The BMD is the dose estimated to cause a predefined increase (e.g. 10%) in the background incidence of tumours. The BMDL is the lower limit of a one-sided 95% confidence interval on the BMD, which can be used as a reference point for calculation of an MOE.



In 2005, an international conference was organised by EFSA and WHO with the support of ILSI Europe on “Risk Assessment of Compounds that are both Genotoxic and Carcinogenic: New Approaches” (Barlow *et al.*, 2006). The conference further explored the various options for risk assessment of genotoxic carcinogens. It reached a consensus that the MOE should be the preferred approach for providing advice to risk managers on genotoxic carcinogens, and concluded that it would be helpful to calculate MOEs for example substances in order to better define the health concern associated with a certain MOE, or range of MOEs. The ILSI Europe Task Force on Risk Assessment of Genotoxic Carcinogens in Food subsequently set up a new expert group, working in collaboration with WHO/IPCS and EFSA, to further elaborate the process of establishing MOEs, using example substances, in order to develop a better understanding of the MOE approach and how MOEs might be interpreted.

The purpose of this workshop was to build on some of the recommendations from the 2005 international conference (Barlow *et al.*, 2006). A draft manuscript prepared by the expert group of the ILSI Europe Task Force on Risk Assessment of Genotoxic Carcinogens in Food, describing the work that had been done on 12 example substances (Benford *et al.*, 2008), was made available to the workshop participants. The presentations at the workshop and the draft manuscript formed the basis for subsequent discussions in working groups and plenary sessions at the workshop. Following the workshop, the expert group of the ILSI Europe Task Force revised and finalised the manuscript and submitted it for publication in a scientific journal. It is suggested that this workshop report is best read in conjunction with the paper from the expert group (Benford *et al.*, 2010).

## OBJECTIVES OF THE WORKSHOP

The overall objectives of the workshop were:

- Ⓜ To critically appraise the MOE approach in light of the worked examples;
- Ⓜ To provide guidance for the application of the MOE approach;
- Ⓜ To further characterise the interpretation of the numerical value of the MOE.

The topics allotted to the three working groups were:

- Ⓜ Choice of cancer data, mode of action;
- Ⓜ Modelling (mathematical) and the selection of reference points;
- Ⓜ Exposure.

Each working group was provided with a brief consisting of a series of questions within the topic to be addressed.

Issues related to the interpretation and communication of the MOE were discussed in plenary session.

## GENERAL INTRODUCTION

The workshop was chaired by **John Larsen** (National Food Institute, Denmark), who welcomed the participants.

The opening session was introduced by **Nico van Belzen** (ILSI Europe, Belgium) who explained that the workshop was supported by the Task Force on Risk Assessment of Genotoxic Carcinogens in Food, which is one of the task forces contributing to the ongoing ILSI Europe programme on Assessment of Benefits and Risks. The objectives of the ILSI Europe Task Force are:

- Ⓢ To improve quantitative hazard characterisation of cancer risk from low-dose dietary exposure to genotoxic carcinogens;
- Ⓢ To provide a critical evaluation and recommendations on the application of risk assessment principles;
- Ⓢ To contribute to the prevention of diet-related cancer.

Dr van Belzen highlighted how improved analytical methods are increasingly able to detect very low concentrations in food of substances, both natural and man-made, that are genotoxic and carcinogenic. Examples include substances such as ethyl carbamate, nitrosamines and heterocyclic amines that can be formed during the processing of food and drink, and naturally occurring substances such as methyleugenol in basil. It was noted that such findings can alarm consumers and that there is a need for international consensus on how to evaluate risks from the presence of such substances in food.

**Josef Schlatter** (Federal Office of Public Health, Switzerland) described the history of the emergence of the MOE concept as an additional tool for risk assessors in developing advice for risk managers on genotoxic carcinogens in food. The MOE approach offers an alternative to the ALARA approach (concentrations of unavoidable genotoxic carcinogens in food should be “as low as reasonably achievable”) and to modelling approaches that require extrapolation of the responses seen in bioassays outside the observed dose-range. The main conclusions on the advantages and disadvantages of the MOE approach that emerged from the 2005 international conference were summarised, together with the various issues on the interpretation of MOE values in terms of risk, on which consensus has not yet been reached. Dr Schlatter noted that:

- Ⓢ Clarification was needed that genotoxic carcinogens should not be deliberately added to food;
- Ⓢ The calculation of an MOE does not exclude advising the use of ALARA (ALARA is a risk management option);
- Ⓢ Transparency is essential.

He considered that further experience with MOEs was needed to develop a rational and consistent approach and consideration of the 12 example substances in the workshop should help take this forward.

**Bernard Bottex** (EFSA, Italy) gave EFSA’s perspective on the MOE approach, summarising the main conclusions from the earlier opinion of its Scientific Committee (EFSA, 2005). He highlighted the need for discussion on two of the recommendations from the 2005 international conference relating to interpretation of MOEs, i.e. to consider ranges rather than single values for MOEs in order to avoid misinterpretation, and to consider banding of MOEs with respect to levels of concern. EFSA was currently developing guidance on the use of the benchmark dose approach and he noted that this would also be very relevant for implementation of the MOE approach. He outlined how EFSA’s Scientific Panel on Contaminants had used the MOE approach in its risk assessment advice on several genotoxic carcinogens and gave examples that illustrated the ranges of MOEs that could be derived from the data:

- Ⓢ For ethyl carbamate, MOEs ranged from 600 to 18,000 for different population subgroups, depending on their alcoholic beverage consumption;
- Ⓢ For benzo a pyrene (BaP), MOEs for the sum of four polycyclic aromatic hydrocarbons (PAHs) and for the sum of eight PAHs all ranged from 9600 to 10,800 for high-end exposures;
- Ⓢ For aflatoxins in nuts and derived products, MOEs did not change much if the maximum limit for aflatoxins was varied between 4 and 10 g/kg, but MOEs did vary by around tenfold depending on whether animal or human data were used to derive the reference point.

Mr Bottex also emphasised the importance of transparency in any risk assessment advice and pointed to EFSA's initiatives in that area.

**Michael Bolger** (Food and Drug Administration, US) presented the WHO perspective, on behalf of **Angelika Tritscher** (Department of Food Safety, WHO, Switzerland). He highlighted some of the main questions concerning provision of risk assessment advice on genotoxic carcinogens:

- Ⓢ What possibilities are there for risk assessment for genotoxic and carcinogenic substances ingested at low levels
- Ⓢ How should the outcomes of the various approaches be interpreted in terms of risks to human health
- Ⓢ To what extent do the available approaches meet the needs of risk managers
- Ⓢ Are the approaches helpful in giving practical options for risk managers in situations where exposure cannot be completely eliminated and the magnitude of risk cannot be readily determined

Considering the approach that uses low-dose extrapolation of the results of animal bioassays outside the observed range, it was noted that there were no internationally agreed methods, that significant modelling expertise is required, and that different models with equally good fits to the data can result in very different estimates of risk. The resulting upper bound estimates of risk can be misinterpreted as actuarial risk. In its discussions during 2005, JECFA had preferred the MOE approach over linear low-dose extrapolation because it was a pragmatic approach that used both intake and potency data, and it could be used to compare and rank substances and to prioritise risk management actions. Detailed guidance on calculation of the MOE was developed (JECFA, 2005), recommending the BMDL<sub>10</sub> as the reference point, modelling according to the advice of IPCS (WHO/IPCS 2004), and providing MOEs for both mean and high (e.g. 90<sup>th</sup> percentile) exposure estimates. Numerical MOEs should be accompanied by a descriptive interpretation to provide context and explanation. This approach was then applied by JECFA to derive MOEs for several contaminants for mean and high exposure estimates; these were, respectively, 75 and 300 for acrylamide, 3800 and 20,000 for ethyl carbamate, 10,000 and 25,000 for polycyclic aromatic hydrocarbons, and 24,000 and 65,000 for 1,3-dichloro-2-propanol (JECFA, 2005, 2007). However, Dr Bolger also noted that because the MOE is a numerical value, not a quantitative risk estimate, it gives no indication of actual risk and so could be difficult to interpret. Because the MOE is a ratio, good intake data are as important as good dose–response data. He further noted that interpretation of the significance of a particular MOE value lies on the borderline between risk assessment and risk management. The following issues were identified as needing further discussion, agreement and possibly guidance:

- Ⓢ Criteria for selection of cancer (or other key) data for modelling;
- Ⓢ Modelling approach, e.g. software to be used, model selection versus model averaging;
- Ⓢ Dietary exposure estimates, e.g. average and high exposure or only high exposure; which percentile to use for high exposure;
- Ⓢ Expression and description of MOE with underlying uncertainties;
- Ⓢ Interpretation of MOE with respect to public health concerns.

## EXPERT GROUP ON MOE

**Diane Benford** (Food Standards Agency, UK), Chair of the ILSI Europe expert group, and members of the expert group summarised the studies performed. The objective was to work through examples of genotoxic carcinogens for which carcinogenicity data and exposure data differed in quality and quantity. Carcinogenicity data were critically evaluated, taking into consideration the quality of the data, uncertainties and limitations and duration of exposure. Mathematical modelling of the data was carried out to derive an appropriate reference point. MOEs were subsequently calculated for each compound on the basis of specified exposure scenarios. A narrative on the assumptions and uncertainties of the data and methodology used for calculating the MOE was provided for each of the examples.

Discussions over the choice of cancer data had focused on the mechanism of action of the substance, and human relevance of the type of tumour observed in animal studies. Key issues were presented by **Gary Williams** (New York Medical College, US). The quality of the data used for dose–response modelling, which statistical models (and which software) should be used to model the data, and how to select the reference point were presented and discussed by **Woodrow Setzer** (Environmental Protection Agency, US) and **Wout Slo** (Dutch National Institute for Public Health and the Environment, RIVM, Netherlands). **Michael DiNovi** (Food and Drug Administration, US) presented the issues related to exposure assessments. **Philanthew** (Unilever Research, UK) discussed how the resulting MOEs can be interpreted.

### Substances investigated

The 12 examples chosen had previously been evaluated by expert groups such as JECFA and EFSA/SCF over the last 15 years. The substances possessed very different chemical structures, but the prerequisite was that they had all been identified as carcinogens with evidence of a genotoxic mechanism of action. Six of the substances are detected in foods due to the nature of the foods and the processing applied: acrylamide (AA), formed in variety of foods rich in carbohydrates; benzo a pyrene (BaP) (representative of the polycyclic aromatic hydrocarbons, PAHs), furan (FU) and 2-amino-1-methyl-6-phenylimidazo 4,5-b pyridine (PhIP) are formed during cooking; ethyl carbamate (EC) is formed in foods and beverages by fermentation; and 1,3-dichloro-2-propanol (DCP) is formed under a variety of conditions during the production of acid-hydrolysed vegetable proteins and of malt- and soy-based products. Aflatoxin B1 (AFB1) is a fungal contaminant, commonly associated with nuts and cereals. Benzene (BZ) is detected in foods as mainly an environmental contaminant. 1-Methylcyclopropene (MCP) is a gas used as a plant regulator, and a maximum residue limit for foods is established based on MCP detection limits. The impurities 1-chloro-2-methylpropene (1-Me-CP) and 3-chloro-2-methylpropene (3-Me-CP), which arise from the chemical synthesis of MCP, are genotoxic carcinogens, and MCP-specific purity criteria have limits of 0.05% for each impurity. Leucomalachite Green (LMG) and Sudan 1 are dyes, and their use in foods is illegal. Human exposure is therefore due only to the fraudulent uses of LMG in farmed fish, and of Sudan 1 in peppers and spices, and is therefore sporadic. Finally, methyleugenol (ME) is a natural constituent of a number of spices, herbs and essential oils.

## Selection of cancer data

Ten of the chemicals had been tested in studies conducted in accordance with current guidelines, seven of which were NTP (US National Toxicology Program) studies. Carcinogenicity assays performed for two chemicals (AFB1 and PhIP) were considered non-conforming and non-standard (small numbers of animals, less than a lifetime dosing), although they provided important information. Only studies with oral administration of the chemical were selected (diet, drinking water, gavage). Exposure through other routes (e.g. inhalation for benzene) was not included. Various durations of dosing were encountered, also with different periods of observation. In cases where dosing and/or exposure had not been for the standard lifetime (104 weeks), "lifetime averaged doses" were derived by adjusting doses by factors of  $w_1$  (duration of dosing)/104, and  $w_2$  (period of observation)/104. In cases where administration was by gavage for 5 days during any one week, the dose was adjusted by 5/7.

A range of potencies of the chemicals in the bioassays were estimated, with AFB1 considered to be of high potency; AA, BaP/PAHs, EC, FU and PhIP of moderate potency; and BZ, DCP, LMG, MCP, ME and Sudan 1 of low potency. Potency was determined by qualitative judgement based on the lowest dose showing an increased tumour incidence (high potency,  $\mu 0.1$  mg/kg bw/day; moderate, 0.1 to  $\mu 10$  mg/kg bw/day; and low, 10 mg/kg bw/day).

The tumour data were critically evaluated in order to choose the relevant datasets for dose–response modelling. Data on some tumour types were excluded from further analysis because of doubtful relevant mechanism of actions or human relevance. For MCP, the two genotoxic residues 1-Me-CP and 3-Me-CP had been tested in bioassays, but the 3-Me-CP data were excluded because tumours were only observed at high doses, which might have been due to contamination of the 3-Me-CP. Forestomach neoplasms from gavage administration of 1-Me-CP were considered to result from irritation. Only the 1-Me-CP nasal tumours were taken for further analysis. Liver cholangiocarcinomas related to FU treatment were considered to be due to oxidative stress. Testes interstitial neoplasms from LMG were considered not to be relevant to humans.

Seventeen sets of tumour data were taken further for dose–response modelling. All treatment-related neoplasms, whether benign or malignant, were taken. Specific target organs were observed for the majority of the chemicals. In the case of BaP, the test material was a mixture of PAHs, which could induce different tumour types to those induced by BaP alone, and so the total number of neoplasms in all affected organs was used. Data for the same tumour type and same target organ for a chemical were combined, also if observed in both sexes. For some substances (AA, DCP, PhIP), tumours were observed in several organs, therefore more than one set of target organ tumour data were used. For two chemicals (AA and PhIP), sex-specific tumours (mammary tumours and peritesticular mesotheliomas, and mammary and prostate carcinomas, respectively) were identified, and these were modelled separately.

Some doubts remained for several of the tumour datasets that were taken for modelling. For some datasets, such as for liver (FU), mammary gland (AA, DCP), thyroid (AA) and kidney (DCP), a genotoxic mechanism had not been established. The relevance of Zymbal gland (BZ) as a relevant organ for humans was questioned. The relevance of tongue tumours observed in DCP studies was questioned because the route of administration via drinking water was not a realistic exposure route for humans. ME is a liver carcinogen in rats, but studies on the mechanism of action suggest that its oxidation to DNA-reactive metabolites is species-specific and, therefore, that the rat liver tumours are of low relevance to humans. 1-Me-CP resulted in nasal tumours; however, 30% of the control animals had suppurative inflammation of the nasal passage, suggesting that the animals might have been very sensitive and casting doubt on the true relevance of the nasal tumours.

## Modelling of cancer data

Conventional dose–response modelling uses several different statistical models. The data from each tumour type (number of tumours and numbers of animals affected) are analysed by each model to estimate the BMD. Each model could result in a different BMD, so either the best fitting model, or the model with the lowest BMDL is selected. The confidence limits resulting from selecting the best fitting model are likely to underestimate the uncertainty of the BMD value, whereas selecting the model with the lowest BMDL might result in an over-estimation of uncertainty.

For the ILSI work, the model-average approach (Wheeler and Bailer, 2007) was used to compute BMDs and their lower confidence bounds for each endpoint modelled. Seven conventional statistical dose–response models (Logistic, Log-logistic, Gamma, Multistage, Probit, Log-Probit and Weibull) were fitted to each dataset (individual tumour types for individual compounds) in turn. A model average was then computed. In brief, this was done by assessing the log-likelihood values associated with the best fit for each model, and these values used to calculate a weighted model-average response. In this method, models that fit well are taken into account more heavily than models that fit less well. The dose where the model-average response is equal to the BMR (e.g. an extra risk of 10%) is defined as the model-average BMD. The Pearson chi-square goodness of fit was calculated. Confidence limits and probability values for the goodness of fit were estimated using the bootstrap method. It was considered that the model-average approach better characterises the uncertainty in the value of the BMD that derives from ignorance of the true (biological) dose–response curve.

The dose–response shape of each of the curves modelled by the individual dose–response models used in the model-averaging was also considered. For some models, a shape parameter is constrained to be greater than 1. When the shape parameter drops below 1, these curves become flatter at higher doses and steeper at lower doses, and the slope of the dose–response curve relative to dose is infinite when the dose is zero (this is biologically implausible). Apparent “plateaus” in a dataset from doses with 100% response can force estimates of shape parameters to be less than 1, especially if there are no doses with lower responses.

For each tumour type for each chemical, a preliminary trend test was performed using the individual models. Out of 304 endpoints modelled, 113 sets showed no trend in the dose response.

In the presence of a significant trend (191 datasets), a complete dose–response analysis of the data was performed using the model-averaging approach. BMDs and their lower 95% confidence limits (BMDLs) were derived. Approximately 16% failed the goodness-of-fit analysis ( $p$ -value of  $\mu 0.05$ ). This was attributed to experimental error or other problems with the data, such as inadequate carcinogenicity models where other competing risks or saturation of metabolism affected the true dose response. In 91% of the datasets calculated BMDs and BMDLs were 0, whereas 5.2% had BMDs and BMDLs  $> 0$ , and 3.7% had BMDs  $> 0$  and BMDLs 0.

In nine datasets (individual compound and endpoint dose–response data), the lowest dose appeared to be too high because there was a 100% response at all doses. In another dataset, all doses yielded approximately the same response level at around 60%, despite covering a substantial dose range. These all resulted in BMD estimates of essentially zero for all response levels. Seven datasets resulted in BMDL<sub>10</sub> estimates of zero; with the number increasing with lower benchmark response levels. The reference points for seven chemicals (1-Me-CP, AFB1, BZ, Fu, ME, PhIP and Sudan 1) were affected by such problems. In these cases, it was considered that the dose–response data were unsuitable for any dose–response analysis, and that a reliable reference point could not be derived.

The BMDs and BMDLs for 1%, 5% and 10% extra risk were calculated. The model-average approach includes model uncertainty and statistical uncertainty in its quantification of BMD uncertainty. This is done by comparison of upper (BMDU) and lower (BMDL) 95% confidence limits on the BMD, e.g. BMDU/BMDL ratios, or log<sub>10</sub> (BMDU/BMDL) ratios. The uncertainty of the ratios varied among endpoints with positive BMDL and significant trend (i.e., with BMDL > 0 and BMDU < ∞). The uncertainty increased as the ratio decreased, with a large jump between 0.05 and 0.01.

It was concluded that modelling is an objective and transparent way to establish reference points for computing MOEs. The model-average approach is practical, and gives results that characterise both statistical uncertainty and uncertainty about the true model (model uncertainty). What would appear to be a failure of the methodology, that is, extremely low BMDs or BMDLs, are in fact useful indicators of poor data quality. The aim of most carcinogenicity studies is hazard identification, rather than estimation of dose–response curves for tumourigenicity. Typically, few doses are used, stepping downward from an MTD (maximum tolerated dose). Whereas it is still possible to estimate a dose–response curve and BMD from such data, the precision might be very uncertain, and would be more reliable if lower dose groups were included in the design of the study. Cancer bioassay designs employing only two treatment doses are generally inadequate for reliable computation of a reference point. A broader class of dose–response models is also needed, for example, to allow for responses where tumour incidence levels off at values below 100% of animals. In the datasets used in the ILSI work, BMD<sub>10</sub> values are the least uncertain, followed by BMD5 values, with BMD1 values being substantially more uncertain.

## Choice of reference point

The resulting BMDL<sub>10</sub> values for the 12 chemicals ranged from 0.00025 to 25 mg/kg bw/day, demonstrating a wide range of potencies, over five orders of magnitude. The T25 (dose at which 25% of animals developed tumours) was also calculated. The problems with using T25 as a reference point are that it uses only part of the data, uses one single (linear) model, ignores uncertainties and is only a rough estimate of a dose that results in tumour incidence in 25% of the animals. On the other hand, the BMDL uses all the data, uses different models, takes uncertainties into account (including sample size and dose response) and ultimately defines a dose where the effect is smaller than the BMR, with a defined confidence (the BMDL<sub>10</sub>). A problem of weak cancer bioassay data cannot be solved by using a weak method such as the T25. A BMD analysis will reveal the potential weaknesses in the data. For example, if high tumour incidence is observed at all doses, then those doses are not suitable for deriving a reference point. The recommended choice of reference point is the BMDL<sub>10</sub>.

It was emphasised that the inherent problem in the MOE approach is how regular exposure profiles in animal bioassays can be accurately extrapolated to highly irregular exposure profiles for the human situation, and how to get from observable risks in animals to an exposure in humans that might be considered tolerable, which in the case of carcinogenic compounds, is extremely small. Tolerable risks to humans are far below the range of observation in animal bioassays, and to ensure that tumour risks smaller than 10% were observable would require a very large increase in the number of animals.

Carcinogenicity studies are generally performed using high doses over a lifetime, with analysis of tumour incidence at end of life (or a standard lifespan of 2 years). The initial direct reaction of a genotoxic chemical with DNA should be linear, but because of other biological factors required for the carcinogenicity process and progression of tumour formation, a wide scatter and a variation in tumour incidence are observed. These same factors affect the latency period required for observable tumours to develop. It was suggested that if bioassays were interrupted at different time points, then the time to tumour could be taken into consideration for the choice of reference point. Using statistical models, this could be extrapolated into

minutes lost out of the lifespan of the animal and, further, into days lost in the life of a human. In general, carcinogenicity studies are not designed for this purpose, or indeed for the purpose of MOE calculations. The example of extensive studies on *N*-nitrosodimethylamine (NDMA), where data from interrupted dose groups is available, could be taken as a model. If this type of analysis, based on the NDMA model, could be validated, then it might be possible to provide an interpretation of an MOE that could be more easily understood from a health perspective. This would, however, require better design of bioassays, and the application or development of appropriate statistical models.

## Exposure assessments

An estimate of dietary exposure to a substance is made by taking the arithmetic product of food type consumption and a concentration of the substance in that food. The exposure estimates provided need to be tailored to answering the question posed. In the case of acute hazards, single meals and high toxin concentrations are used, whereas for chronic hazards such as genotoxic carcinogens, usual food consumption and mean toxin concentrations are often taken.

Exposure assessments depend on the availability and choice of food consumption databases as well as the choice of target population. Estimates can be made for average intakes over the whole population, or specific groups such as consumers only (including averages and high-end consumers), or for populations with known sensitivity to the biological effects of the substance in question. Variability in exposure assessments results from the different consumption habits in the population and concentrations of chemicals in the foods. The nature of the exposure might be nearly continuous, in many food categories; or less frequent, restricted to only certain food groups; or only occasional, as with illegally added substances. Assessments are affected if data are taken from a single meal or from chronic exposure. The availability of occurrence data depends on whether routine or widespread testing has been performed, or if only limited, non-representative samples have been taken.

For the cases taken in the examples, clear differences in the availability and quality of data affected the reliability and relevance of the exposure assessments, and ultimately the value of the derived MOE.

Extensive databases on occurrence in relevant food categories were available for several of the chemicals (AA, AFB1, DCP, EC, FU, PhIP), some from several countries. For BZ, food occurrence data are poor because most of the exposure comes from inhalation and there are only a few food sources that have been analysed, such as beverages containing benzoates. In this case, data quality issues and sources of exposure limit the relevance of MOE calculations. For BaP and PAHs, cooking practices have a strong influence on the concentrations present in food.

For illegal contaminants, data were limited. Some data were also not available in the public domain. Good, but limited, surveillance data were available for LMG because exposure is only sporadic, coming from its illegal use in aquacultured fish. There is a very poor database for MCP impurities. Exposure estimates were extrapolated from the theoretical levels of impurities encompassed within the regulatory MCP limits, and so would be considered as a worst-case situation. Only a very poor dataset is available for Sudan 1 because contamination events are random and the occurrence levels vary considerably and inconsistently. Consumption data on spices and spice mixtures are also highly variable.

ME is a natural constituent of herbs such as basil. Many uncertainties were identified because occurrence data are affected by the multiple natural cultivars of the herb and the extensive natural variation of the ME content, and because most consumption is from foods seasoned with the herb, which is again very variable. The estimated daily intake (EDI) for high-end consumers was greatly different from that for average consumers or for the total population. Therefore, the resulting MOE is very uncertain because it was derived from many assumptions in the EDI.

In conclusion, MOEs are very calculation-dependant, and the assumptions made for both food consumption and occurrence data can dramatically affect the resulting estimates of dietary exposure. The type of hazard (acute or chronic) and the duration of dietary exposure (intermittent or continuous) need to be considered in the interpretation of the data. In essence, the MOE should be considered as a “snapshot in time”, because it can change depending on the data used to derive the EDI.

## Presentation and interpretation of the MOE

In the ILSI work, MOEs were derived from the tumour type that was considered relevant for humans and that exhibited the lowest BMDL. MOEs were derived from the BMDL<sub>10</sub> and for average and high-end exposure estimates. In view of the various uncertainties in the data used, MOEs can only be considered as rough estimates. The MOEs were rounded to two significant figures, although it was questioned whether a single figure would be more appropriate. MOEs ranged over seven orders of magnitude, from 15 for high-end continuous exposure to ME, up to 110,000,000 for intermittent exposure to carcinogenic impurities in MCP.

In order to express the level of concern and priorities for risk management for the resulting MOEs, the previous opinion from EFSA (2005) was considered, which stated that *an MOE of 10,000 or higher if it is based on the BMDL from an animal study could be of low concern from a public health point of view and might be considered as a low priority for risk management actions*. This value of 10,000 was based on consideration of the various uncertainties, taking account of the default uncertainty factors often used, such as 10 for interspecies extrapolation and 10 for intraspecies extrapolation. However, there are additional uncertainties, specifically for substances that are both genotoxic and carcinogenic, because of the inter-individual human variability in cell-cycle control and DNA repair, which influence the carcinogenic process. In addition, the reference point is not equivalent to a NOAEL (no observable adverse effect level) and effects can occur at lower doses. The dose–effect relationship below the reference point, and the dose level below which cancer incidence is not increased are unknown, representing additional uncertainties. In the opinion from EFSA (2005), it was considered that an additional 100-fold difference would allow for these additional uncertainties. The availability of data and rationale to support the judgement that an MOE of 10,000 would be of low concern has been questioned, in particular whether applying an additional default factor onto a BMDL<sub>10</sub> and for inter-individual human variability in cell-cycle control and DNA repair is justified.

For the group of contaminants, MOEs for high-end consumers of AA, AFB1 and FU, were derived at 40–1300; for PAHs and EC at 2200–3100; and for PhIP and DCP at 24,000–74,000. This rough banding suggests different priorities for risk management actions. BZ and MCP were considered to be a very low priority for risk management, with a high-end consumer MOEs of 500,000 and 110,000,000 respectively.

For the illegal substances, Sudan 1 had a broad range of MOEs of 31–3,800,000, based on spice consumption and adulteration level. Large MOEs of 410,000–4,100,000 were derived for LMG. In these cases, the risk management actions need to take into account the regulatory and legal context, since genotoxic carcinogens should not be deliberately added to foods. The MOE could be used as a basis for communicating the level (or lack) of any health concern.

The smallest MOE of 15 was calculated for high-end consumers of ME. Because this is a normal plant constituent, risk management options are limited and it was therefore considered crucial to understand the mode of action, particularly the importance of metabolism in bio-activation, and the wide variation in dietary exposure levels.

Various issues had arisen during the work relating to the inherent uncertainties of the MOE values derived for the examples taken. These included the degree of certainty that the tumour selected for modelling was caused by a direct-acting genotoxic mechanism; the quality of the tumour data used and whether the T25 or BMDL was used as the reference point: the degree of (un)certainly in exposure estimates, e.g. if they were prepared for different population groups (aged, children) or if the length of exposure (episodic contamination versus lifetime) was important.

These uncertainties were not comparable between the different case studies, resulting in difficulties in comparing the MOEs for risk communication and risk management purposes. Approaches are required to quantify and describe those uncertainties. Future work is also needed to expand on the banding approach for communicating on the MOE.

**Dr Benford** concluded that:

- ® The value of an MOE depends on the quality of the carcinogenicity data used to derive a BMDL and of the estimated dietary exposure;
- ® Limitations to exposure assessment are not unique to substances that are genotoxic and carcinogenic;
- ® Depending on the tumour endpoint selected, the bioassay protocol and the ways in which the data are analysed, it is possible to generate vastly different reference points to calculate the MOE, and hence vastly different values of the MOE.

Hence, transparency is essential.

## WORKING GROUPS AND PLENARY DISCUSSIONS

**I**n the derivation of MOEs using cancer dose–response data from animal bioassays, various issues need to be considered. These include the heterogeneity and interpretation of the available data, their relevance for humans, the underlying mode of action, the suitability of the data for modelling and the selection of appropriate models. Once these issues have been considered, it is necessary to select the data most relevant for deriving a reference point and decide on what type of human exposure estimates are needed in order to calculate the MOE(s). These key issues were considered as a series of questions in the three working groups and subsequent plenary sessions, both in relation to the work of the ILSI Europe expert group on the 12 example substances and in the context of further development and use of the MOE approach.

### **A: CHOICE OF CANCER DATA, MODE OF ACTION**

The working group on this topic was chaired by **Iona Pratt** (Food Safety Authority, Ireland) and the rapporteur was **Brett Jeffery** (Mars, UK). The plenary discussions were led by **Rolaf van Leeuwen** (National Institute for Public Health and the Environment, RIVM, Netherlands).

*If the compound produces tumours at a number of sites, should those sites where there is evidence for a significant non-genotoxic mode of action also be used to calculate a reference point for the MOE?*

It was agreed that carcinogenic effects for which there is evidence of a genotoxic mode of action are the most appropriate endpoints for calculating MOEs. Where treatment-related tumours occur at several sites, the narrative should explain which endpoint was finally chosen for deriving an MOE and why. Tumour sites where there is evidence for a non-genotoxic mode of action should not be modelled for derivation of an MOE. In this context, it was suggested that the framework to apply should be that developed by the IPCS Harmonization Project (WHO/IPCS 2008) that looks at mode of action on a case-by-case basis.

In considering the weight of evidence for a genotoxic mode of action, all positive results from genotoxicity studies could be considered potentially relevant to humans, but the accompanying narrative on the MOE should indicate whether a precautionary approach has been taken (e.g. whether there are positive in vitro data but no in vivo genotoxicity data).

The default approach would be to model all relevant datasets unless a genotoxic mode of action could be discounted, and then identify the tumour site with the lowest BMDL<sub>10</sub>. It was acknowledged that this would include the modelling of datasets for which the mode of action is unknown and that this could result in low MOEs for tumours that were, in fact, non-genotoxic in origin. Risk managers might then conclude that these were high-risk exposures. The narrative accompanying the MOE would need to explain these uncertainties.

The narrative should also give guidance to risk managers in cases where knowledge of the background biology indicates that a low MOE is not necessarily a cause for concern. This is exemplified by methyleugenol, which causes tumour formation at high doses following saturation of a metabolic pathway and a shift towards formation of a DNA-reactive metabolite, but human exposures are below this saturation point. This illustrates the importance of avoiding too simplistic an interpretation of numerical values of MOEs.

***Should tumours that are known not to be relevant to humans also be modelled or used to calculate a reference point?***

It would be relevant to consider, case by case, not only the tumour type and site in animals but also whether the pathogenesis was relevant to humans. There is a need to consider what is known about the species and strain used, especially if there is a high background incidence of tumours that are not considered relevant to humans (e.g. testicular tumours in F344 rats). However, investigations on whether the pathogenesis of a particular rodent tumour is relevant to humans have not been widely undertaken, and there is no consensus among pathologists on the significance of certain rodent tumours (e.g. liver tumours) for human risk assessment. If data for a particular tumour type are discarded, reasons should be given in the narrative. The reasons might relate for example to species differences in anatomy (e.g. tumours of the Zymbal gland) or to pathogenesis (e.g. forestomach tumours induced by irritation from gavage administration), but even those sites require careful consideration before being discarded if the mode of action of the substance is genotoxic, and some considered it more precautionary to include data from such sites.

***Should only site (organ)-specific cancer data be modelled or should the data on total numbers of tumour-bearing animals also be modelled to calculate a reference point?***

It was agreed that normally only site (organ)-specific cancer data should be modelled. In the work of the ILSI Europe expert group, neither the total number of tumour-bearing animals nor the total number of tumours of all types were included in the modelling as there can be a high background incidence of tumour-bearing animals in controls, making the dose–response assessment less sensitive. However, the expert group did make an exception for PAHs. Since exposure is to mixtures of PAHs, which individually might affect different sites, the total number of tumour-bearing animals was modelled to give the lowest BMDL. Modelling the total number of tumours of all types should not be done as it could unbalance the analysis by multiple scoring; only primary tumours and not metastases should be modelled.

***Should data on benign tumours and/or preneoplastic lesions (e.g. hyperplasia) be used as the basis for calculating a reference point?***

Data on benign tumours should be included since, in most cases, benign tumours are likely to develop into malignant tumours and pathologists have long considered both adenomas and carcinomas when advising on the carcinogenicity of a substance. It was suggested that for genotoxic carcinogens, both adenomas and carcinomas should be combined.

In the case of Sudan I, there were no carcinomas and unless the data on adenomas were used, an MOE could not be derived. The workshop participants considered that this approach was reasonable because Sudan I is genotoxic both in vitro and in vivo, and because bioassays on other azo dyes have resulted in both adenomas and carcinomas.

The ILSI Europe expert group did not use any data on preneoplastic lesions for the 12 example substances. It was noted that IARC does use such information in its weight-of-evidence approach for classification, but the workshop participants agreed that the use of such data was not appropriate for risk assessment. If all lesions involved in the pathogenesis of malignancy were to be considered, there would probably be practical difficulties. For instance, hyperplasia would need to be defined; the timing of conversion from one pathogenic stage to the next can be substance-specific; focal hyperplasia of the liver would present particular problems because it is difficult to quantify its potential for malignancy, which is generally low.

***How many dose groups with an increased tumour incidence are needed as a minimum for modelling to calculate a reference point?***

It was not possible to give a general answer to this question as it depends on the nature of the data. The fewer the number of dose groups, the less constraints there will be on the number of plausible models, which will increase uncertainty. Bioassays conducted in the past have not been designed with dose–response modelling in mind. The workshop participants discussed the suggestion that a minimum of three dose groups (including controls) is necessary for dose–response modelling, whereas five dose groups would be better suited to the calculation of a reference point (more dose groups will provide more information on the shape of the dose–response curve, even when the total number of animals used remains unchanged, i.e. fewer animals per dose group). Data from bioassays with few dose groups in which there is a maximal response in all treated groups are difficult to model; such problems can be overcome by including more lower-dose groups in study designs.

***Should dose levels be excluded from modelling under certain circumstances (e.g. early mortality, several dose groups with a saturated tumour incidence, apparently abnormal incidence)?***

This was an important issue, especially in the not-uncommon situation of early mortality in a high-dose group. There are also situations in which tumour incidence in the highest-dose group is less than that in the second-highest-dose group. The reduction might not be significant and is probably a reflection of the response reaching a plateau. In such cases, a model that allows for plateauing should be used.

Deciding on exclusions is a difficult issue that involves expert judgement. If any dose groups are excluded, a transparent rationale for the exclusion should be provided in the narrative.

***Should the data for males and females be combined in the model if there is no statistically significant sex difference in incidence?***

Currently, it is not routine to compare sex differences in incidence, but this can be done. The analysis should compare BMDs and not BMDLs. It was noted that using sex as a covariate in the analysis would be best, but modelling software does not currently allow this and sex differences have to be analysed separately. If responses differ, the sexes should be treated separately, but if the incidence of a particular tumour type is similar in both sexes, the data from males and females can be combined. This could decrease the size of the confidence interval, as was the case for ethyl carbamate in the analysis by the ILSI Europe expert group. A more complex situation arises when there is a sex difference in background incidence of a tumour but the slope of the response to the substance is similar. If males and females have been given different doses and the responses are similar, then combining the data from the two sexes will be advantageous because it will increase the number of dose groups for modelling.

***Should the data from different studies be combined?***

This is a similar question to the one above. From a statistical point of view it is possible to combine data from separate studies. If the same model is fitted, assuming the same shape but a different slope, then combining data can yield additional information. The ILSI Europe expert group did not combine data from different studies. In the modelling on acrylamide by JECFA (2005), data from the two available bioassays were combined, with bioassay as covariate. This was possible with the USEPA software (BMDS) but the model-averaging software does not allow combining of data from different studies.

***Should dose levels be corrected for duration of treatment (e.g. gavage only on 5/7 days, study duration of less than 2 years)?***

It was agreed that corrections should be made for duration of treatment and that there is a need to harmonise on what corrections should be applied. The pattern of human exposure (e.g. continuous or intermittent) should also be taken into consideration in interpretation of MOEs derived from animal data. The ILSI Europe expert group, in the case of furan, made a correction for gavage on only 5 days/week. However, the corrections that might be applied in the case of very short exposures of 3–6 months were unclear; the situation was complicated because when doses approach the maximum tolerated dose, even short exposures might give high tumour incidences.

***Use of epidemiological data***

To date, data from cancer bioassays conducted in animals have mostly been used to calculate MOEs. This is because there are few epidemiological data that are suitable for the MOE approach. The ILSI Europe expert group had considered whether to use the human data available on AFB1 and AA, but concluded that in the case of AA they were inadequate for MOE calculations because the datasets were not large enough to provide the necessary sensitivity. Modelling of human data was likely to produce very large confidence intervals. It would also be difficult to rank carcinogens using MOEs derived from both animal and human data because the reference points are unlikely to be comparable.

## ***B: MODELLING AND THE SELECTION OF REFERENCE POINTS***

The working group on this topic was chaired by **Lutz Edler** (German Cancer Research Centre, DFKZ, Germany) and the rapporteur was **John O'Brien** (Food Safety Authority, Ireland). The plenary discussions were led by **Michael Bolger** (Food and Drug Administration, US).

***What level of transparency or level of detail in the description of the various steps in the modelling is required? Is a general description of the modelling and presentation of final results (e.g. BMD, BMDL) sufficient or is more required?***

The workshop participants considered that there was a need for further guidance on modelling, beyond that currently available (e.g. from WHO/IPCS, 2004; WHO/IPCS 2009; USEPA, 2009). New USEPA guidance is expected to be published soon following extensive peer review. EFSA guidance on the use of the benchmark dose approach has been published recently (EFSA, 2009).

It was noted that the ILSI Europe expert group had used a model-average approach (Wheeler and Bailer, 2007, 2008). This was considered to be a good approach because it better quantifies uncertainty, but, unlike the USEPA BMDS, the software for the model-average approach has not been  $\beta$ -tested or validated. However, the expert group had also compared the outputs of BMDS with model-averaging for each substance and, with the exception of ethyl carbamate, the BMDLs were in the same range. The workshop participants therefore concluded that the work of the expert group was robust enough to be regarded as proof of principle.

To ensure transparency, the final paper from the ILSI Europe expert group should include a narrative to explain the rationale for using model-averaging and the differences between models (e.g. in the case of ethyl carbamate, why the model-averaging approach gave a lower BMDL than any of the individual models contributing to the average). The narrative also needs to address the major sources of uncertainty and express the degree of confidence in the reference point. The paper should also include summaries of the raw data on tumour incidence for each substance and describe the model constraints so that others can independently reproduce the BMDLs. Modelling should always be accompanied by a critical analysis of the input data.

On the wider question of whether model-averaging should be the preferred approach, it was considered crucial that appropriate and user-friendly software be readily available. Workshop participants had reservations about recommending model-averaging as the preferred approach because, although the software is available online (Wheeler and Bailer, 2008), it is not in an executable form and needs adjustment after downloading in order to use it. So far, few have experience with it and there is no guidance on its application. A benefit of the model-average approach is that the resulting BMDL avoids the need for selecting a single (usually the lowest) BMDL from the range of models used in the BMDS approach. However, in practice the results of model-averaging and BMDS are not very different, then continuing to use the more familiar BMDS might be preferable.

***What criteria are needed to be considered for model parameter values that will optimise the model fit to the data? Should this include the consideration of threshold parameters, which generally do not improve model fit and result in large confidence intervals (CIs)?***

It needs to be investigated whether model-averaging will overcome this problem. It would also be worth exploring whether there are biological thresholds that would be more rational to use than the threshold parameters used in the mathematical models. Although it is possible to fit many models, this is not considered to be a good approach because not all models that are statistically plausible are also biologically plausible.

***Should biologically based models be considered for use in dose–response modelling?***

Current mathematical and statistical models are not biologically based, and that includes time-to-tumour models. Where biologically based models are available (e.g. for formaldehyde) then they should be used (Conolly, 2002; Schlosser *et al.*, 2003).

***If all models fit poorly because of undetected error and data variation, and the model-averaging technique does not account for it, what could be done to address this problem?***

Reasons for failures in data fitting vary and, in such cases, analysis is needed to determine why the model fit is poor. It is usually due to a problem with the data itself, rather than a limitation in the modelling.

If a broad suite of models has been tried initially, it is unlikely that further enlargement of the number of models would solve the problem. In such cases, it will not be possible to find a reference point based on a BMD. In the past, it has been suggested that the T25 could be used as an alternative reference point, but the working group concluded that if the data are insufficient to determine a BMD, then they will also be insufficient to determine a T25 with any reasonable confidence. Where the data are adequate for deriving either a BMDL or a T25, the T25 would give a rough estimate, but if computing facilities are available then the BMD approach is preferable.

***How critical is significance of trend?***

Pre-screening the data to assess the significance of trend in the dose–response is important and allows better selection of the critical data for modelling. However, significance of trend might not be the only consideration as there is also a need to take into account tumour type (e.g. rare tumours could be relevant but might not show a significant trend).

***What are the criteria for the selection of relevant and appropriate mathematical and/or statistical model(s)? Is there a standard set of models that should always be used?***

A broad set of models (such as the seven used in the ILSI work) should be used to start with, plus a model that allows a maximum response of less than 100%. For the model-average approach, performance criteria for selecting appropriate models have been described (Wheeler and Bailer, 2007, 2008). No model should have more parameters than the number of dose groups to be modelled.

***Bootstrapped confidence limits have an inherent uncertainty that can be decreased with increasing numbers of bootstrap samples. What is the minimum level of confidence acceptable for BMDL values?***

It is usual to estimate the 95% confidence limits on the BMD. Confidence intervals do not become narrower with bootstrapping, but confidence in the BMDL increases. Around 2000 bootstrap samples should be adequate.

The report of the 2005 international conference suggested that when there is a factor of more than 100 between the values of the BMD and the BMDL it reflects considerable uncertainty and such BMDLs should not be used as reference points to derive MOEs. In the example substances explored by the ILSI Europe expert group using model-averaging, the ratios of the BMD to the BMDL<sub>10</sub> were all below 10. The Workshop participants did not reach a consensus on whether there should be a limit on the magnitude of the ratio of the BMD to the BMDL beyond which the data should not be used to derive an MOE. If wide confidence intervals are obtained (e.g. in cases where the lowest dose administered gives a maximum tumour response), the uncertainty that is implied needs to be explained in the narrative. The data should be examined to see if there is any way to reduce the uncertainty, but they should not be discarded because they may represent the most sensitive endpoint. If MOEs are being compared, it should at least be explained that an MOE derived from a BMD with a small confidence interval has lower uncertainty than an MOE derived from a BMD with a large confidence interval. Similar explanations in the exposure estimates used to calculate the MOEs should be given.

***What response level should be used for the benchmark response (BMR) and since it is not always clear what BMR can be considered adverse, what criteria need to be considered to make such a determination?***

Modelling should only relate to data in the observable range. The workshop participants agreed with the ILSI Europe expert group that a BMR of 10% for cancer endpoints was preferable, because modelling of lower values results in greater uncertainty. In general, BMRs of 5% and 1% for cancer endpoints should be avoided.

## **C: E P O S I T I O N**

The working group on this topic was chaired by **Andy Hart** (Central Science Laboratory, UK) and the rapporteur was **Gunna Würtzen** (Consultant for Coca-Cola Europe, DK). Plenary discussions were led by **Michael DiNovi** (Food and Drug Administration, US).

### ***Adequacy of available databases for performing exposure assessments.***

Food consumption data are available at both national and international levels, from developed and developing nations. The type of information contained in the databases, and the quality of the data, can vary considerably. Food commodity data are available from studies on regional and cluster diets. In general, population data are more readily available than individual data, which is useful for the particular case of genotoxic carcinogens where average intake is an important measure. The overall quality of the exposure data is strongly influenced by occurrence data. Analytical data are affected by limits of detection or maximum residue limits.

### ***What determines the type of exposure assessments required?***

To determine what data should be taken for assessing exposure, the purpose of the calculation of the MOE should first be clearly understood because this affects what type of exposure estimates are needed. What level of assessment and which databases are used depend on whether the purpose is for comparison of risks from more than one genotoxic carcinogen and setting priorities at national or international level, for screening purposes, or for estimates of risk in whole populations, toxicologically sensitive subgroups or groups with special intake patterns.

It should be clearly defined whether acute or chronic data (one meal or multiple day averages) are needed, depending on the nature of the toxicity and exposure patterns (e.g. intermittent or continuous). Data from the total population or consumers only may be taken. Estimates can be provided for median, or mean plus high-end consumers for the general population, and any sub-groups of interest. There is a need for consistency, aiming for a specific common percentile for high consumers, when using the MOE for priority setting or comparative assessments.

### ***Does averaging exposure over a broader population underestimate the risks to key groups, and how does this impact the MOE? How should MOEs for sensitive sub-populations be applied?***

For genotoxic carcinogens, both average and high intakes should be given. Since effects are usually only seen after a long period of exposure, average intake is a useful measure. This is in contrast to acute toxicants, for which a worst-case scenario is often necessary. High-end exposure is also important for genotoxic carcinogens because it encompasses higher chemical occurrence data. Different MOEs could be calculated for different situations. This must be clearly presented for risk-management decisions. It was acknowledged that data on average values for the whole population (per capita data) are more readily available than individual data. However, it was considered that average intake should preferably be calculated for consumers only, which would provide a more realistic picture. This is exemplified by ME, for which high-end consumers of pesto might have tenfold higher exposures than others.

Difficulties were acknowledged in comparing chemicals for which there is almost continuous exposure (e.g. AA) and for cases where exposure is intermittent (e.g. Sudan 1). This is especially important when comparing derived MOEs for priority setting for risk management actions. It is currently not clear how to relate irregular exposures to cancer risks, and it was acknowledged that the simplest way of comparing data is by using the arithmetic mean of all daily exposures. The choice of time period for which exposure is estimated should be decided on by consultation with toxicology experts.

Population groups with toxicological sensitivity should be distinguished from those with special intake patterns. An example of the former is individuals with hepatitis B, who are more biologically at risk from the carcinogenic effects of the liver carcinogen AFB1. Groups with special intake patterns might include high-end consumers, where the choice of food items containing the genotoxic carcinogen influences risk, rather than any biological sensitivity.

***Can intakes of genotoxic breakdown products and/or process contaminants be estimated, and how can these be effectively reflected in the MOE?***

Many food consumption values are conservative because the foods considered are raw commodities, whilst the concentrations of process-derived contaminants (such as AA) apply to specific processed foods. Exposure estimates are perfectly feasible for these substances, as long as data are available on which foods are affected, consumption of affected foods and concentration in these foods. The intake data should reflect the products as consumed.

***What is the utility and relationship between external exposure or dose and internal biomarkers of exposure?***

Participants agreed that when an MOE is calculated as a prioritisation measure for food safety, other exposure routes such as inhalation should not be included. Total exposure would, however, be necessary for general risk assessment purposes.

The use of biomarkers in estimating (internal) exposures was discussed. Where such data are available, they should be incorporated into estimates. However, the biomarker would have to be validated and it might be difficult to obtain sufficient, good quality data (interventions would be required), and to back-calculate to food occurrence and intake data. Currently, the resulting estimates would be uncertain. It is easier and cheaper to collect distribution and intake data.

The utility of prospective epidemiological studies to quantitatively assess exposure (MOEs based on animal versus human data) was discussed. The limitation of a prospective study to define relationships between cancer incidence and exposure is that it takes many years, whereas the purpose of the MOE paradigm is to define priorities for immediate risk management actions. If good epidemiological data are available, then it is always better to estimate cancer risk directly than to calculate an MOE derived from animal carcinogenicity data.

***Should MOEs only be compared if there is an equivalent data level and if exposure or cancer data are obtained using equivalent study methodologies?***

Participants agreed that MOEs can only be comparable if equivalent data are used in the derivation of the MOE. The examples in the ILSI manuscript are not equivalent because the exposure estimates do not use the same percentile of the population. In some cases, the high estimate is a different population rather than a high percentile of the same population. The difficulty of comparing MOEs based on differing

exposure scenarios and uncertain data is clearly highlighted in the cases of Sudan 1 and ME. For each population, an average and a high percentile should be estimated. In order to achieve comparability, the estimate should aim at the same percentile for each assessment. There are three aspects to be decided: which percentile, whether the mean or median estimate should be used (where the estimate is probabilistic), and whether non-consumers should be included. True comparisons can only be made if these aspects are the same for all cases included in the exercise.

***How can the variability and uncertainties in country-specific exposure data, which depend on consumption patterns and methodology used, be effectively reflected in the MOE value?***

Variability in exposure data can be described by presenting average and high-end estimates. This also allows a comparison between country-specific data.

A description and list of the uncertainties in occurrence and consumption patterns should be included in a narrative attached to the MOE. Some members of the working group thought that it might sometimes be useful to provide alternative estimates of exposure that indicate the assessors' evaluation of uncertainty. It was noted that in exposure assessments the uncertainty is always in one direction, and the estimates are, by the nature of the methodologies used, already conservative.

The group did not consider appropriate the suggested ILSI Expert group approach of including numerical uncertainty or modifying factors into the exposure calculation. Alternative approaches could be the WHO tiered methodology (WHO/IPCS, 2008), or qualitative description as suggested by EFSA (2006). It was agreed that further exploration on how to identify, evaluate and quantify uncertainties is needed.

Uncertainties in the quality of the data do not mean that the data should not be used, rather that an initial (conservative) estimate can be made, and then refined as needed. The first MOE does not have to be "perfect". In the first instance, a best estimate or ranges for the general population could be given, and then further refined for relevant sub-populations. In cases where data are poor, a conservative estimate of the MOE based on, for example, 95th percentiles can be made. If this extreme worst-case situation results in a low MOE, then this could already indicate a potential concern, in the first instance triggering a refinement of the MOE before any regulatory action.

## INTERPRETATION AND COMMUNICATION OF MOE

**Orlando Galli** (University of Milan, Italy) presented and led plenary discussions between all participants of the workshop on issues related to the interpretation and communication of the MOE approach.

Risk assessors need to develop scientifically based tools and forms of advice that help inform risk managers about the possible magnitude of health concerns, so that risk managers can set relative priorities for urgent actions or allocation of resources. The MOE has the potential to differentiate between exposure situations of larger, intermediate and lesser concern and can be used as a tool for prioritisation of risk management measures. It provides useful guidance for the application of risk management options, including ALARA. It can be used to set targets for risk reduction strategies, and also can help in cases where regulatory limits are exceeded.

The MOE is calculated as a function of potency in animal carcinogenicity assays and the human dietary exposure of the chemical in question. Participants agreed that if there are considerable uncertainties in the data used to derive the MOE, it can be regarded as only a rough estimate. To assist in its interpretation, it must be accompanied by an explanation of how it was derived, including all the various uncertainties in the data used. This narrative is needed to provide perspective and context. The narrative should be established case-by-case, on the basis of expert judgement. A framework is needed for the risk assessors to better present the (un)certainties and the degree of confidence in the MOEs so that risk managers are better advised on the level of concern.

Participants agreed that a structured approach on how to derive an MOE was required. Guidance is required on how to determine the adequacy of the toxicity and exposure data, including whether the data are so uncertain that the MOE approach should not be used. Knowledge on the mechanism of action of carcinogenicity is required, with explanations. Some concern was expressed on the potential over-extrapolation of biological data when modelling. Not yet fully covered is the relevance of the pathogenicity observed in the animal models, which in some instances was very questionable, to the severity of the different cancer types in humans.

The difficulty of comparing MOEs for different chemicals was discussed. In the worked ILSI examples, the MOEs derived for ME ranged from 15 to 790; for AA, MOEs of 40 and 160 for mammary tumours and of 96 and 430 for peritesticular mesothelioma; and for AFB1 the MOEs were 96–630. The MOEs for these chemicals have similar orders of magnitude, all less than 1000, and all could initially be considered to have high priority for risk management. Once the narrative and uncertainties in the data used are presented, it is clear that, for example, in the case of ME, there is considerable uncertainty in the relevance of the cancer data used and in the substantial variation in possible exposure scenarios. Similarly, concern was expressed that two chemicals might have similar MOEs, but that the relevance of the tumour types observed in animals to the human situation could question the value of the comparison.

As an example of mixtures, the PAHs were included in the ILSI work. It was queried whether the MOE approach could also be developed further for evaluating aggregate exposures, e.g. for dietary exposure to several hepatocarcinogens.

To compare MOEs in order to set priorities for risk management actions, it is crucial that the same reference point (e.g. the BMDL<sub>10</sub>) be used, and that average and high-end consumers be considered for each of the chemicals in the comparison.

The wisdom of communicating an MOE when too many uncertainties are apparent was questioned. Going through the exercise to derive an MOE would establish the weaknesses in the data, but if there are too many uncertainties, the MOE might be misleading, and should not be used for risk management purposes. The mechanism of action should be considered up-front and if there is little confidence that the end-point is related to genotoxicity, then the comparative MOE approach should not be used.

As proposed by EFSA, an MOE of 10,000 or higher, if it is based on the  $BMDL_{10}$ , might be viewed as a low priority for risk management. There was concern expressed by participants on the potential for misuse and misinterpretation of MOE values of 10,000 or more. There is a danger that if an MOE was greater than 10,000, then it could be interpreted that there was no concern for that chemical and no further action needed to be taken. A crucial aspect would be the grey area below the value of 10,000, where many MOEs will fall. Also, the value of 10,000 should not be considered as a target value nor as a threshold value for concern as this is also dependent on the confidence in the calculated MOE. All participants agreed that in its simplest terms, a high MOE is of a lower concern than a low MOE.

The MOE approach can be used both as a prioritisation tool for risk management purposes and as a risk assessment tool for communication purposes. For example, it is useful to support communications on low concerns (e.g. in the case of low level sporadic exposures from illegal contaminants, as shown in the ILSI examples of LMG and Sudan 1), or to highlight that not enough is known about a specific chemical and that more data are needed. It was emphasised that the MOE is not a numeric measure of risk; it is a tool to enable a description of the level of concern, and should not be used to extrapolate to a risk estimate.

Participants agreed that an MOE presented as an abstract number on its own has no value. Ways in which the level of concern could be presented were discussed. A possibility is to use a banding approach. Recommendations could then be made for risk management options depending on the category (or level of concern). Banding could be based on logarithmic intervals, i.e. 1–100, 1000–10,000, 10,000–100,000, etc. A further difficulty is then to describe what level of concern these bandings represent and where to place the boundaries. Transparency of the choice of allocating MOEs into such banding or categories would be required, together with presentation of the degree of confidence in the MOE.

It was suggested that at this current time, banding might not be useful because not enough is known about the uncertainties surrounding the derivation of the MOEs. In addition, a variable range of MOE values can be derived for the same substance depending on the data used, which is difficult for comparison purposes. However, in a risk assessment there should be an indication of the level of concern, based on the results of the tumour modelling and the commentary in the accompanying narrative.

## SUGGESTIONS ON FUTURE DEVELOPMENTS AND OTHER RECOMMENDATIONS

**Sue Barlow** (Consultant, UK) presented for discussion some suggestions that had emerged during the workshop discussions on issues that still needed to be elaborated or resolved and on possible future developments.

### *Should an MOE calculation be performed at all?*

There is a need for guidance on when it is appropriate to perform an MOE calculation. This would involve both toxicological considerations (e.g. metabolism information) and modelling considerations (e.g. if the data do not allow a BMDL estimate to be made, or the resulting estimate is very uncertain).

### *How to select critical endpoints for MOEs?*

There was a need for consensus and guidance on the biological and toxicological considerations in deciding which endpoints (tumour types) are potentially relevant for humans, and on how to select the critical endpoint when there is a range of tumour types to choose from.

### *How to deal with intermittent vs. continuous exposure?*

Assessing the risks of intermittent versus continuous exposure has not been widely explored. It is an issue for both toxicologists and exposure assessors. It requires consideration of how to make bioassay datasets more closely comparable, the extent to which intermittent human exposure might alter the potential risks, and how to best gather human exposure data. It is likely that case-by-case consideration will be needed.

### *Are we advocating model-averaging as the gold standard for now?*

The workshop had not expressed a clear preference for the model-averaging approach and there were concerns that to do so might inhibit some from trying the MOE approach. The model-averaging approach might offer better precision, but given the uncertainties in risk assessment of carcinogens, this might not be as important as ease of use. To encourage use of the model-averaging approach, there is a need to ensure that executable software is made available and that there is guidance on applying it.

### *Is there a need for further exploration of optimal study design issues?*

Currently, most cancer bioassays are designed to answer the classification question, "Is the substance a carcinogen or not?" The workshop had made some suggestions for optimising study designs so that the results were better suited for deriving BMDs suitable for MOE calculations, but many current datasets can be modelled and it was questioned whether it was realistic to expect standard protocols to be adjusted to better answer MOE questions.

***For what purposes can MOEs be used?***

There was a consensus on the utility of MOEs for ranking of carcinogens and prioritisation of risk management actions, but there is still a debate on the utility of stand-alone MOEs in the context of risk assessment advice on an individual carcinogen – yet it is advice on individual carcinogens that is most often requested. More work was needed on interpretation of individual MOE values.

***Should MOEs be banded and, if so, how?***

Banding of MOEs into broad numerical ranges associated with differing levels of concern could be helpful as background information when advice is given on an individual carcinogen. Banding would also promote a wider understanding that the numerical value of an MOE is not a precise value. There was agreement that MOEs of particular numerical values should not be described as equating with particular levels of risk. However, suitable phrases for conveying differing levels of concern, that do not stray into the area of risk management, are difficult to agree upon. Further, as yet, there is no agreement on numerical cut-off values for banding. These issues need pursuing in discussions between risk assessors and risk managers.

***When should dialogue start between the various stakeholders?***

The international conference in 2005 flagged up the need for a dialogue on the MOE approach between risk assessors, risk managers, risk communicators and consumers, but this has not yet occurred (except in specific instances like AA). There is a need to know whether risk managers find the MOE a useful tool, whether the approach can be readily explained to the public, and what the public understand by it, bearing in mind that people might not make much distinction between carcinogens in terms of either potency or exposure.

## CONCLUSIONS OF THE WORKSHOP

The overall chair, **John Larsen** (National Food Institute, Denmark), summarised the discussions in the workshop. He reviewed the context of the workshop in the light of previous discussions that had taken place at earlier international meetings on how to advise on the risks of genotoxic carcinogens. He highlighted the research that is continuing on the underlying mechanisms by which genotoxic agents can cause damage at the molecular level and the extent to which such damage remains fixed or is repaired. These variables indicate there are likely to be deviations from linearity in carcinogenic responses, and hence risk, at low doses. On the key issues, he summarised conclusions from the workshop as follows.

### *Choice of cancer data and mode of action*

- Ⓜ Model all datasets considered relevant to humans, unless a genotoxic component can be discounted.
- Ⓜ Cancer data for sites for which there is evidence of a significant non-genotoxic component should not be used to derive the reference point.
- Ⓜ Benign tumours (based on morphological classification) should be assumed to progress into carcinoma and should be used together with the malignant counterpart as the basis for calculating the reference point for genotoxic compounds.
- Ⓜ Preneoplastic lesions should not be used for deriving an MOE.
- Ⓜ Normally site (organ)-specific data should be used, but data on the number of tumour-bearing animals might be relevant for mixtures and/or when they provide the lowest BMDL.
- Ⓜ Data from males and females for the same tumour type can be combined in the modelling if there is no statistically significant sex difference in incidence.
- Ⓜ Data from different studies should only be combined in special cases and, if done, a strong and transparent rationale should be provided.
- Ⓜ Exclusion of data from a particular dose level in the modelling might be appropriate under certain circumstances but it can be difficult to provide a transparent rationale for doing so.
- Ⓜ Dose levels should be corrected for duration of treatment (e.g. gavage only on 5/7 days, study duration of less than 2 years) but correction for short study durations is still a matter of debate.

### *Modelling and the selection of reference points*

- Ⓜ The BMDL<sub>10</sub> should be used as a reference point on the dose–response curve. It represents the lower bound of a 95% confidence interval on the benchmark dose that corresponds to a 10% increase in tumour incidence (BMD<sub>10</sub>).
- Ⓜ The issue of how small the confidence intervals on the BMD should be in order to use the data is not yet resolved.
- Ⓜ If the data can be modelled to derive a BMD or BMDL, the T25 should not be used.
- Ⓜ Pre-screening for selection of end-points with significant trend should be carried out to determine which data will be useful for modelling.
- Ⓜ Initially, a broad family of models should be used, such as seven models plus a model to allow a maximum response of less than 100%.
- Ⓜ Reporting on model performance criteria, including determination of goodness-of-fit, is essential.
- Ⓜ The model-average approach could be used in the future for calculating the BMDL; however, some concern was expressed that the software is not yet validated and that an executable file version is not available. In the meantime, use of the BMDS was equally acceptable.

## Exposure

- Ⓢ Different exposure scenarios should be provided, e.g. for the whole population and/or for specific groups of the population, depending on the substance under consideration and its presence in the diet.
- Ⓢ Both toxicologically sensitive groups and groups with special dietary intake patterns might need to be considered.
- Ⓢ For genotoxic carcinogens, average intake is an important measure (not only high intake). Mean and median exposures should be given, as well as exposure in highly exposed individuals.
- Ⓢ Population data are more widely available than individual data. Should it be the average exposures for consumers only that are considered or the more frequently available average values for the whole population
- Ⓢ Exposure estimates should be presented with their inherent uncertainties.
- Ⓢ The issue of how to deal with intermittent versus continuous exposure needs further consideration.
- Ⓢ Overall quality of an MOE is strongly influenced by the availability of occurrence data.
- Ⓢ When exposure data are poor, a conservative estimate should be made initially and, if necessary, further refined in a type of tiered approach.
- Ⓢ When MOEs are calculated in order to assess priorities for food safety measures, other routes of exposure should not be included. For general risk assessment, total exposure from all routes should be looked at.
- Ⓢ Biomarkers can be used in theory, but in practice their use can be difficult and uncertain. It might be cheaper, better and easier to collect distribution and intake data.

## Interpretation and communication of MOE

- Ⓢ The MOE approach is both a prioritisation tool and a risk assessment tool, but the MOE is not a numerical measure of risk. Therefore, it should be communicated in terms of concern rather than risk.
- Ⓢ In general, small MOEs indicate high concern, and large MOEs low concern.
- Ⓢ The MOE should always be accompanied by a narrative to explain the background and the uncertainties in the reference point and exposure estimates.
- Ⓢ Better communication tools on the concept of the MOE approach and resulting levels of concern need to be developed. This needs cooperation between risk assessors, risk managers and risk communicators. Consumer perceptions about these tools have not yet been addressed adequately.

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## LIST OF ACRONYMS

AA	Acrylamide
AFB1	Aflatoxin B1
ALARA	As low as reasonably achievable
BaP	Benzo a pyrene
BMD	Benchmark dose
BMDL	Lower limit of the 95% confidence interval on the benchmark dose
BMDS	EPA benchmark dose software
BMDU	Upper limit of the 95% confidence limit on the benchmark dose
BMDL <sub>10</sub>	Lower confidence limit on the benchmark dose associated with a 10% change in response
BMR	Benchmark response
BZ	Benzene
CI	Confidence interval
DCP	1,3-Dichloro-2-propanol
EC	Ethyl carbamate
EDI	Estimated daily intake
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FU	Furan
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LMG	Leucomalachite Green
MCP	Methylcyclopropene
ME	Methyleugenol
3-Me-CP	3-Chloro-2-methylpropene
MOE	Margin of exposure
MTD	Maximum tolerated dose
NOAEL	No observable adverse effect level
PAHs	Polycyclic aromatic hydrocarbons
PhIP	2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine
T25	Dose at which 25% of the animals developed tumours
USEPA	US Environmental Protection Agency
WHO/IPCS	World Health Organization/International Programme on Chemical Safety

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# Other ILSI Euro e Pu t o

## Co eMo o r h

a h- l r- a c  
 a S g u - a  
 h- a- c- c- p- c-  
 c- N  
 c- N h- a  
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## Re ort

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*Escherichia coli* eh ec-  
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 g- m- o-  
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 f- v- a e- p-  
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*M. cobacterium avium paratuberculosis* map-  
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