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Principles and Methods for the Risk Assessment of Chemicals in Food

Chapter 7
RISK CHARACTERIZATION

A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization

Food and Agriculture Organization of the United Nations

World Health Organization

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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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7.1 Introduction

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers (Renwick et al., 2003). The Codex Alimentarius Commission (CAC) has defined risk characterization as "The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment" (FAO/WHO, 2008).

Historically, different approaches have been used for the risk characterization of toxic effects considered to have a threshold and for those considered to have no threshold. Health-based guidance values have been used by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for substances that produce

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For acronyms and abbreviations used in the text, the reader may refer to the list of acronyms and abbreviations at the front of this monograph. Definitions of select terms may be found in the glossary at the end of the monograph.

threshold effects (see chapter 5). In the risk characterization for these types of substances, the health-based guidance values are compared with estimated or measured human exposure. In circumstances where the data are not sufficient to propose a health-based guidance value for a substance producing threshold effects, JECFA and JMPR may comment on the margin of exposure (MOE) between the doses at which effects are seen in animals and the estimated human dietary exposure.

Substances that are both genotoxic and carcinogenic would generally not be considered acceptable for use as food additives, pesticides or veterinary drugs. For those substances that are genotoxic and carcinogenic, the traditional assumption is that there may not be a threshold dose and that some degree of risk may exist at any level of exposure. Thus, health-based guidance values have not been developed by JECFA for substances, such as certain contaminants, that are known to be both genotoxic and carcinogenic. It should be noted, however, that some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms, and establishing a health-based guidance value would be appropriate for such chemicals. The types of risk characterization advice that have been developed for substances that are genotoxic and carcinogenic include:

1) a recommendation that the exposure should be as low as reasonably achievable (ALARA);
2) quantification of the risk at different levels of exposure (e.g. aflatoxin) (FAO/WHO, 1999, 2007b); and
3) ranking of compounds producing similar hazards according to their estimated risk (e.g. substances that are genotoxic and carcinogenic) (FAO/WHO, 2006a).

It is recognized that the advice in approach 1 is of limited value, because it does not take into account either human exposure or carcinogenic potency and does not allow risk managers to prioritize different contaminants or to target risk management actions.

While approach 2 can provide advice for risk management of a specific substance, it does not provide the information necessary to prioritize different contaminants.

Approach 3 includes the MOE approach, which is the ratio between an amount of a substance producing a small but measurable effect in

laboratory animals or humans and the estimated human exposure (see section 7.4). For substances that are both genotoxic and carcinogenic, this approach provides advice to inform risk managers of how close human exposures are to those anticipated to produce a measurable effect in laboratory animals or humans. In addition, MOEs for different substances can be compared to assist risk managers in prioritizing risk management actions (EFSA, 2005a; FAO/WHO, 2005; O'Brien et al., 2006).

7.2 Risks at estimated levels of exposure

7.2.1 General considerations

The calculation of health-based guidance values was discussed in chapter 5. In risk characterization of substances exhibiting threshold effects, health-based guidance values are compared with estimates of dietary exposure. If exposures are below the relevant value, then no further information on risk characterization need be provided. However, in cases where exposures exceed health-based guidance values, the values themselves do not provide the risk manager with advice on the possible extent of the risk to those exposed to these higher amounts.

A first consideration should take into account the fact that health-based guidance values themselves incorporate safety or uncertainty factors (see chapter 5). A small or occasional dietary exposure in excess of a health-based guidance value based on a subchronic or chronic study does not necessarily imply that adverse health effects will occur in humans. If further advice is required on the possible health consequences for those exposed to amounts greater than the health-based guidance value, then the toxicity database needs to be considered with respect to the lowest-observed-adverse-effect levels (LOAELs), the nature and severity of the effects observed, the shape of the dose–response curve in the observed range (chapter 5) and whether acute toxicity, including developmental toxicity, is an issue. In the case of acute toxicity, the possible consequences of an estimated dietary exposure in excess of the acute reference dose (ARfD) should also be considered on a case-by-case basis. The option of refining the dietary exposure estimate may also be explored (see chapter 6).

JECFA has taken an MOE approach in characterizing risks associated with certain contaminants in food for which the available data were insufficient to establish a health-based guidance value, such as polybrominated diphenyl ethers (FAO/WHO, 2006a) and temephos (FAO/WHO, 2006b). Consideration of whether the identified MOE presents a concern for human health follows a process similar to selection of appropriate uncertainty factors to be used in establishing a health-based guidance value (e.g. factors of 10 for interspecies differences, 10 for human variability and additional factors for important gaps in the database). Other examples of applying an MOE for effects considered to have a threshold include the JECFA evaluation of the neurotoxic and reproductive effects of acrylamide, for which a health-based guidance value could not be proposed because of its additional genotoxic and carcinogenic properties (FAO/WHO, 2006a). JECFA also applied an MOE approach in considering risks of carrageenan in infant formula (FAO/WHO, 2007b), as a health-based guidance value cannot be assumed to be sufficiently protective for infants under the age of 12 weeks.

Another type of risk characterization output from dose–response modelling is the prediction of risks at specified exposure levels. This output can take the generic form of predicting "X number of health-impacted individuals at exposure Y". An example of such estimates is the case of aflatoxins, where JECFA predicted the additional cancer risk at different levels of exposure (FAO/WHO, 1999, 2007b). In the optimal case, such estimates are supported by parallel assessments that describe the uncertainty in such estimates by providing additional information on the range of estimates, rather than a single value. The risk manager can then make statements such as "Up to X number of individuals may be adversely affected by exposure Y". As discussed in chapters 5 and 6, assumptions inherent in such estimates can influence risk management decisions. These include choice of models, choice of end-points and limitations in initial data sets that were extrapolated.

These types of assessments have also been performed for lead (FAO/WHO, 2000), fumonisins B1 and B2 (Humphreys et al., 2001), methylmercury (Carrington et al., 2004) and cadmium (FAO/WHO, 2006a). In this context, it may be desirable to create a statistical model that estimates the range of effects expected for a population. Availability of such estimates can provide additional information for

risk managers to conduct cost–benefit analyses, risk–benefit assessments and evaluations of public health interventions.

7.2.2 Uncertainty and variability analysis

Uncertainty refers to limitations in the knowledge of the risk assessor about the data and models used. Variability reflects the inherent biological heterogeneity, either in exposure or in response. Thus, although both uncertainty and variability can be characterized using probability distributions, they are different concepts. Uncertainty can be decreased as the quantity or quality of the information available improves. In contrast, modelling variability is an exercise in descriptive statistics that results in a model of a population rather than an individual. Characterization of the variability in dietary exposure in the population, as an example, can be improved by better information, but the variability cannot be eliminated.

Uncertainty analysis can be applied to both exposure data and health effects data, but so far it has been applied mainly to exposure estimates. In an uncertainty analysis (EFSA, 2005b; IPCS, 2008), each component of a model may have its own uncertainties. If the assessor's knowledge were perfect, then the exposure estimates for specific members of the population (e.g. the median individual or the 95th-percentile individual) could be characterized as a single value. This is never the situation, so an uncertainty analysis is an important part of a probabilistic model and should portray the limits of current knowledge by generating a range of estimates that cover the range of plausible interpretation. More typically, knowledge is imprecise, and exposures for representative individuals must be reported as a range of values. The uncertainty analysis is ideally a quantitative exercise where feasible. This serves two basic purposes. First, it gives decision-makers an idea of the overall confidence associated with the estimation process. Second, it facilitates research planning by giving researchers a formal target.

A formal uncertainty analysis is not always necessary. Two good reasons for omitting a formal representation of uncertainty are that 1) the uncertainties involved are relatively small and 2) it is known beforehand that either a most likely case or worst-case scenario will drive the decision process. However, even in these cases, a rationale for determining that these assumptions are true should be given.

The basic notion underlying a "statistical" uncertainty is that the uncertainty about an unidentified (or random) individual or event is characterized by the known frequency distribution of a population or series. Thus, the same distribution may function as either a frequency distribution or an uncertainty distribution, depending on whether it is being used to make a prediction about a population or about an individual.

The concept of statistical sampling error is another important frequency-based uncertainty. Sampling error depends not only on the number of samples taken but also on the variance within the total population from which the sample is taken—that is, the larger the variance, the more samples are required to correctly describe the population. The description of uncertainty involves the use of a statistical distribution to express the doubt that a small sample accurately represents a population. The underlying distribution used is speculative and is usually assumed to be the normal distribution. Confidence intervals for parameter estimates usually reflect sampling error.

Formal representation of uncertainty may utilize statistical concepts of uncertainty, such as measurement and sampling error. In addition, probability trees (Hacking, 1976; Rescher, 1993) may be used to represent uncertainties associated with the use of alternative plausible model forms or alternative surrogate data sets.

For many public health issues, it may be desirable to characterize the uncertainty associated with population estimates for a value that varies among individuals. For example, dietary exposure estimates are often made for a series of individuals in a survey, and hence those population estimates are uncertain. In these circumstances, each inference may have distributions that describe the range of population values and distributions or probability trees that represent uncertainty. An uncertainty analysis may also alleviate concerns over the accuracy of a simulation method for estimating the tails of the frequency distributions by demonstrating that the uncertainties associated with the extreme values are larger than the errors introduced by the simulation method. In order to integrate these different elements into the conclusions, a two-dimensional simulation is useful.

The discussion of variability and uncertainty here is intended to provide a general framework for thinking about the characterization of

population dietary exposure. In practice, the emphasis of public health risk assessments is on the characterization of population variability. Nonetheless, it is useful to keep in mind that the population estimates developed are not certain and that, ideally, the assessor should provide some indication of the plausible range of values for various representative members of the population.

For both exposure and health effects, the risk assessment should include a narrative evaluation of uncertainty. As indicated above, uncertainty can be assessed qualitatively, semiquantitatively or quantitatively. Whereas a complete quantitative assessment would involve probabilistic approaches with sensitivity analysis, this will often not be necessary or even feasible. As a minimum, the major sources of uncertainty in a risk assessment should be identified. Where possible, some idea of their magnitude should be provided, even if only semiquantitatively (e.g. small, moderate, large), together with an indication of whether they tend to increase or decrease the conservatism of the assessment. Such information can provide a guide to which studies would contribute most to helping refine any further risk assessment. Sources of variability should be identified and, where possible, some indication of their magnitude provided.

7.2.3 Sensitivity analysis

Risk assessment models may become very complex. An uncertainty analysis (see above) may reveal that there are substantial uncertainties in an estimate without indicating from where those uncertainties arise. That is, it may not be apparent which of the uncertainties in the assumptions give rise to the uncertainty in the model predictions. Sensitivity analysis refers to quantitative techniques that may be used to identify those aspects of the inputs (concentration or food consumption data) that contribute the greatest extent to the uncertainty. Analyses that evaluate inputs identified as the most important sources of uncertainty may be expected to be the most useful.

There are many different sensitivity analysis techniques (Cullen & Frey, 1999; Frey & Patil, 2002). The simplest of these vary each uncertain input one at a time, with all the other values held at some nominal (i.e. central or most likely) value. The resulting range in the output is then compared for each of the inputs. Although they are invariably

more calculation intensive, the more sophisticated sensitivity analysis methods analyse correlations among input distributions.

Sensitivity analysis is also sometimes used to evaluate frequency distributions (Frey & Patil, 2002). In this case, the relationship of the inputs used to describe population variability and the output distribution for the population estimate are examined. This type of analysis may be useful for identifying food chemical control strategies.

7.3 Risks from exposure to multiple substances

7.3.1 General considerations

There is an increasing awareness by those involved in risk assessment and by the general public of the need to consider any risks associated with combined exposure to mixtures of substances, both human-made and naturally occurring. This has been the focus of considerable risk assessment activity around the world (FSA, 2002; IPCS, 2009b; see also http://www.epa.gov/pesticides/cumulative/).

Given the numbers of human-made and naturally occurring chemical substances to which humans are exposed, there is a very large number of possible binary, tertiary, quaternary, etc. combinations. In consequence, direct experimentation cannot resolve this risk assessment issue, and research has focused on understanding the basic science of combination toxicology. In recent years, there have been major advances in understanding mechanisms of combination toxicology, and a significant theoretical and experimental database has been developed (Ito et al., 1995a,b; Jonker et al., 1996, 2004; Groten et al., 2000, 2001; Feron & Groten, 2002; Feron et al., 2002). In principle, combination effects could occur as a result of different chemicals present in food at the same time or at different times, depending on the rate of clearance of the chemicals from the body. There are four types of combined effect or interaction:

- *Dose addition* occurs when substances produce toxicity via the same mechanism of action. For substances that have a threshold in their dose–response relationships, the total activity of the mixture is the sum of the exposures for each component multiplied by its relative potency. A consequence of this is that a biological effect

may be produced if there is exposure to a mixture that contains a large number of substances that have the same mechanism of action, even though the exposures to each substance are too low to individually elicit a response. This mechanism is the basis for the group acceptable daily intake (ADI) approach for structurally related additives and pesticides (see chapter 5, section 5.2.8) and the use of toxic equivalency factors (TEFs) to derive an overall tolerable intake (TI) for structurally related contaminants (see section 7.3.2). A review of approved food additives with numerical ADI values has shown that dose addition might arise only rarely for structurally unrelated substances (Groten et al., 2000). Dose addition is the basis for recent considerations of pesticides that share the same mode of action by the Pesticide Residues Committee (2007) in the United Kingdom, in which simultaneous exposures to different acetylcholinesterase (AChE) inhibitors are assessed on the basis of summing each exposure as a fraction of the relevant ADI (this method assumes that each ADI is based on inhibition of AChE).

- *Response addition* is possible when two or more substances produce the same response or effect by different mechanisms. If the dose–response models used to estimate effects have thresholds, only those substances present in amounts above the threshold are relevant.

- *Synergism* occurs when the effect of the combination is greater than predicted by the summed activity of each component individually at the same level of exposure that occurs in the mixture. Synergism may arise from either toxicokinetic or toxicodynamic interactions. Toxicokinetic interactions are possible when one compound alters the metabolism of the potentially toxic component to increase the internal dose of or systemic exposure to the active form of the toxic component (parent compound or metabolite). Such an interaction can increase the activity of the toxic component and is the basis for the addition to pesticide formulations of synergistic compounds, which enhance the desired pesticidal activity of the formulation in the target organism. Synergism could result in an otherwise inactive level of exposure to a potential toxicant producing an effect when it is present in combination with sufficient amounts of another component to influence

its activity. Thus, synergism typically occurs when at least one of the components is present in sufficient amounts to affect the biological system in some way. In consequence, synergism is much less likely in an exposure scenario in which the exposure to each component in a mixture is below their respective health-based guidance values.

- *Antagonism* may arise from either toxicokinetic or toxicodynamic interactions, but usually requires that each substance is present at active doses or concentrations. Such an interaction would reduce the toxicity of the active component and therefore would not result in a possible health concern. Antagonism would occur if a substance with a low efficacy, such as a partial agonist, were to compete for a site of action with a high-efficacy compound, such as a full agonist. Such interaction may well occur in the application of TEFs (see section 7.3.2) and would make the assumption of full dose additivity a conservative approach.

One of the major lessons learnt from research to date is that exposure to mixtures of chemicals at levels that are non-toxic for each individual chemical generally will not result in a health risk, but dose addition is an important exception to this.

Evaluations of mixtures have been undertaken by JECFA and JMPR for some food additives, pesticides and veterinary drugs that are produced and tested as mixtures and some co-occurring mixtures of certain contaminants, such as polyhalogenated dibenzodioxins. As the testing of all possible combinations of substances that can occur in food is virtually impossible, substances are usually tested for toxicity singly in order to optimize hazard identification and characterization. Combinations are considered when substances are closely related structurally and co-exposure is likely. Examples are the use of data on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for the risk characterization of mixtures of dioxin-like compounds and the use of data on related substances for flavourings evaluated by the JECFA Procedure for the Safety Evaluation of Flavouring Agents (see JECFA reports from the forty-fourth meeting onwards).

For pesticides and veterinary drugs that are mixtures, JMPR and JECFA, respectively, base the ADI for the residues on the mixture as

tested. In some cases, a group ADI (see chapter 5, section 5.2.8) has been allocated. JECFA has also used the group ADI for certain food additives that are metabolized to a common potentially toxic metabolite and a group tolerable daily intake (TDI) for closely related contaminants that occur as mixtures.

When considering a substance that is a member of a series of compounds that are very closely related chemically (e.g. fatty acids or esters of allyl alcohol), but for which toxicological information is limited, it may be possible to base the safety evaluation on a group ADI established for the series of substances. This procedure can be followed only if a great deal of toxicological information is available on at least one member of the series and if the known toxic properties of the various substances can be predicted to fall along a well-defined continuum. Apart from the evaluation of flavouring substances by JECFA, consideration of mixtures represents one of the few situations in which the Committee has used structure–activity relationships in its safety assessments.

7.3.2 Toxic equivalency factor (TEF) approach

An approach that takes account of dose additivity is the TEF approach. The strategy of the TEF approach is to scale the exposure for each component of a mixture relative to the potency of an index chemical. In principle, TEFs can be used for a toxic end-point or a readily measured biomarker of a toxic response, such as binding affinity to the aryl hydrocarbon receptor or induction of cytochrome P-450 1A1. The biochemical effects used as an index of potency should be associated with subsequent toxic responses. The TEF estimates can be based on the results of in vivo and in vitro studies or a combination of both. The scaled concentrations are added, and the dose–response curve of the index chemical is used to generate a health-based guidance value, which is used as the response estimate for the sum of scaled concentrations. For this dose addition, the same mode of action and similarly shaped dose–response curves across the components are assumed. This method requires both toxicity and exposure data on the components of the mixture and sufficient data on one well-studied component to estimate a health-based guidance value. This component is typically chosen because it has a high relative potency or has been best characterized with respect to its effects and dose–response relationship.

The TEF approach is often complicated to use, is data intensive and requires some statistical modelling and expert judgement. A major disadvantage in the TEF approach is that the use of single point estimates for TEFs incompletely addresses the temporal issues when half-lives of the compounds in question differ considerably and there is a large degree of variability in the time intervals between exposure to the various compounds in the mixture (Mileson et al., 1999). A TEF method may not be appropriate when there are significant non-additive interactions among chemicals within the mixture (Krishnan et al., 1997).

The TEF approach has been developed by WHO (Van den Berg et al., 1998, 2006) and used by JECFA for the evaluation of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) (FAO/WHO, 2002) and has also been considered for possible application in the evaluation of polycyclic aromatic hydrocarbons (PAHs) (FAO/WHO, 2006a).

7.3.3 Surrogate approach

The surrogate approach to mixture evaluation uses a single component as the measure of concentration in relation to the response of the whole mixture. It assumes that the risks associated with each of the components of the complex mixture are proportional to the level of an indicator or index chemical in the mixture. The surrogate approach can be used for a series of compounds that are very closely related chemically (e.g. PAHs) but for which toxicological information on some members is limited. This procedure can be applied with confidence only if a great deal of toxicological information is available on at least one member of the series and if the known toxic properties of the various compounds fall along a well-defined continuum.

JECFA used the surrogate approach for the evaluation of PAHs (FAO/WHO, 2006a). The Committee noted that the TEFs that had previously been proposed for PAHs were derived from studies involving parenteral administration or in vitro approaches and that no data on oral administration were available that were suitable for this purpose. The Committee concluded that a surrogate approach should be used for the evaluation of mixtures of PAHs administered by the oral route, with benzo[*a*]pyrene being used as a marker of exposure to, and carcinogenicity of, the genotoxic and carcinogenic PAHs.

7.4 The formulation of advice on compounds that are both genotoxic and carcinogenic

JECFA has established procedures for determining health-based guidance values, such as the ADI or TI, for chemicals that produce adverse effects that are thought to show a threshold in their dose–response relationships. That is, there is considered to be no appreciable risk at intakes below the health-based guidance value. Some chemicals increase the incidence of cancer in experimental animals by non-genotoxic mechanisms; for these, establishing a health-based guidance value such as a provisional tolerable weekly intake (PTWI) would be appropriate. However, for substances that are both genotoxic and carcinogenic, dose levels that do not show a carcinogenic effect may simply represent the limit of detection in that bioassay, rather than an estimate of a possible threshold. Therefore, JECFA and JMPR do not establish health-based guidance values for compounds that are both genotoxic and carcinogenic using the no-observed-adverse-effect level (NOAEL) approach (see chapter 5). In the absence of evidence on the influence of non-linearity on the incidence of cancer at low levels of exposure, the advice given previously by JECFA on compounds that are both genotoxic and carcinogenic has been that intakes should be as low as reasonably achievable (ALARA). Such advice is of limited value, because it does not take into account either human exposure or carcinogenic potency and has not allowed risk managers to prioritize different contaminants or to target risk management actions. In addition, ever-increasing analytical sensitivity means that the numbers of chemicals with both genotoxic and carcinogenic potential detected in food will increase.

At its sixty-fourth meeting (FAO/WHO, 2006a), JECFA considered a number of contaminants for which genotoxicity and carcinogenicity were important issues and discussed possible approaches to the formulation of advice that would better inform risk managers about the possible magnitude of health concerns at different levels of intake in humans. Hazard identification would normally be based on data from studies on genotoxicity and from cancer bioassays. As described in chapter 5, hazard characterization (dose–response assessment) of substances that are both genotoxic and carcinogenic would be based on the available dose–response data for cancer, which would be derived mostly from studies in rodents given daily doses many orders of magnitude greater than

the estimated intakes in humans. If available, dose–response data from studies of epidemiology may also be used for hazard characterization and would avoid the necessity for interspecies comparisons and extrapolation over many orders of magnitude. An International Programme on Chemical Safety (IPCS) 2004 workshop recommended the use of the lower one-sided confidence limit of the benchmark dose (BMDL) as a starting point for hazard characterization based on data from a bioassay for cancer in experimental animals when the data are suitable for dose–response modelling (IPCS, 2009a).

The dose metric used for modelling could be a biomarker, providing that it was critically related to the process by which cancer arises and had been validated in relation to the external dose or intake. For carcinogenesis, selection of the dose–response data for modelling will need to consider both site-specific incidences of tumours, especially for the site showing the greatest sensitivity, as well as combined data (e.g. numbers of tumour-bearing animals) for compounds that do not show clear organ specificity. Analyses based on the numbers of tumour-bearing animals may also be appropriate under other circumstances—for example, in the assessment of complex mixtures of compounds that are both genotoxic and carcinogenic. Dose–response characterization should aim to define the BMDL for the carcinogenic responses of relevance to human health, at the lowest level of response (the benchmark response [BMR]) that reliably defines the bottom end of the observed experimental dose–response relationship. A BMR of a 10% incidence is likely to be the most appropriate for modelling of data from cancer bioassays, because the values for different mathematical models show wider divergence at incidences below 10%. The consistent use of the same BMR (i.e. 10%) will facilitate comparisons of the risks associated with different compounds that are both genotoxic and carcinogenic.

Exposure (intake) assessment for a compound that is both genotoxic and carcinogenic is no different from that for other types of contaminants. Risk characterization involves comparison of the estimated exposure with the identified BMDL. In principle, this can take different forms (FAO/WHO, 2006a):

- *Calculation of the MOE for substances that are both genotoxic and carcinogenic*. The MOE is the ratio between a point of departure (POD) or reference point (such as the BMDL) on the

dose–response curve from experimental animal or epidemiological studies and the estimated human exposure. The MOE can be used to prioritize different contaminants, providing that a consistent approach has been adopted. The acceptability of an MOE depends on its magnitude and is ultimately a risk management decision (IPCS, 2009a). To aid that decision, the risk assessor should provide information on the nature and magnitude of uncertainties in both the toxicological and exposure data. Although the risk assessor should not provide an assessment of the acceptability of the MOE, guidance should be given on its adequacy, taking into account the inherent uncertainties and variability (Barlow et al., 2006).

- *Dose–response analysis outside the observed dose range.* Quantitative dose–response analysis could be used to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans or the exposure associated with a predetermined incidence (e.g. 1 in 10^6). In order to provide estimates of the possible carcinogenic effect at the estimated exposure for humans, mathematical modelling would need to take into account the shape of the dose–response curve between the high doses used in the cancer bioassay and much lower intakes by humans. This requires extrapolation outside the observed dose range. In the future, it may be possible to incorporate data on dose–response or concentration–response relationships for the critical biological activities involved in the generation of cancer, such as metabolic bioactivation and detoxification processes, deoxyribonucleic acid (DNA) binding, DNA repair, rates of cell proliferation and apoptosis, into a biologically based dose–response model for cancer that would also incorporate data on species differences in these processes. However, such data are not currently available. At present, any estimate of the possible incidence of cancer for humans has to be based on extrapolation of cancer bioassay data by application of empirical mathematical equations that may not reflect the complexity of the underlying biology. A number of mathematical equations have been proposed for low-dose extrapolation. The resulting risk estimates are dependent on the mathematical model used; the divergence increases as the dose decreases, and the output from different equations can differ by orders of magnitude at very low incidences (see also chapter 5).

- *Linear extrapolation from a POD.* Because the estimated risks at low doses are model dependent, linear extrapolation from the BMDL, which is conservative and simple to apply, has been used as a matter of policy by some scientific bodies or authorities in order to calculate levels of exposure associated with different theoretical incidences of cancer. The incidence used is regarded as an upper-bound estimate for lifetime risk of cancer, and the actual risk may lie anywhere between zero and the calculated upper-bound estimate. Calculation of the intake associated with an incidence of 1 in 10^6 from the BMDL for a 10% incidence using linear extrapolation is simply equivalent to dividing the BMDL by 100 000, and this approach is therefore no more informative than calculation of an MOE.

Of the three options given above, the MOE and linear extrapolation from a POD are the most pragmatic and usable at the present time. Linear extrapolation from a POD offers no advantages over an MOE, and the results are open to misinterpretation, because the numerical estimates may be regarded as quantification of the actual risk. The sixty-fourth JECFA meeting (FAO/WHO, 2006a) therefore decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The strengths and weaknesses inherent in the data used to calculate the MOE should be given as part of the advice to risk managers, together with advice on its interpretation.

7.5 Subpopulations at risk

It is preferable for risk management and enforcement purposes to set a health-based guidance value, such as an ADI, PTWI, provisional maximum tolerable daily intake (PMTDI) or ARfD, for a substance that will cover the whole population. These values are normally established to protect the most sensitive subpopulation, based on the most sensitive critical health outcome. The use of safety or uncertainty factors has been generally assumed to take into account the differences in sensitivities in human populations, particularly from genotypic and phenotypic variations (Renwick et al., 2003).

However, it is recognized that the most sensitive critical health outcome may not always be relevant to some population subgroups. For example, it is particularly important to ensure that any health-based

guidance value is adequate to protect the embryo or fetus from possible effects in utero. While a health-based guidance value derived from developmental (embryo/fetal) effects would necessarily apply to women of childbearing age, it is recognized that such a value may be unreasonably conservative and not relevant to other population subgroups. Thus, in some situations in which a developmental or other subpopulation-specific end-point determines the health-based guidance value for a substance exhibiting no other toxicity at the developmental or other subpopulation-specific NOAEL, risk managers might request advice regarding a second (higher) value based on another end-point relevant to the rest of the population, as, for example, in the case of methylmercury in fish (FAO/WHO, 2007a).

The critical risk assessment issue that should be considered in recommending different health-based guidance values for different population subgroups is whether the most sensitive critical health outcome is irrelevant for a significant part of the whole population.

The advice provided to risk managers should include the following considerations:

- If a higher health-based guidance value is established based on another end-point, can the exposure be controlled for the sensitive population subgroup?

- Are there potential benefits, such as beneficial food components, for less sensitive populations that would be adversely affected by a health-based guidance value that is based on the most sensitive critical health outcome?

In deciding on the applicability of a health-based guidance value, it should also be considered whether there are particular subpopulations that may be at risk because they are allergic or intolerant to a substance that may be present in food. Examples include the need for individuals with phenylketonuria to avoid sources of phenylalanine, such as the artificial sweetener aspartame, or individuals with hereditary intolerance to fructose, sucrose and sorbitol who should also avoid D-tagatose (FAO/WHO, 2005).

Very young infants are a particularly sensitive subgroup because their metabolic capacities are not yet fully developed. It should be

noted that health-based guidance values are not considered applicable to infants under the age of 12 weeks who might be at risk at lower levels of exposure. Accordingly, risk characterization of exposure of such infants to chemicals (e.g. in infant formula or occurring as contaminants) has to be considered on a case-by-case basis. This is in accordance with similar advice in EHC 70 (IPCS, 1987), where the scientific rationale for this conclusion was originally set out. EHC 237, which provides a systematic analysis of the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct stages of development, is a useful reference in this regard (IPCS, 2006).

7.6 References[1]

Barlow S, Renwick AG, Kleiner J, Bridges JW, Busk L, Dybing E, Edler L, Eisenbrand G, Fink-Gremmels J, Knaap A, Kroes R, Liem D, Müller DJG, Page S, Rolland V, Schlatter J, Tritscher A, Tueting W & Würtzen G (2006) Risk assessment of substances that are both genotoxic and carcinogenic—Report of an international conference organized by EFSA and WHO with support of ILSI Europe. Food Chem Toxicol, **44**: 1636–1650.

Carrington CD, Montwill B & Bolger PM (2004) An intervention analysis for the reduction of exposure to methylmercury from the consumption of seafood by women of child-bearing age. Regul Pharmacol Toxicol, **40**: 272–280.

Cullen AC & Frey HC (1999) Probabilistic techniques in exposure assessment. New York, NY, Plenum Press.

EFSA (2005a) Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic (Request No. EFSA-Q-2004-020). Adopted on 18 October 2005. EFSA J, **282**: 1–31 (http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_ej282_gentox_en3,0.pdf).

EFSA (2005b) Opinion of the Scientific Committee on a request from EFSA related to exposure assessments (Request No. EFSA-Q-2003–107). Adopted on 22 June 2005. EFSA J, **249**: 1–26 (http://www.efsa.europa.eu/en/efsajournal/doc/sc_op_ej249_exposure_en2.pdf).

FAO/WHO (1999) Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 884; http://whqlibdoc.who.int/trs/WHO_TRS_884.pdf).

[1] Internet links provided in these references were active as of the date of final editing.

FAO/WHO (2000) Evaluation of certain food contaminants. Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 896; http://whqlibdoc.who.int/trs/WHO_TRS_896.pdf).

FAO/WHO (2002) Evaluation of certain food contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 909; http://whqlibdoc.who.int/trs/WHO_TRS_909.pdf).

FAO/WHO (2005) Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 928; http://whqlibdoc.who.int/trs/WHO_TRS_928.pdf).

FAO/WHO (2006a) Evaluation of certain food contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 930; http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf).

FAO/WHO (2006b) Pesticide residues in food—2006. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper, No. 187; http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2006_rep/report2006jmpr.pdf).

FAO/WHO (2007a) Evaluation of certain food additives and contaminants. Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 940; http://whqlibdoc.who.int/trs/WHO_TRS_940_eng.pdf).

FAO/WHO (2007b) Evaluation of certain food additives and contaminants. Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, World Health Organization (WHO Technical Report Series, No. 947; http://whqlibdoc.who.int/publications/2007/9789241209472_eng.pdf).

FAO/WHO (2008) Codex Alimentarius Commission procedural manual, 18th ed. Rome, Food and Agriculture Organization of the United Nations, Codex Alimentarius Commission (ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_18e.pdf).

Feron VJ & Groten JP (2002) Toxicological evaluation of chemical mixtures. Food Chem Toxicol, **40**: 825–839.

Feron VJ, Cassee FR, Groten JP, Van Vliet PW & Van Zorge JA (2002) International issues on human health effects of exposure to chemical mixtures. Environ Health Perspect, **110**(Suppl 6): 893–899.

Frey HC & Patil SR (2002) Identification and review of sensitivity analysis methods. Risk Anal, **22**: 553–578.

FSA (2002) Risk assessment of mixtures of pesticides and similar substances. London, United Kingdom Food Standards Agency, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (http://www.food.gov.uk/multimedia/pdfs/reportindexed.pdf).

Groten JP, Butler W, Feron VJ, Kozianowski G, Renwick AG & Walker R (2000) An analysis of the possibility for health implications of joint actions and interactions between food additives. Regul Toxicol Pharmacol, **31**: 77–91.

Groten JP, Feron VJ, Sühnel J (2001) Toxicology of simple and complex mixtures. Trends Pharmacol Sci, **22**: 316–322.

Hacking I (1976) The emergence of probability. Cambridge, Cambridge University Press.

Humphreys SH, Carrington CD & Bolger PM (2001) A quantitative risk assessment for fumonisins B1 and B2 in corn. Food Addit Contam, **18**: 211–220.

IPCS (1987) Principles for the safety assessment of food additives and contaminants in food. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 70; http://www.inchem.org/documents/ehc/ehc/ehc70.htm).

IPCS (2006) Principles for evaluating health risks in children associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 237; http://www.inchem.org/documents/ehc/ehc/ehc237.pdf).

IPCS (2008) Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. In: Uncertainty and data quality in exposure assessment. Geneva, World Health Organization, International Programme on Chemical Safety (Harmonization Project Document, No. 6; http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf).

IPCS (2009a) Principles for modelling dose–response for the risk assessment of chemicals. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria, No. 239; http://whqlibdoc.who.int/publications/2009/9789241572392_eng.pdf).

IPCS (2009b) Risk assessment of combined exposures to multiple chemicals: a WHO/IPCS framework (Harmonization Project Draft Document for Public and Peer Review). Geneva, World Health Organization, International Programme on Chemical Safety (http://www.who.int/ipcs/methods/harmonization/areas/combinedexposure.pdf).

Ito N, Hasegawa R, Imaida K, Kurata Y, Hagiwara A & Shirai T (1995a) Effect of ingestion of 20 pesticides in combination at acceptable daily intake levels on rat liver carcinogenesis. Food Chem Toxicol, **33**: 159–163.

Ito N, Hagiwara A, Tamano S, Hasegawa R, Imaida K, Hirose M & Shirai T (1995b) Lack of carcinogenicity of pesticide mixtures administered in the

diet at acceptable daily intake (ADI) dose levels in rats. Toxicol Lett, **82–83**: 513–520.

Jonker D, Woutersen RA & Feron VJ (1996) Toxicity of mixtures of nephrotoxicants with similar or dissimilar mode of action. Food Chem Toxicol, **34**: 1075–1082.

Jonker D, Freidig AP, Groten JP, de Hollander AE, Stierum RH, Woutersen RA & Feron VJ (2004) Safety evaluation of chemical mixtures and combinations of chemical and non-chemical stressors. Rev Environ Health, **19**: 83–139.

Krishnan K, Paterson J & Williams DT (1997) Health risk assessment of drinking water contaminants in Canada: the applicability of mixture risk assessment methods. Regul Toxicol Pharmacol, **26**: 179–187.

Mileson B, Faustman E, Olin S, Ryan PB, Ferenc S & Burke T eds (1999) A framework for cumulative risk assessment. Washington, DC, ILSI Press.

O'Brien J, Renwick AG, Constable A, Dybing E, Müller DJG, Schlatter J, Slob W, Tueting W, Van Benthem J, Williams GM & Wolfreys A (2006) Approaches to the risk assessment of genotoxic carcinogens in food: a critical appraisal. Food Chem Toxicol, **44**: 1613–1635.

Pesticide Residues Committee (2007) Pesticide residues monitoring report. Second quarter report 2007. Quarter ended June 2007. York, Pesticide Residues Committee, pp 46–47 (http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PRC/Report_Q2_2007_13_Dec.pdf).

Renwick AG, Barlow SM, Hertz-Picciotto I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Grieg JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher A, Tuijtelaars S, Van den Brandt PA, Walker R & Kroes R (2003) Risk characterization of chemicals in food and diet. Food Chem Toxicol, **41**: 1211–1271.

Rescher N (1993) Probability logic: a non-truth-functional system. In: Many-valued logic, 2nd ed. Aldershot, Gregg Revivals, pp 184–187.

Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, Van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F & Zacharewski T (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect, **106**: 775–792.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N & Peterson RE (2006) The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci, **93**: 223–241.