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Principles and Methods for the Risk Assessment of Chemicals in Food

SUMMARY (English)

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

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organization or the World Health Organization.

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SUMMARY

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) follow the same general principles and methods for chemical risk assessments, which are published in the reports of both committees. In response to recommendations made by JECFA and JMPR in the 1980s to review the validity of the evaluation procedures then in place, the International Programme on Chemical Safety (IPCS) sponsored the preparation of Environmental Health Criteria monographs (EHCs) on Principles for the Safety Assessment of Food Additives and Contaminants in Food (EHC 70) and Principles for the Toxicological Assessment of Pesticide Residues in Food (EHC 104). These monographs and the principles laid out in subsequent reports have served as the basis for the assessments that have been performed by JECFA and JMPR.

Although much of the guidance set out in EHC 70 and EHC 104 remains valid, there have been significant advances in chemical analysis, toxicology, dietary exposure assessment and risk assessment approaches for chemicals in food since these monographs were prepared. Accordingly, FAO and WHO initiated a project to update, harmonize and consolidate principles and methods used by JECFA and JMPR for the risk assessment of food additives, food contaminants, natural toxicants and residues of pesticides and veterinary drugs. This monograph is the outcome of that project.

The purpose of this monograph is 2-fold: 1) to provide descriptive guidance for JECFA and JMPR to ensure the continuation of transparent and sound expert evaluations of scientific data for risk assessments of chemicals in food; and 2) to be informative for users of the outputs from JECFA and JMPR, such as risk managers and other risk assessment bodies in Member countries and authorities.

The monograph addresses the key issues considered by JECFA and JMPR in their food chemical risk assessments, as summarized below.

Risk assessment and its role in risk analysis

Risk analysis consists of three components: risk assessment, risk management and risk communication. Risk assessment is the central component of risk analysis and provides a scientific basis for risk management decisions on measures that may be needed to protect human health. It takes into account all available relevant scientific data and identifies any uncertainties in the knowledge base. Risk assessment comprises the four steps of hazard identification, hazard characterization (including dose–response assessment), exposure assessment and risk characterization. It is a conceptual framework that, in the context of food chemical safety, provides a mechanism for the structured review of information relevant to assessing possible health outcomes in relation to exposures to chemicals present in food.

Risk assessment of chemical substances present in or on food forms the core work of JECFA and JMPR. Based on the advice from these two committees, food safety measures are taken in the risk management executed by countries nationally and by the Codex Alimentarius Commission (CAC) internationally. Whereas JECFA and JMPR base their evaluations on scientific principles and ensure necessary consistency in their risk assessment determinations, CAC and its respective committees that deal with chemicals in food are responsible, as risk managers, for the final decisions on establishing maximum limits for pesticide residues, veterinary drug residues, contaminants and additives in food and adopting other related measures.

Although it is desirable to separate the functional activities of risk assessment from those of risk management in order to ensure scientific independence, it is acknowledged that risk managers should communicate and interact with risk assessors during the process to establish the scope of the analysis, particularly during problem formulation. Thus, the relationship between risk assessment and risk management is an interactive, often iterative, process.

Chemical characterization, analytical methods and the development of specifications

This section of the monograph describes the chemical information that is required for risk assessment. Such information is also a prerequisite for surveillance and control of chemical substances in food.

Proposed analytical methods are reviewed by JECFA and JMPR for their suitability for international use. Analytical methods are necessary, for example, for the speciation of contaminants, for determination of the concentrations of a chemical and its metabolites in pharmacokinetic, toxicokinetic and residue depletion studies, and for the reliable determination of the concentrations of contaminants and of incurred residues of veterinary drugs and pesticides in foods. The monograph describes the key features of suitable analytical methods and the validation criteria for such methods.

Food additive specifications

Specifications of identity and purity are necessary products of JECFA safety assessments for food additives. Evaluations of food additives by JECFA depend on studies performed with a chemical substance or product of defined identity, purity and physical form. The safety assessment is valid only for products that do not differ significantly in identity and quality profile from the material used to generate the data used in the evaluation.

Pesticides

The Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) establishes specifications for technical-grade material and formulations. JMPR takes the JMPS specifications into account during the safety assessment. JMPR evaluates the analytical methods used for generation of residue data to check that the methods are suitable for the relevant analytes and sample types. JMPR also reports information on methods that are suitable for enforcement of maximum residue limits (MRLs) and whether particular compounds are suitable for analysis by multiresidue methods.

Veterinary drug residues

JECFA must be assured that any veterinary drug it evaluates is well characterized, with details of its chemical and physical properties and the identity and concentrations of any major impurities. In addition, the manufacturing process should be described and the consistency and quality of the final products demonstrated.

The form and the distribution of the residues that result from each authorized mode of application in each species should be determined,

and the depletion of the residues from edible tissues or animal-derived foods should be studied. A marker residue should be identified, which is usually the form of the drug (parent compound or metabolite) that is found at the highest concentration for the longest period. The relationship of this marker residue to the total residue of the drug is determined.

Contaminants

The data required for the characterization of a contaminant should include its concentrations in foods and the total diet from as many countries as possible. Data should be formatted using the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) to facilitate the collation and quality control of the data. The data should be accompanied by additional details on sampling plans and analytical methods used to generate the data.

Substances consumed in large amounts

Thorough chemical analyses should be performed on high-consumption substances, such as bulk additives, to identify potential impurities and to provide information on nutritional adequacy, especially when such substances replace traditional food. Because exposure to undesirable impurities (e.g. heavy metals) concomitant with the intake of high-consumption materials is potentially high, special effort should be made to identify and quantify such impurities.

Hazard identification and characterization: toxicological and human studies

Scope and choice of test methods

Toxicological studies may be broadly divided into 1) in vitro studies, using cultured organisms or cells or tissue preparations from laboratory animals or humans; and 2) in vivo studies in laboratory animals or humans. Such studies serve a number of purposes, including the identification of potential adverse effects (hazard identification), definition of the exposure conditions necessary to produce the effects and the assessment of dose–response relationships for the adverse effects (hazard characterization). JECFA and JMPR consider data from both types of study in their risk assessments.

It is widely accepted that animal testing should be reduced, refined or replaced as far as is practicable, and this has led to an increased use of alternative approaches and to improved study designs. It is equally important that scientifically sound methods and approaches are used for the safety testing of food chemicals. Hence, although advances are being made in the development of in silico and in vitro approaches, at the present time these do not permit the replacement of animal testing for most end-points of concern. Although no experimental species is an ideal model for humans, there is evidence that studies in animals generally provide an effective means for evaluating the potential toxicity of substances in food, provided that the data are interpreted critically.

Several internationally recognized organizations, such as the Organisation for Economic Co-operation and Development (OECD), provide guidance on minimum standards for the design and conduct of toxicological studies. All studies used in the risk assessment of a substance in food are assessed for adequacy of design and conduct and should preferably be conducted according to the principles of Good Laboratory Practice. The monograph also discusses promising recent developments in testing protocols that have not yet been formally accepted by OECD.

Study of the absorption, distribution, metabolism and excretion (ADME) of a substance at an early stage of testing is important in aiding the selection of appropriate test species and test doses for toxicity studies. Where possible, investigation of any qualitative or quantitative differences in ADME between the test species and humans will provide important information for characterization of the hazard.

The extent of toxicological testing required depends on the nature and use of the substance under consideration. Not all of the tests discussed in the monograph will necessarily need to be conducted in order to reach a conclusion on the risk assessment for a particular substance. Tiered testing approaches are also discussed in which screening tests or a limited number of standard toxicity studies are conducted, which may be sufficient for risk assessment or may trigger necessary further investigations.

Short-term and long-term tests for general systemic toxicity are usually conducted. These identify target organs for toxicity and may

indicate the need for additional or more specific testing (e.g. for neurotoxicity or immunotoxicity). The effects of the test substance on a wide range of end-points indicative of toxicity, including observational, functional, biochemical and pathological end-points, are examined. Studies are typically conducted in two species, either a rodent and a non-rodent species or two rodent species, and in both sexes, to maximize the opportunity to find any effects (hazard identification). Long-term testing often also includes carcinogenicity testing in two rodent species. The use of an alternative method in place of one rodent species may be acceptable on a case-by-case basis; a variety of alternative tests for carcinogenicity have been introduced in which tumorigenic responses are enhanced and the duration of bioassays is thereby reduced, including initiation/promotion models, the neonatal mouse model and transgenic mouse models.

Testing should be conducted in a manner that best relates to human exposure scenarios. Dose selection should take into account the anticipated human exposure, the frequency of exposure and the duration of exposure. For substances present in foods, administration of the substance in repeated-dose animal studies is usually by diet, gavage or drinking-water. Ideally, the dose levels selected are such that toxic effects, but not death or severe suffering, are produced at the highest dose level, with lower dose levels producing graded responses and no adverse effects at the lowest dose level. The study design should be adequate to determine a reference point for hazard characterization, also known as a point of departure (POD), such as a no-observed-adverse-effect level (NOAEL) or a benchmark dose (BMD), which is a dose producing a low but measurable adverse response.

For all study designs, careful consideration needs to be given to dose spacing and number of study groups, maximum dose utilized, number of animals per sex in each dose group, choice of controls, dosing regimen, confirmation of dose administered compared with nominal dose, and dose ingested (e.g. palatability, wastage of food).

In addition to tests for general systemic toxicity, the potential genotoxicity of a substance should be evaluated using a range of appropriate in vitro and, if necessary, in vivo tests. For comprehensive coverage of the potential genotoxicity of a substance, information on

the ability to induce gene mutations, structural chromosomal aberrations and aneuploidy is required. A small number of well-validated in vitro assays are usually selected to cover the different genetic endpoints. Commonly used test batteries include a gene mutation test in bacteria (i.e. the *Salmonella*/microsome assay) and one or two tests in mammalian cells detecting point mutations or chromosome damage (clastogenicity/aneugenicity).

Effects of the substance on reproductive performance of both males and females and on the prenatal and postnatal development of offspring are also usually determined. The purpose of reproductive and developmental toxicity studies is to assess 1) possible effects that may be expressed through reduced fertility or fecundity in either the parents or offspring as a result of morphological, biochemical, genetic or physiological disturbances and 2) whether there is normal growth and development of the offspring. However, tests for reproductive and developmental toxicity do not necessarily cover the full range of effects that might be induced by chemicals that interfere with the endocrine system. Development of a battery of screening tests that can evaluate chemicals that interact with the estrogen, androgen and thyroid signalling pathways is still ongoing at the time of the publication of this monograph.

There should also be consideration of the need for acute toxicity testing. Some substances (e.g. certain metals, mycotoxins, veterinary drug residues, pesticide residues) could give rise to acute health effects in relation to short periods of intake. JECFA includes in its evaluations an assessment of acute effects and, where appropriate, the possibility of acute effects in sensitive individuals. JMPR also now routinely considers the need to set an acute reference dose (ARfD) for all pesticides it evaluates. JMPR has developed guidance for a single-dose study in experimental animals, with the aim of enabling more accurate derivation of ARfDs; this guidance serves as the basis for an OECD test guideline currently under development.

Additional testing may also be required for nutritional effects, neurotoxicity, including neurobehavioural effects both in adults and during development, and immunotoxicity. The need for such additional testing may be evident from the results of the standard tests described above. Specific studies on mechanism of toxicity or mode of action may provide additional useful data for the evaluation.

Interpretation of findings

Critical evaluation of study designs and their findings and interpretation of the results are the most important steps in risk assessment. The findings from treated groups are usually compared with those from concurrent controls. Comparison of test data with data from historical controls, particularly in the case of carcinogenicity and developmental toxicity, may also be necessary to understand the significance of a particular finding.

For the assessment of many toxicological end-points, a weight of evidence approach is necessary, utilizing the data from all the available studies in which the same or functionally related fluids, cells, tissues or organs have been studied. Similar findings across different studies and evidence of dose–response relationships give added weight to the hazard characterization.

Determination of whether or not a compound is genotoxic should be based on an overall assessment of the available data. Completely negative results in an in vitro test battery are normally considered sufficient to conclude that a substance is devoid of genotoxic potential, unless there are reasons for special concern (e.g. high or sustained human exposure, structural considerations). Conversely, one or more positive in vitro tests normally require follow-up by in vivo genotoxicity testing. The outcome of the genotoxicity tests may then be considered alongside experimental results from rodent carcinogenicity bioassays, as the results of short-term tests alone do not provide a reliable prediction of whether or not a chemical is a carcinogen in rodents. Positive genotoxicity studies do provide knowledge about mode of action for substances that are carcinogenic and influence the approach used in the subsequent risk characterization. Positive findings in rodent cancer bioassays require careful interpretation in relation to mode of action, possible interspecies differences in background incidence and in response, and the issue of high dose to low dose extrapolation. IPCS has developed a conceptual framework on the evaluation of the mode of action for chemical carcinogenesis in animal test species, which was subsequently extended to address the issue of human relevance of animal cancer data. Mechanisms relevant to humans include deoxyribonucleic acid reactivity or genotoxicity. Some mechanisms were identified not to be relevant to humans,

including α2u-microglobulin-induced rat nephropathy and peroxisome proliferation.

In interpreting data from reproductive and developmental toxicity studies, it is important to look for biologically related patterns of response and the relationship of outcomes across end-points and to relate any findings to the toxicological data available from other studies. As standard study designs require that the top dose exerts some minimal indication of maternal toxicity, it may be difficult to assess whether a developmental effect seen at such a dose is a direct result of the action of the chemical on the embryo or fetus or an indirect result of altered maternal homeostasis. Although there have been several examples of the latter, it is important not to infer causation from an association of developmental toxicity with maternal toxicity without additional testing and evaluation.

Food allergy and other food hypersensitivities

Food allergies are a consequence of the undesired or uncontrolled immune response to a food antigen in susceptible individuals. They are based on the body's aberrant interpretation of certain dietary proteins as "foreign", which leads to a heightened response of the immune system. Allergy develops through the process of sensitization. During the sensitization phase, exposure to the food allergen stimulates production of antigen-specific immunoglobulin E.

Food allergy risk assessment is a relatively new discipline, and there is no general consensus on how it should be conducted, although several approaches have been suggested. For example, there is no current consensus regarding a threshold dose below which sensitization to food allergens would not occur. To predict the potential allergenicity of novel food proteins, such as in genetically modified foods, decision tree strategy approaches have been described.

General principles of studies in humans

Data from human studies are of potential importance in identifying and characterizing hazards and evaluating the risks of food additives, contaminants and residues of veterinary drugs and pesticides. The information may come from controlled experiments in human

volunteers, surveillance studies, epidemiological studies (e.g. eco-logical studies, case–control studies, cohort studies, analytical or intervention studies) of populations with different levels of exposure, experimental or epidemiological studies in specific subgroups of peo-ple, or clinical reports (e.g. poisoning) or case-studies of individuals. End-points may include examination of safety or tolerance, nutritional and functional effects of foods or food components, the metabolism and toxicokinetics of the substance, mode of action, possibly using biomarkers for effects identified in animal studies, and adverse health effects from unintentional exposures (e.g. to a contaminant).

Critical issues for any experimental study in humans are the ethical, professional and basic legal controls that govern whether a study in humans is necessary and the circumstances under which it may be properly performed. The numbers of subjects entered into a study should be sufficient to realize the aims of the investigation. Consideration needs to be given to when the use of human tissues ex vivo or in vitro might be sufficient. Experiments on human cells or tissues or using other preparations containing or expressing human enzymes, receptors and other subcellular factors in vitro are funda-mentally different from studies in people, because they do not take account of absorption, distribution, aspects of integrated metabolism and excretion. However, an advantage is that they permit mechanistic studies under controlled conditions not feasible in the clinic, and these techniques are of considerable value in suggesting metabolic pathways and response mechanisms that may be important in humans and may be worth studying as biomarkers of exposure or effect.

Gastrointestinal tract considerations, including effects on the gut microflora

Interactions that may occur between chemicals in food and the bac-terial flora of the gastrointestinal tract should be considered in terms of both the effects of the gut microflora on the chemical and the effects of the chemical on the gut microflora.

In vivo methods for studying the role of the gut microflora in the metabolism of a substance include 1) parenteral administration of the compound, which should result in decreased microbial metabolism of poorly absorbed polar compounds, compared with oral dosing; 2) studies on animals in which the bacterial flora are reduced by the use

of antibiotics; and 3) studies on germ-free animals and on (formerly) germ-free animals inoculated with known strains of bacteria (gnotobiotic animals). A number of factors may influence the metabolic activation of foreign chemicals by the host microflora, including host species, diet, medication and metabolic adaptation. In addition, various in vitro and in vivo methods exist to test the potential of a substance to induce resistance in the gut microflora as a result of ingesting substances or residues with antimicrobial properties.

Dose–response assessment

Dose–response assessment is a major part of the hazard characterization within the risk assessment paradigm. Dose–response assessment is used to develop risk assessment advice and to derive health-based guidance values.

Approaches generally take one of two forms: 1) analyses that provide a quantitative or qualitative estimation of risk; and 2) analyses that establish health-based guidance values, such as an acceptable daily intake (ADI) or tolerable daily intake (TDI), which are levels of human exposure considered to be "without appreciable health risk". The TDI is used for contaminants, whereas the ADI is used in cases where exposure can be controlled, such as for food additives and residues of pesticides and veterinary drugs in foods. The approaches to dose–response assessment applied to data from studies in animals have been discussed in EHC 239 on Principles for Modelling Dose–Response for the Risk Assessment of Chemicals.

One of the primary components of a risk assessment is determination of the presence or absence of a cause–effect relationship. If there is sufficient plausibility for the presence of such a relationship, then dose–response data are essential. Dose–response data may be derived from in vivo studies in laboratory animals or humans, which usually provide the basis for risk characterization. In each case, interpretation of the data on effects usually requires recognition of the levels of exposure that do not produce a measurable effect and the relationship between the increase in incidence, severity or nature of the effect with increase in exposure.

Dose–response modelling can be described by six basic steps. The first four steps (data selection, model selection, statistical linkage and

parameter estimation) relate to the analysis of the dose–response data. In this analysis, the observed dose–response data are modelled in a way that allows prediction of the likely magnitude of the response at a given dose, either within or outside the observed dose–response range, or prediction of the likely dose causing a given magnitude of response. The last two steps deal with implementation and evaluation of the results of the analysis.

Extrapolation is a necessary part of all risk assessments. In most cases considered by JECFA and JMPR, the data used for dose–response assessment come from experiments in laboratory animals administered doses significantly exceeding the potential human exposure. For such dose–response analyses, there are two issues of extrapolation: 1) extrapolating from the test species to humans; and 2) allowing for possible human differences in response. The methods employed for these extrapolation issues are discussed in the monograph and are varied, ranging from the use of uncertainty factors to more complicated modelling schemes based upon differences in toxicokinetics and toxicodynamics between humans and experimental animals and variability between different human individuals.

Derivation of health-based guidance values

The setting of health-based guidance values provides quantitative information from risk assessment, enabling risk managers to make decisions concerning the protection of human health. Health-based guidance values are derived from the dose–response assessment for the most relevant end-point in the most relevant species. The first approach, which is the one still most commonly used by JECFA and JMPR to derive health-based guidance values in order to protect against effects considered to have a threshold, is to define the NOAEL or sometimes a lowest-observed-adverse-effect level (LOAEL) as the POD. The other approaches that have been used by JECFA and JMPR are to use the lower one-sided confidence limit of the BMD (the BMDL) as the POD for the derivation of a health-based guidance value or for calculation of a margin of exposure (MOE). Dose–response assessment is occasionally used to define the dose associated with a negligible (e.g. 1 in a million) increased response over background.

For food additives and for residues of pesticides and veterinary drugs in food, the health-based guidance value is termed the ADI. JECFA and

JMPR determine ADIs based on all the known facts at the time of the evaluation. JECFA generally sets ADIs on the basis of the lowest relevant NOAEL in the most sensitive species. The ADI is expressed in amount (e.g. mg) per kilogram of body weight, usually as a range from 0 to an upper limit. ADIs are normally expressed numerically using only one significant figure. When appropriate, JMPR and JECFA develop ARfDs, an estimate of the amount of a substance in food and/or drinking-water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less, without appreciable health risk to the consumer, on the basis of all the known facts at the time of the evaluation.

For food contaminants that are generally unavoidable, JECFA has used the term "tolerable" for health-based guidance values, as it signifies permissibility for the intake of contaminants associated with the consumption of otherwise wholesome and nutritious food. Principles in deriving tolerable intake levels are the same as for ADIs: either the NOAEL or BMD approaches can be used as the POD to set health-based guidance values for contaminants. Food contaminants include heavy metals, environmental contaminants such as dioxins and mycotoxins, impurities arising in food additives, solvents used in food processing, other substances arising from food processes such as heating, substances migrating from food contact materials and residues arising from the use of animal feed additives or the non-active components of veterinary drug formulations. Guidance values may be expressed as a TDI, provisional maximum tolerable daily intake (PMTDI), provisional tolerable weekly intake (PTWI) or provisional tolerable monthly intake (PTMI). The use of the term "provisional" expresses the tentative nature of the evaluation, when there is a paucity of reliable data on the consequences of human exposure at levels approaching those with which JECFA is concerned. PMTDIs are established for food contaminants that are known not to accumulate in the body. For contaminants that may accumulate within the body over a period of time, JECFA has used the PTWI and PTMI.

The critical steps in the NOAEL approach to deriving health-based guidance values are selection of the appropriate data and determination of the NOAEL. In calculating the health-based guidance value, a safety or uncertainty factor is applied to the NOAEL to provide a conservative margin of safety because of the inherent uncertainties in extrapolating toxicity data from experimental animals to potential

effects in humans as well as variation within the human species. The terms "safety factor" and "uncertainty factor" are often used interchangeably, "safety factor" having been used historically, but the preference now is to use "uncertainty factor". The concept of chemical-specific adjustment factors has been introduced to allow the use of specific data on species differences or human variability in either toxicokinetics or toxicodynamics to derive data-driven uncertainty factors instead of the use of default factors, where possible.

The BMD approach has been introduced as an alternative to the NOAEL approach. This method defines a level of exposure producing a low but measurable effect size or level of response as the POD for risk assessment. The BMD method has a number of advantages, including the use of the full dose–response data in the statistical analysis, which allows quantification of the uncertainty in the data. Higher uncertainty in the data—for example, due to small group sizes or high variation within a group—would be reflected in lower health-based guidance values.

There are occasions when JECFA and JMPR consider the setting of an ADI in numerical terms not to be appropriate, such as when the estimated consumption of the additive is expected to be well below any numerical value that would ordinarily be assigned to it. Under such circumstances, the term ADI "not specified" is used.

There may be situations where either the body of available data on a substance is limited on some aspects or the safety of a chemical for which JECFA or JMPR had previously assigned an ADI was brought into question by new data. When JECFA or JMPR feels confident that the use of the substance is safe over the relatively short period of time required to generate and evaluate further safety data, but is not confident that its use is safe over a lifetime, it often establishes a "temporary" ADI, pending the submission of appropriate data to resolve the safety issue within a defined time-line.

For veterinary drugs and pesticides, the ADI is used to confirm the safety of proposed MRLs when the substances are applied in accordance with good practices. In establishing the ADI for a veterinary drug or a pesticide residue, the toxicities of the parent drug and of its main metabolites are considered, and the ADI is based on the toxicological end-point of the compound of most concern.

If a veterinary drug can affect the human gut microflora at exposures lower than those causing toxicological effects, then this end-point is used as the basis for establishing the ADI. An internationally harmonized decision tree approach, for which the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) has developed a guideline, is used to determine the need to establish a microbiological ADI. The first three steps consider whether 1) residues of the drug and/or its metabolites are microbiologically active against representatives of the human intestinal flora, 2) residues enter the human colon and 3) the residues entering the human colon remain microbiologically active. If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are considered: 1) disruption of the colonization barrier and 2) increase of the populations of resistant bacteria.

If several substances that produce similar toxic effects or share a common toxic metabolite are to be considered for use as food additives, pesticides or veterinary drugs or occur as contaminants, it may be appropriate in establishing a health-based guidance value to consider the substances as a group in order to limit their overall intake. For this procedure to be feasible, the substances should have a similar mode of action and a similar range of toxic potency.

It is preferable to set health-based guidance values 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. 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. 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, advice regarding a second (higher) value based on another end-point relevant to the rest of the population may be provided.

Dietary exposure assessment of chemicals in food

In the assessment of dietary exposure to chemicals, food consumption data are combined with data on the concentration of chemicals in

food. The resulting dietary exposure estimate may then be compared with the relevant health-based guidance value or with the toxicological POD (NOAEL; BMDL) for the food chemical of concern as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures. Dietary exposure assessments should cover the general population, as well as critical groups that are vulnerable or are expected to have exposures that are significantly different from those of the general population (e.g. infants, children, pregnant women, elderly, vegetarians).

In principle, dietary exposure assessments need to be performed for all identified chemicals present in the diet that are subject to risk assessment. Similar methods are appropriate for contaminants, pesticide and veterinary drug residues, food additives (including flavourings), processing aids and other chemicals in foods. A stepwise approach is recommended, in which screening methods can be applied to identify, among the large number of chemicals that may be present, those of no safety concern, using minimal resources in the shortest possible time. A refined exposure assessment is not needed for such substances. Further steps to allow the refinement of the dietary exposure assessment should be designed in such a way that potential high dietary exposure to a specific chemical is not underestimated.

Sources of information on concentrations of chemicals in food include proposed maximum levels (MLs) or MRLs, proposed manufacturers' use levels, monitoring and surveillance data, total diet studies (TDSs), the GEMS/Food database, veterinary drug residue depletion studies, highest and mean residues from supervised trials for pesticides, and the scientific literature. The most accurate data are obtained from the measurement of chemical concentrations in foods as consumed. Programmes to generate data on concentrations of chemicals in food require validated sampling plans and analytical methods. There are two main approaches to analysing foods when generating analytical data from surveys: 1) analysis of food group composites; and 2) analysis of individual foods (either as single samples or as composites).

Food consumption information can be obtained from food balance sheet data, which include the amounts of foods available for human consumption derived from national statistics on food production,

disappearance or utilization. They are generally available for most countries. The GEMS/Food consumption cluster diets developed by WHO are based on selected FAO food balance sheets and represent average per capita food consumption. The consumption cluster diets replace the five regional diets previously developed by WHO.

Food consumption data should be available in a format that allows matching of the consumption data with the concentration data used in the dietary exposure assessment. Data collected using population-based methods are generally compiled and reported for raw or semi-processed agricultural commodities, and they represent the total annual amount of a commodity available for domestic consumption per year. Data from individual food consumption surveys are often not publicly available in raw format (i.e. at the individual respondent level), and risk assessors have to rely on published summary statistics. Market share corrections can be applied to food consumption data for processed foods or percentage of treated crops. The approach is used mainly when the substance being evaluated has been deliberately added to the food.

The available methods for estimating dietary exposure have been divided into those that provide single (point) estimates and those that characterize the full distribution of consumer exposures. Point estimates include 1) screening methods, 2) exposure methods that rely on crude estimates of consumption, such as the theoretical added maximum daily intake (TAMDI) and other model diets, and 3) more refined exposure methods based on actual consumption data and chemical concentration data, such as TDSs, selective studies of individual foods and duplicate portion diets. A deterministic or point estimate of dietary exposure is simply a single value that describes some parameter of consumer exposure (e.g. the average exposure of a population). Characterizing the full distribution of consumer exposures is the most resource-intensive assessment, as data are required that characterize the range of food consumption practices as well as the range of chemical concentrations in the foods that are eaten. The extent to which estimates of dietary exposure need to be refined will depend, in part, on the nature of the substance and the toxicity profile.

Screening methods overestimate dietary exposure of high consumers using conservative assumptions in terms of food consumption and

chemical concentrations. Their aim is not to assess true dietary exposure but to identify food chemicals for which a more comprehensive dietary exposure assessment is necessary. Screening methods include poundage data (for food additives, including flavours), the budget method (which has been used to assess the theoretical maximum daily dietary exposure to some food additives) and model diets (which are constructed from available information on food consumption and are designed to represent a typical diet for the population whose exposure is to be considered).

Point estimate modelling may also be appropriate as a second step in a tiered approach. The model selected can be more or less conservative, depending upon the purpose and the available information. Model diets for high consumers can be developed on the basis of published data from food consumption surveys as an alternative to the budget method or as an additional step in the screening process. Food consumption amounts and dietary exposures for high consumers can also be derived from distributional data. The tendency of consumers to repeatedly purchase and consume the same food products, sometimes termed consumer loyalty, may need to be considered and a range of concentrations may need to be used to generate dietary exposure estimates to cover various scenarios of consumer behaviour.

For substances requiring further refinement beyond screening methods or point estimates of exposure, a probabilistic analysis of exposure variability can be conducted. Approaches to developing probabilistic models for dietary exposure assessments include simple empirical distribution estimate, developing probabilistic models from data sets, stratified sampling, random sampling (Monte Carlo simulation) and Latin hypercube.

For a probabilistic exposure assessment, the readily available distributions of food consumption data are from short-term studies and are not representative of true long-term consumption. Approaches that have been used to estimate long-term consumption have included methods combining food frequency data with information on amounts consumed and statistical models that use the correlations among the days of consumption to estimate the "usual" intake of the substance under consideration.

Exposures to food chemicals through other routes may occur, and exposures to chemicals or drugs sharing the same mechanism of action (toxicity) may also be encountered. Consideration of combined exposures to a single chemical across multiple routes (oral, dermal, inhalation) and across multiple pathways (food, drinking-water, residential) is known as aggregate exposure. Consideration should also be given to the assessment of risks from exposure to multiple pesticide residues that have a common mechanism of toxicity, and the exposure estimate for that situation is termed cumulative exposure. Guidance for estimating aggregate exposure has been issued.

Risk characterization

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. Historically, different approaches have been used for the risk characterization of toxic effects considered to have a threshold for the observed adverse effect and those considered to have no threshold. Health-based guidance values are set by JECFA and JMPR for substances that produce effects exhibiting a threshold. In the risk characterization for these types of substances, the health-based guidance values are compared with estimated or measured human exposure.

In cases where exposures exceed health-based guidance values, the values themselves do not provide risk managers 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. 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.

In circumstances where the data are not sufficient to propose a health-based guidance value for a substance or the mode of action cannot be assumed to exhibit a threshold, JECFA and JMPR may comment on the MOE between the doses at which effects are seen in animals and the estimated human dietary exposure.

Risk characterization should include consideration and description of uncertainty and variability. 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. 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. The risk characterization should include a narrative evaluation of uncertainty for both exposure and health effects. Sensitivity analysis refers to quantitative techniques that may be used to identify those aspects of the inputs (e.g. concentration or food consumption data) that contribute the greatest extent to the uncertainty.

There is an increasing awareness by those involved in risk assessment of the need to consider any risks associated with combined exposure to mixtures of substances. There are four types of combined effect or interaction: dose addition, response addition, synergism and antagonism. 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. 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 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 TDI for closely related contaminants that occur as mixtures. An approach that takes account of dose additivity is the toxic equivalency factor (TEF) approach, which scales the exposure for each component of a mixture relative to the potency of an index chemical (e.g. for dioxins and dioxin-like chemicals).

For 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

that are known to be both genotoxic and carcinogenic. Some chemicals, however, induce cancer in experimental animals by non-genotoxic mechanisms that have a threshold, and for these, health-based guidance values can be established.

Substances that are both genotoxic and carcinogenic would generally not be considered acceptable for use as food additives, pesticides or veterinary drugs. JECFA has considered a number of contaminants that have been demonstrated to be both genotoxic and carcinogenic and has 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. Exposure (intake) assessment for a compound that is both genotoxic and carcinogenic is no different from that for other types of contaminants. Risk characterization can take different forms: 1) calculation of the MOE between the dose causing a low but defined incidence of cancer (usually in animal bioassays) and estimated human exposure; 2) dose–response analysis outside the observed dose range of animal bioassays to calculate the incidence of cancer that is theoretically associated with the estimated exposure for humans or the exposure associated with a predetermined incidence of cancer (e.g. an increased risk of cancer over a lifetime of 1 in a million); and 3) linear low-dose extrapolation from a POD such as the BMDL. Of these three options, the MOE and linear low-dose extrapolation from a POD are the most pragmatic and usable at the present time. JECFA has decided that advice on compounds that are both genotoxic and carcinogenic should be based on estimated MOEs. The monograph emphasizes that strengths and weaknesses inherent in the data used to calculate MOEs should be described in the advice to risk managers, together with advice on interpretation of the MOEs.

Maximum residue limits for pesticides and veterinary drugs

MRLs for pesticide residues and residues of veterinary drugs are the maximum concentrations of residues to be permitted in or on a food. International standards on MRLs are adopted by CAC on recommendation by the respective Codex committees, the Codex Committee on Pesticide Residues (CCPR) and the Codex Committee on Residues

of Veterinary Drugs in Foods (CCRVDF). These recommendations are based on advice provided by JMPR and JECFA. Both JECFA and JMPR have similar requirements for the identification and characterization of a substance that is under review for the establishment of an ADI, ARfD and MRLs.

JMPR evaluates pesticide residue data resulting from pesticide use according to Good Agricultural Practice (GAP) to estimate maximum residue levels in food and feed commodities. JMPR evaluates animal (livestock) and crop metabolism studies as the prime determinants of the residue definition in food and feed commodities. The recommended maximum residue levels in various crops depend mainly on the data from supervised residue trials conducted in line with maximum registered uses within GAP. The trials should cover the range of conditions expected to occur in practice, including application methods, seasons, cultural practices and crop varieties. If residue levels in the processed commodity exceed the residue levels in the raw agricultural commodity by a margin sufficient to require an MRL higher than the raw agricultural commodity MRL, it is necessary for JMPR to estimate a maximum residue level for the processed commodity. The pesticide residue dietary burden for livestock is derived from supervised residue trials for feed commodities multiplied by standard animal diets based on OECD livestock feed tables. Estimated maximum residue levels as well as highest residues (HRs) found in the supervised trials and supervised trial median residues (STMRs) derived from external animal treatments are compared with those derived from exposure through the feed. The recommended maximum residue levels, HRs and STMRs are based on whichever values are higher from this comparison. Estimates of chronic exposure are based on the STMRs from the supervised trials and food processing studies and long-term food consumption. For short-term exposure assessment, estimates of high intake of pesticide residue on a single day are based on the HRs from the supervised trials.

For veterinary drugs, JECFA evaluates residue depletion studies with radiolabelled parent drug as well as additional studies with unlabelled parent drug in intended target animal species for recommending MRLs in raw commodities of animal origin. Data from the studies using radiolabelled substance are used to estimate the time course of the concentration of the total residue of concern and to determine a marker residue. The derived MRLs are defined on the basis of the

marker residue. The marker residue may be the parent drug, a major metabolite, a sum of parent drug and metabolites, or a reaction product formed from the drug residues during analysis. It is not necessarily a residue of toxicological or microbiological concern, but is useful for monitoring purposes. Data from the studies using unlabelled substance are used to estimate the time course of the concentration of the marker residue in raw commodities of animal origin under approved practical conditions of use (i.e. Good Practice in the Use of Veterinary Drugs or GPVD). The relationship between the marker residue and total residues is used for the conversion of concentrations of the marker residue into concentrations of total residues of concern for the purpose of estimation of dietary exposure.

MRLs are generally recommended for several edible tissues and products, as appropriate for the intended use—for example, for muscle, liver, kidney and fat of slaughter animals, for fat and skin of poultry (and, where appropriate, of pigs) in natural proportions, for muscle and skin of fish in natural proportions, as well as for milk, eggs and honey.

For veterinary drugs, JECFA now develops recommendations for MRLs based on chronic intake estimates calculated from the median residue levels and a theoretical food basket (consisting of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat, 1500 g milk, 100 g eggs and 20 g honey), to estimate a conservative daily intake of residues, known as the estimated daily intake (EDI). The formerly used theoretical maximum daily intake (TMDI) utilized the MRL per se as the point estimate, which is a single value representing the upper limit of a high percentile of the distribution of residues. JECFA concluded that this method was not realistic and that all concentrations in the distribution of residues should be considered in the estimation of chronic intake. In cases where the quality of the data is not sufficiently robust to estimate a median residue level or intake, the TMDI may be used to provide a conservative intake estimate.

JECFA may make full recommendations for MRLs of a veterinary drug in appropriate food animal species and tissues on the basis of an ADI and adequate residue data. Temporary MRLs may be recommended either when there is an ADI but adequate residue or analytical method performance data are lacking or when the ADI is temporary. The Committee may recommend MRLs "not specified"

or "unnecessary" when there is a very wide margin of safety between estimated consumption of residues and the ADI.

Principles related to specific groups of substances

Many of the substances evaluated by JECFA are present in food at low concentrations. Examples include flavouring substances, processing aids, extraction solvents and enzymes used in food production. For the evaluation of such substances, it may be more appropriate to use the approaches described in this section of the monograph.

One such approach is the threshold of toxicological concern (TTC) concept. The knowledge that toxicity is a function of both chemical structure and the extent of exposure is the basis of the TTC concept. The TTC concept allows risk assessors to provide science-based advice when there is a high probability of negligible harm based on low dietary exposure and chemical structure alone. It is not intended to replace established risk assessment procedures used by JECFA and JMPR for substances on which extensive toxicity data are available.

The TTC approach, as applied by JECFA, utilizes human exposure threshold values (TTC values) for three structural classes of chemicals, below which there is a very low probability of any appreciable risk to human health. These TTC values have been derived from existing toxicity data on chemicals that have been classified into one of three structural classes. The TTC values for structural classes I, II and III are 1800, 540 and 90 μg/person per day, respectively. As the human exposure threshold values are compared with known or anticipated exposure, the TTC approach requires sound estimates of human exposure.

A decision tree approach (the Procedure for the Safety Evaluation of Flavouring Agents) has been developed by JECFA for the application of the TTC concept to flavouring substances. When the Procedure was first adopted, JECFA decided that a practical and realistic approach to derive estimated dietary exposures for consumers of flavouring agents was to use annual production volume data for different regions. This estimate, termed the maximum survey-derived intake (MSDI), was derived from figures for the total annual production of flavouring agents, adjusting for the fact that not all the chemical produced

would be reported and assuming that the flavouring agent would be consumed by only 10% of the population considered.

JECFA noted that use of the MSDI might result in an underestimation of dietary exposure to a flavouring agent for regular consumers of certain foods containing that flavouring agent. An additional new method of estimating dietary exposure for flavouring agents was therefore elaborated, termed the single portion exposure technique (SPET). The SPET estimate assumes a daily consumption of a single portion of food containing the flavouring agent, based on added use levels provided by the industry. The SPET identifies all food categories likely to contain the flavouring agent, assigns an added use level to a single "standard" portion of each of these categories and then identifies the single food category that is likely to contribute the highest dietary exposure. The standard portion is taken to represent the mean food consumption amount for consumers of that food category, assuming daily consumption over a long period of time. The standard portion does not reflect high food consumption amounts reported in national dietary surveys for the food category and is therefore a more realistic prediction of long-term consumption patterns. JECFA has concluded that the MSDI and SPET dietary exposure estimates provide different and complementary information. The higher value of the two dietary exposure estimates (MSDI or SPET) will be used within the Procedure.

JECFA has considered applying the TTC approach for the risk characterization of not only flavouring substances, but also other substances present in the diet in small amounts. For further application of the TTC approach, the Committee noted that it should be used in conjunction with conservative estimates of dietary exposure and that additional data on the toxicity of structurally related substances might be required. It further recommended that guidance be drawn up on application of the approach with regard to substances present in the diet in small amounts, such as certain residues of processing aids, packaging materials and contaminants, to provide advice on the risk assessment of substances for which full toxicological data sets are not available or are unnecessary.

The safety assessment of food packaging materials presents special problems because of the very large number of them in use and

the anticipated low level of migration of substances from food contact materials and consequent low dietary exposure. In principle, two alternatives exist for performing safety assessments on food contact materials. One is to require toxicological data regardless of the level of potential dietary exposure so that a safety assessment can be performed. A second option is to apply a tiered approach in which the number of toxicological data required is related to the extent of anticipated exposure as measured by migration studies.

Processing aids are composed of diverse substances, including, but not limited to, carrier or extraction solvents and enzymes used in food processing. JECFA has elaborated and periodically updated principles and procedures for the safety assessment of enzyme preparations.

The safety assessment of substances that are consumed in relatively large amounts, such as bulk sweeteners, modified starches, nutrients and related substances, and non-traditional whole foods, presents a number of special problems. The safety assessment of such substances differs from that for other food additives because of high dietary exposure, and minor constituents and processing impurities may assume greater than usual significance.

The increased use of fortified foods, dietary or food supplements, specially formulated foods and so-called "functional foods" has increased the intake of nutrient substances around the world. JECFA evaluates only the safety of these ingredients in accordance with the principles and methods in this monograph and has expressed the view that the evaluations should not be interpreted as an endorsement of the use of these substances for their claimed nutritional or health benefits.

Nutrient substances are biologically essential or have a demonstrated favourable impact on health at specified levels of intake. This consideration influences approaches applied to adjust for uncertainty associated with the data used to estimate a health-based guidance value and necessitates that the homeostatic mechanisms specific to essential nutrient substances be taken into account. Therefore, modifications to the classic non-nutrient risk assessment approach are needed. Internationally, guidance for risk assessment of nutrients and related substances recommended the use of the upper level of intake (UL),

in addition to a minimum intake for various strata of the population necessary to avoid nutritional deficiencies. The UL is the estimate of the highest level of regular intake that carries no appreciable risk of adverse health effects. The UL can be derived for nutrients using the principles of risk assessment similar to those that have been developed for biological and chemical agents.

Foods from novel sources include traditional and non-traditional foods, novel foods and foods for special dietary uses. Specifications are necessary to ensure that levels of potentially hazardous contaminants, such as mycotoxins and heavy metals, are kept to a minimum. The influence of the introduction of the new substance on the nutrient composition of the diet as a whole should be identified, particularly with respect to groups such as children, the elderly and "captive populations" (e.g. hospital patients and schoolchildren). The nutritional value of the novel food should be assessed initially from its chemical composition with respect to both macronutrients and micronutrients, taking into account the effects of any further processing and storage. Depending on the nature and intended uses of the novel food, studies in laboratory animals may be needed to supplement the chemical studies. Human studies on novel foods need to be designed on a case-by-case basis. Human experience is an essential part of the data collection in the history of use. For novel foods, exposure will need to be estimated from proposed uses. For the risk characterization of novel foods, the MOE approach may be suitable.