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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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Principles and Methods for the Risk Assessment of Chemicals in Food

Chapter 9
PRINCIPLES RELATED TO SPECIFIC GROUPS OF SUBSTANCES

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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9. PRINCIPLES RELATED TO SPECIFIC GROUPS OF SUBSTANCES

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.

9.1 Special considerations for substances consumed in small amounts

Many of the substances evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) are present in food at low concentrations. Examples include flavouring substances, which are added to food to enhance organoleptic appeal, processing aids, extraction solvents and enzymes used in food production. Also included are residues migrating into food from packaging materials, environmental contaminants, such as lead, cadmium, mercury and chlorinated organic chemicals, and residual amounts of pesticides and veterinary drugs used in livestock production. Residues in food from pesticides and veterinary drug use are not considered further here, as they have been discussed in detail in chapter 8.

9.1.1 Threshold of toxicological concern (TTC)

The establishment of safe exposure levels for food chemicals typically involves the first two steps of the risk assessment process, in which no-observed-adverse-effect levels (NOAELs) are determined, either from laboratory animal studies or from human observations, and translated into acceptable exposure levels or health-based guidance values, such as an acceptable daily intake (ADI) (see chapter 5). This traditional approach, which has been in constant use for over 50 years, generally requires that toxicological data on each chemical substance are available in order to perform a safety assessment.

The toxicological potency of the chemicals to which humans are exposed via the diet varies up to 6 or more orders of magnitude. This means that the exposure at which adverse effects are triggered, in terms of the amount of substance ingested per unit body weight, varies considerably between substances. Many factors influence the in vivo toxicity of chemicals, including chemical reactivity, metabolism and toxicokinetics, and the nature and magnitude of their interaction with molecular targets (toxicodynamics). Among organic chemicals, the principal determinant of toxicity is chemical structure; information accumulated over time indicates that the presence of functional groups on a molecule is a primary determinant of inherent toxicity. For example, for most chemical carcinogens, the structural features

leading to deoxyribonucleic acid (DNA) reactivity and subsequent carcinogenesis have been elaborated (Ashby & Tennant, 1991).

The knowledge that toxicity is a function of both chemical structure and the extent of exposure is the basis of the concept of the threshold of toxicological concern (TTC). The TTC approach can be used to facilitate risk assessment of substances present at low levels in the diet for which there are few or no toxicity data. The approach is based on the concept that a human exposure threshold value can be determined for substances, below which there is a very low probability of any appreciable risk to human health (Munro et al., 1996). The TTC concept has been developed and refined (Kroes et al., 2000, 2004).

Regulatory agencies have long had an interest in this concept, because humans may be exposed to very small amounts of an enormous number of naturally occurring and human-made chemicals from a wide variety of sources. The TTC concept was initially proposed by Rulis (1986, 1989, 1992) as a way for the United States Food and Drug Administration (USFDA) to remove unnecessary requirements for testing of components of packaging materials that could migrate in extremely low amounts into foods.

Based on the assumption that carcinogenicity would be the most critical effect at low exposures, Rulis (1986, 1989, 1992) applied a mathematical approach to the development of a threshold of concern for food contact materials. Rulis (1986) transformed the potencies (expressed as tumorigenic dose for 50% of test species, or TD_{50} values) of 343 orally administered carcinogens, compiled by Gold et al. (1984), into a distribution of exposures calculated to present a theoretical lifetime cancer risk of 1 in 1 million by simple linear extrapolation. His analysis indicated that it was highly probable that dietary exposures to organic chemicals at levels of 0.05 µg/kg of diet or less would not present a carcinogenic risk to humans, regardless of chemical structure, and therefore it was not necessary to obtain laboratory animal toxicity data to evaluate such exposures.

Munro (1990) reanalysed the data assessed by Rulis (1986) using the same methodology and also applied a probabilistic approach to three alternative data sets, consisting of 1) carcinogens from the updated database of Gold et al. (1989), 2) the United States National

Toxicology Program (NTP) carcinogens as defined by Ashby & Tennant (1988) and Ashby et al. (1989) and 3) carcinogens selected using conservative biological criteria. Overall, the results of the reanalysis indicated that there was low probability that exposure to a substance of unknown toxicity at a level of 1 μg/kg of diet would present a greater than 1 in 1 million risk of cancer.

On the basis of this work, the USFDA established a "threshold of regulation" for indirect food additives (the term used by the USFDA for migrants from food contact materials) of 0.5 μg/kg total diet (USFDA, 1995). This is equivalent to a daily dietary exposure of 1.5 μg, assuming consumption of 3 kg of food and liquid per day. The USFDA stated that this threshold of regulation would be applied to indirect food additives that are not known to be carcinogens and that do not contain structural alerts indicative of carcinogenicity. Substances meeting these criteria and with intakes less than the TTC would not require toxicological testing.

It should be noted that the threshold of regulation adopted by the USFDA was based on a presumption that migrating packaging material components might be carcinogenic. Assuming that 1 in 10 compounds assessed might be a carcinogen, a TTC value of 1.5 μg/person per day was derived from the distribution of TD_{50} values in the Gold et al. (1989) carcinogen database: at this intake, there is a 96% probability that the risk of cancer would be 1 in 1 million or less. If carcinogenic potential could be ruled out, presumably higher threshold values could be generated for non-carcinogenic components. To this end, the analyses conducted by the USFDA (1995), Rulis (1986, 1989, 1992) and Munro (1990) were further developed by Munro et al. (1996) through compilation of a database consisting of over 600 reference substances from which distributions of no-observed-effect levels (NOELs) were derived. The reference database presented the toxicity in terms of NOELs for a wide variety of organic chemicals of diverse structure, similar to the efforts of the previous workers but, in this case, grouped into three general classes based on chemical structure using the decision tree of Cramer et al. (1978). The use of a structural classification is based on the well-accepted tenet that inherent toxicity is related to chemical structure. This reference database was used to derive a threshold of human exposure that would be without safety concern for each

of the three structural classes and that can be applied to substances lacking toxicity data.

Munro et al. (1996) plotted the distribution of NOELs for 600 chemical substances, which included food additives, drugs, industrial chemicals and pesticides, arranged according to the three structural classes of Cramer et al. (1978). The 5th percentile of the distribution of NOEL values was calculated for each of the three structural classes. These 5th-percentile NOELs were then transformed into human exposure threshold values, referred to as TTCs, by dividing the 5th-percentile NOEL for each structural class by a 100-fold uncertainty factor. The TTC values for Cramer et al. (1978) structural classes I, II and III were 1800, 540 and 90 µg/person per day, respectively. As the TTC approach compares human exposure threshold values with exposure data, it requires sound estimates of human exposure.

Subsequent work conducted by Kroes et al. (2000, 2004) attempted to further evaluate the appropriateness of the thresholds proposed by Munro et al. (1996) to the distributions of NOELs for various specific forms of toxicity, such as developmental toxicity, neurotoxicity and immunotoxicity. With the exception of neurotoxicity induced by organophosphorus compounds, none of the end-points examined produced TTC values less than the TTC for Cramer et al. (1978) structural class III of 90 µg/person per day, and all classes of substances examined (including endocrine disrupting chemicals) would be accommodated within the TTC based on the carcinogen database of 1.5 µg/person per day.

Kroes et al. (2004) developed a decision tree for the application of the TTC concept for substances in structural classes I, II and III. The decision tree also includes a TTC for potential genotoxic carcinogens, based on the carcinogenic potencies associated with 730 compounds, mostly drawn from the Gold et al. (1989) carcinogen database (Gold & Zeiger, 1997). Analyses by Cheeseman et al. (1999) had indicated that the TD_{50} values for different structural alerts could be used to identify the most potent genotoxic carcinogens. Kroes et al. (2004) incorporated into their decision tree (Figure 9.1) a TTC value of 0.15 µg/person per day for those compounds that contained certain structural alerts for genotoxicity. They excluded substances with aflatoxin-like, azoxy- and nitrosamine groups, because such substances would

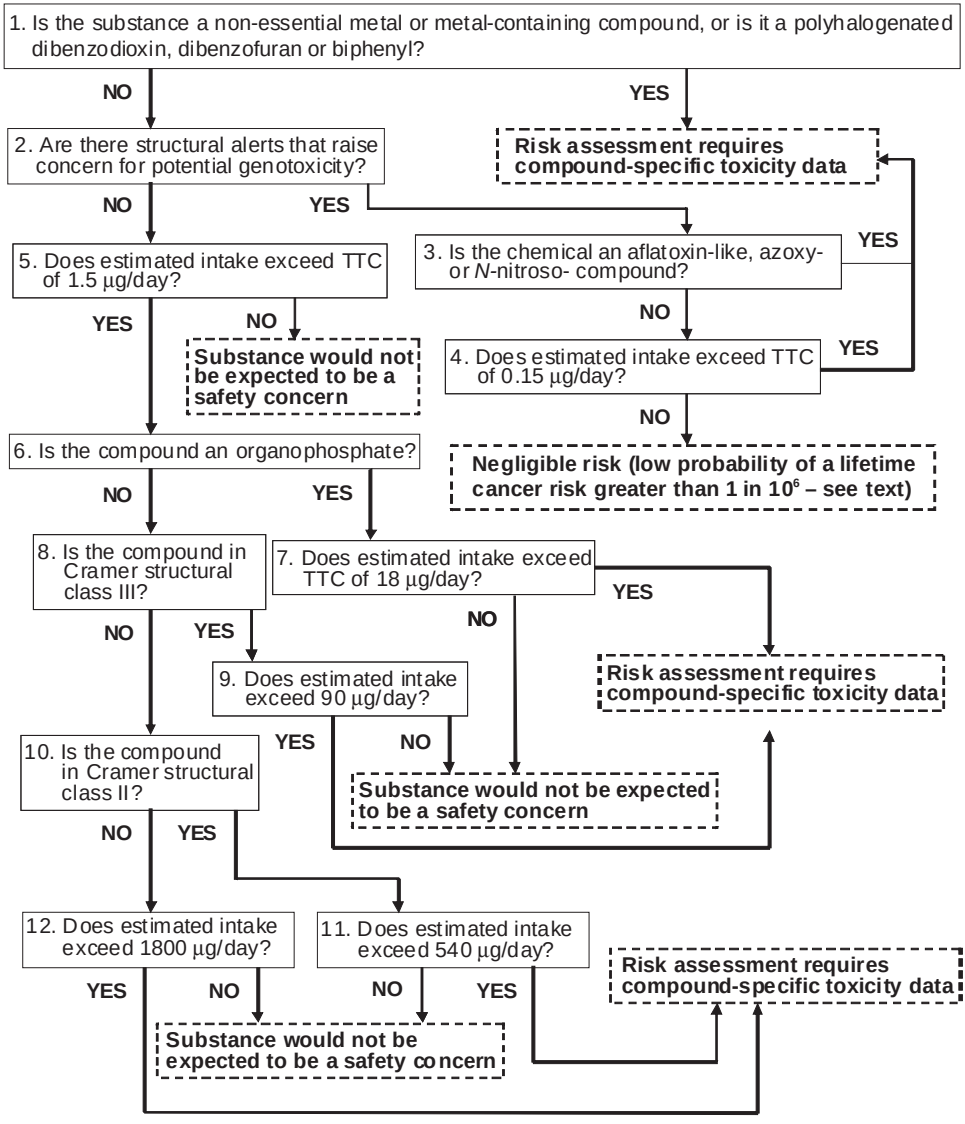

Fig. 9.1. Decision tree of Kroes et al. (2004) for application of the TTC approach

give a high probability of a theoretical lifetime cancer risk greater than 1 in 1 million at such an intake, whereas other substances with structural alerts for genotoxicity would present a 95% probability of less than 1 in 1 million risk. They also excluded metals and metal-containing compounds and proteins, because the database from which the TTC values were derived did not include these types of substances. Polyhalogenated dibenzodioxins, dibenzofurans or biphenyls were also excluded because of their long half-lives and wide species differences in toxicokinetics; in addition, such substances would be evaluated by the toxic equivalency factor (TEF) approach, so the TTC concept would not be appropriate. The rationale for the TTC value of 0.15 µg/person per day applicable to compounds with certain structural alerts for genotoxicity is similar to that for the TTC value of 1.5 µg/person per day (discussed previously), except that it was assumed that all compounds with such structures could be potential DNA-reactive carcinogens, rather than 1 in 10, as used in the derivation of the higher value. The TTC value of 0.15 µg/person per day is designed to allow the formulation of timely advice to risk managers about the possible risk due to very low levels of a compound with a structural alert for genotoxicity or with positive evidence of genotoxicity and is not intended to provide a rationale for the deliberate addition of such a compound to the food supply.

A major advantage of the TTC concept is that it presents a method for focusing resources on public health problems of greatest significance. Substances having exposures below the relevant TTC have low potential for human harm and low priority for testing. The procedure provides confidence that substances consumed in very small amounts present only a minimal potential for risk. Moreover, the TTC provides a reasonable and science-based alternative to laboratory animal testing of substances with innocuous structures and minimal exposure.

At its sixty-fifth meeting in 2006 (FAO/WHO, 2006a), JECFA considered the application of approaches involving the TTC, not only for the risk characterization of flavourings, for which the TTC concept had been used by JECFA for a decade (see section 9.1.2), but also for other substances present in the diet in small amounts. The Committee noted that the following considerations should be taken into account for further application of TTC approaches:

- The approaches should be used in conjunction with conservative estimates of dietary exposure.

- 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 TTC concept was introduced to allow risk assessors to provide science-based advice when there is a high probability of negligible harm based on dietary exposure and chemical structure alone. It is not intended to replace established risk assessment procedures used by JECFA and JMPR for substances such as food additives and pesticide residues, which undergo prior approval based on the generation of a comprehensive database. Also, the TTC approach would not replace the established procedures for dioxin-like compounds or certain heavy metals or where there are sufficient data to allow the establishment of a health-based guidance value.

9.1.2 Flavouring agents

9.1.2.1 The JECFA procedure for safety evaluation

For flavouring agents, JECFA has noted that in most cases dietary exposure to these substances is low and self-limiting, and the majority of flavours are metabolized rapidly to innocuous end-products (FAO/WHO, 1995). This fact limits the need for toxicological testing of many flavouring agents, and therefore metabolic data (e.g. hydrolysis of esters) and structure–activity relationships can play a key role in their safety evaluation.

Flavouring agents are composed of divergent groups of materials, including:

- artificial substances unlikely to occur naturally in food;
- natural materials not normally consumed as food, their derived products and the equivalent nature-identical flavourings;

- herbs and spices, their derived products, and the equivalent nature-identical flavourings; and
- natural flavouring substances obtained from vegetable and animal products and normally consumed as food, whether processed or not, and their synthetic equivalents.

The safety evaluation of flavouring agents presents a special challenge. Flavouring substances are generally consumed in low amounts, and there are several thousand individual flavouring substances in commercial use worldwide. All of the existing individual flavouring substances can be arranged into about 40 groups comprising substances with related chemical structures and similar known or predicted metabolic fates. Testing all these substances for toxicity using classical toxicological approaches would present a formidable challenge and require a massive use of resources. The safety evaluation of flavours presents an opportunity to combine data on intake, metabolic fate and toxicity, including the application of the TTC concept (see section 9.1.1), to perform assessments of flavourings in related structural groups.[1]

The current JECFA Procedure for the Safety Evaluation of Flavouring Agents (the "Procedure") was first considered in 1995 (FAO/WHO, 1995), based on work subsequently published by Munro et al. (1999). The Procedure was adopted by JECFA for the evaluation of flavouring agents at its forty-sixth meeting in 1997 (FAO/WHO, 1997) and has since been modified several times (FAO/WHO, 1999, 2006a, 2009), as outlined in chapter 1. At the sixty-fifth JECFA meeting in 2005 (FAO/WHO, 2006a), the Committee reaffirmed the use of the TTC approach in the evaluation procedure for flavouring agents. The Procedure is outlined in Figure 9.2.

The approach incorporates a series of criteria designed to provide a method to evaluate flavouring substances in a consistent and timely manner. The criteria take account of available information on dietary exposure from current uses, structure–activity relationships and known or predicted metabolism, plus any available toxicity data on

[1] A JECFA number is assigned consecutively to every flavouring substance specified and evaluated by JECFA.

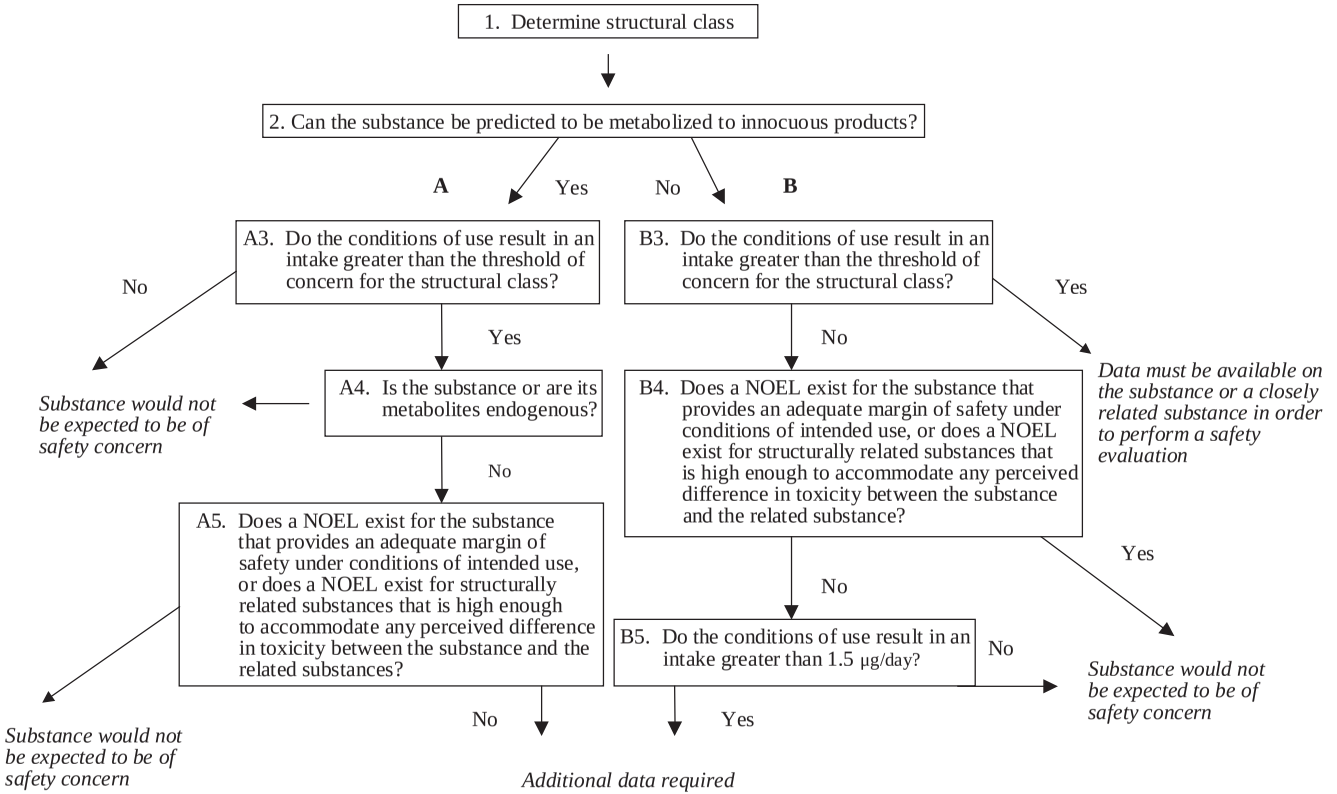

Fig. 9.2. Procedure for the Safety Evaluation of Flavouring Agents adopted by the Committee at its sixty-fifth meeting (FAO/WHO, 2006a)

the compound or structurally related compounds. The use of these criteria provides a means of sorting flavouring substances in terms of the presence or absence of safety concerns and provides guidance on the nature and extent of the data required to perform a safety evaluation.

The criteria take advantage of the fact that some flavouring agents occur as normal constituents of mammalian tissues or are metabolized to form such constituents and are then completely metabolized to innocuous end-products, such as carbon dioxide and water. Flavouring agents with these characteristics are considered to be safe for consumption if dietary exposure is below the threshold of concern for the structural class, but are evaluated on the basis of toxicity data if dietary exposure is above the threshold of concern for the structural class. This safety evaluation may involve the use of toxicity data on the individual substance concerned or may rely, at least in part, on toxicity data on substances of closely related structure.

For flavouring agents that are not known or predicted to be metabolized to innocuous end-products, the safety evaluation must be based on toxicity data, even if estimated dietary exposure is low. In such cases, there must be an adequate margin of safety between dietary exposure to the flavouring agent and the NOEL/NOAEL for the substance or the NOEL/NOAEL for a substance of closely related structure on which the safety evaluation relies. Flavouring agents currently in use for which no toxicity or metabolic data exist, and for which estimated dietary exposure is extremely low, less than 1.5 μg/day, could be considered not to present a safety concern provided they do not contain structural alerts for genotoxicity.

It has been noted that the safety evaluation procedure is not intended to be applied to flavouring agents with existing unresolved problems of toxicity. As with any scheme, its application calls for judgement, and it should not replace expert opinion; JECFA therefore reserved the right to use alternative approaches when data on specific flavouring agents warranted such action.

It was noted that a key element of the Procedure involves determining whether a flavouring agent and the products of its metabolism are innocuous or endogenous substances. The Committee considered that these terms require definition. It recommended that *innocuous*

metabolic products should be defined as products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent, whereas *endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated dietary exposure to a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

JECFA has noted that ADIs had previously been established for some flavouring agents or groups of flavouring agents and recommended that these should be retained, as the information on which they are based is relevant to an evaluation of their safety and, in addition, they may have uses other than as flavouring agents (e.g. as food additives).

9.1.2.2 *Consideration of dietary exposure estimates*

When the Procedure for the Safety Evaluation of Flavouring Agents was first adopted at its forty-sixth meeting in 1996 (FAO/WHO, 1997), 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 (60–80% reported) and assuming that the flavouring agent would be consumed by only 10% of each population considered. MSDI estimates were originally based on production and population data for the United States of America (USA) and Europe, but now include data from Japan, with a requirement for recent production data to be submitted by the industry to each meeting. At the sixty-eighth meeting (FAO/WHO, 2007b), a correction factor of 0.8 was applied to the annual production volumes reported in the surveys from Europe, Japan and the USA.

Although JECFA re-endorsed the MSDI approach at meetings subsequent to the forty-sixth meeting, it also discussed limitations to the use of the MSDI for estimating dietary exposure to flavouring agents

(FAO/WHO, 2001, 2005, 2006a, 2007b, 2009). Specific concerns were that low production volume flavouring agents may be added at high levels to certain foods and that high production volume flavouring agents could be present in a large number of foods at different added use levels. The uneven distribution of added use levels for some flavouring agents across different food categories and within food categories and the consequent uneven distribution of dietary exposures to a flavouring agent could not be taken into account in the MSDI estimate. 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.

At its sixty-fifth meeting (FAO/WHO, 2006a), JECFA reviewed existing model diets for estimating potential dietary exposure to flavouring agents based on generally recognized as safe (GRAS)[1] levels published in the USA or added use level data. These models for dietary exposure estimation assume daily consumption of large portions of several food categories containing the same flavouring agent (possible average daily intake [PADI], theoretical added maximum daily intake [TAMDI]) (see chapter 6, section 6.3.4.1). However, the dietary exposure estimates from these model diets were not considered to be realistic estimates of dietary exposure to flavouring agents as a result of the conservative assumptions made and therefore were not suitable for use in the Procedure. JECFA therefore recommended that there should be further consideration of the most appropriate approach for evaluating the safety of flavouring agents.

JECFA considered further information on recommended use levels supplied by industry on flavouring agents evaluated at subsequent meetings (FAO/WHO, 2007a,b, 2009). An additional new method of estimating dietary exposure for flavouring agents, using the single

[1] GRAS, or generally recognized as safe, is a regulatory concept specific to the United States Federal Food, Drug, and Cosmetic Act. Any substance added to food requires a food additive regulation for its use, unless its intended use is GRAS. Food ingredients whose use is GRAS are not required by law to receive USFDA approval before marketing. The Flavour and Extract Manufacturers Association (FEMA) has been publishing lists of flavouring substances and associated use levels at or below which they have deemed their use to be GRAS for over 30 years.

portion exposure technique (SPET), was agreed upon in 2008 (FAO/WHO, 2009).

The SPET estimate assumes a daily consumption of only a single portion of food containing the flavouring agent, based on added use levels provided by the industry, rather than FEMA GRAS levels.[1] It aims to represent the chronic dietary exposure for a regular consumer who consumes a specific food product containing the flavouring agent of interest daily and not a high consumer of these foods.

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.

A summary of an analysis of MSDI and SPET estimates for 225 flavours for which added use level and production data for one of the three geographic regions (Europe, Japan and the USA) were available was reported at the sixty-ninth meeting of JECFA (FAO/WHO, 2009). In nearly all cases (>90%), the SPET estimate was above the MSDI, and the SPET estimate was more likely than the corresponding MSDI to be above the TTC of the relevant structural class. The SPET estimate was most frequently above the TTC in class III, but this also occurred in classes I and II.

JECFA concluded that the MSDI and SPET dietary exposure estimates provide different and complementary information (FAO/WHO, 2009). Inclusion of the SPET estimate in the Procedure addressed previous concerns about the MSDI estimate of dietary exposure, because the SPET estimate takes account of the possible

[1] Lists of flavouring substances and associated use levels at or below which they have deemed their use to be GRAS are published regularly by FEMA.

uneven distribution of dietary exposures to a flavouring agent for consumers of foods containing that substance. The higher value of the two dietary exposure estimates (MSDI or SPET) will be used within the Procedure.

As it was not possible to elaborate criteria, based on structure, production level or group of flavouring agents, to identify the flavouring agents for which the MSDI underestimated dietary exposure and SPET estimates should be used, JECFA concluded that it was necessary to incorporate SPET estimates into the Procedure for all flavouring agents considered at future meetings. JECFA also noted that the addition of the SPET dietary exposure estimate, where it was higher than the MSDI, to the relevant steps A3 and B3 of the decision tree in the Procedure (see Figure 9.2) would be likely to lead to a more extended evaluation in only a limited number of cases. It was not considered necessary to re-evaluate flavouring agents already assessed using the Procedure.

9.1.3 Food contact materials/packaging migrants

Many food contact materials are made from polymers that are usually inert biologically as a result of their high molecular weight. However, constituents of these polymers, such as monomers, additives, catalysts and other substances used in their manufacture, are low molecular weight substances, which theoretically could migrate from the food contact material into foods. The same can be said for other constituents of the food contact materials, such as inks used in labelling. Migration may occur during storage and be enhanced during food preparation, such as heating, microwave cooking or processing with ionizing radiation. Also, the food matrix may affect the degree of migration, such that fat-soluble substances will migrate more readily into fatty foods, whereas water-soluble substances will migrate more readily into aqueous foods.

The safety evaluation 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. JECFA (IPCS, 1987) has previously set out criteria for the evaluation of these substances, noting that the following information is required:

- the chemical identity and toxicological status of the substances that enter food;
- the possible exposure, details of which can be derived from migration studies using suitable extraction procedures and/or the analysis of food samples; and
- the nature and amount of food contact with the packaging materials, and the intake of such food.

These criteria define the fundamental data required to identify those substances that migrate, the amounts that may be present in food and consequent exposures.

In principle, two alternatives exist for performing safety evaluations on food contact materials. One is to require toxicological data regardless of the level of potential dietary exposure so that a safety evaluation can be performed. A second option is to apply a tiered approach in which the number of toxicological data required are related to the extent of anticipated exposure as measured by migration studies. As discussed previously (see section 9.1.1), in 1995, the USFDA adopted a "threshold of regulation" for food packaging migrants such that a substance would be exempt from USFDA regulation if exposures were less than 1.5 µg/person per day, provided the migrant was not carcinogenic or did not contain structural alerts for carcinogenicity (USFDA, 1995). Given the large number of food contact materials in commerce, such an approach provides a reasonable alternative to requiring that all such migrating substances be tested for toxicity.

Models for estimating potential dietary exposures to packaging materials are discussed in chapter 6 (section 6.3.4.1).

9.1.4 Processing aids

Processing aids are composed of diverse substances, including, but not limited to, carrier or extraction solvents and enzymes used in food processing.

9.1.4.1 Solvents

Extraction solvents are used in, for example, the extraction of fats and oils, defatting fish and other meals, and decaffeinating coffee and tea. They are chosen mainly for their ability to dissolve the desired

food constituents selectively and for their volatility, which enables them to be separated easily from the extracted material with minimum damage. The points raised by their use relate to:

- the toxicity of their residues;
- the toxicity of any impurities in them;
- the toxicity of substances such as solvent stabilizers and additives that may be left behind after the solvent is removed; and
- the toxicity of any substances produced as a result of a reaction between the solvent and food ingredients.

Before any extraction solvent can be evaluated, information is required on:

- the identity and amount of impurities in the solvent (including those that are formed, acquired or concentrated owing to continuous reuse of the solvent);
- the identity and amount of stabilizers and other additives; and
- the toxicity of residues of solvents, additives and impurities.

Impurities are particularly important, because there are wide differences in the purities of food-grade and industrial-grade solvents. The food use of extraction solvents is frequently much less than the industrial use, and considerable problems may arise in their evaluation if toxicological data exist only on the industrial grade of the solvent, which contains potentially toxic impurities that may not be present in the food-grade material. For example, when evaluating the solvents 1,1,1-trichloroethane, trichloroethene and tetrachloroethene, it was noted that the toxicological data indicated the presence of certain known toxic and carcinogenic substances. The interpretation of these data became extremely difficult because industrial-grade material had been used in the studies. Only food-grade material should be used in toxicological studies, and the impurities in the material should be fully identified.

Carrier solvents raise somewhat different issues. They are used for dissolving and dispersing nutrients, flavours, antioxidants, emulsifiers and a wide variety of other food ingredients and additives. With the exception of carrier solvents for flavours, they tend to occur in food at levels higher than those of extraction solvents, mainly because some

of them are relatively non-volatile. As carrier solvents are intentional additives and are often not removed from the processed food, it is important to evaluate their safety together with the safety of any additives or stabilizers in them.

Section 9.1.4.2

Enzymes

Second edition

(2020)

CONTENTS

This text updates section 9.1.4.2 of Chapter 9, Principles Related to Specific Groups of Substances, of Environmental Health Criteria 240 (EHC 240), which was originally published in 2009. It was developed through an expert meeting of a working group established to consider the evaluation of enzyme preparations used in the manufacture of foods, held in December 2018. The text was available for public comment in December 2019, and the final version was discussed and approved at the eighty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in June 2020.

For abbreviations used in the text, the reader may refer to the list of abbreviations at the front of this section. Definitions of select terms may be found in the glossary in Annex 1 of EHC 240 (http://www.inchem.org/documents/ehc/ehc/ehc240_annex1.pdf).

List of abbreviations

ADI	acceptable daily intake
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
CAS	Chemical Abstracts Service
DNA	deoxyribonucleic acid
EC/IUBMB	Enzyme Commission/International Union of Biochemistry and Molecular Biology
EHC 240	Environmental Health Criteria 240
FAO	Food and Agriculture Organization of the United Nations
FASTA	FAST-All
GMP	Good Manufacturing Practice
IgE	immunoglobulin E
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MOE	margin of exposure
NOAEL	no-observed-adverse-effect level
rDNA	recombinant deoxyribonucleic acid
RNA	ribonucleic acid
SCF	Scientific Committee on Food
SDS PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
USA	United States of America
WHO	World Health Organization

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9.1.4.2 *Enzymes*

(a) Introduction

The history of enzyme use in food applications – especially in the making of bread, cheese, wine and beer, where enzymes are part of the manufacturing or maturation processes – is long and well known. Enzymes used in the food industry are produced from animal tissues, plants and microorganisms. However, most commercial enzymes are produced from microorganisms that are enhanced through natural selection, classical strain improvement techniques (e.g. mutagenesis and selection), recombinant DNA technologies and gene editing. Microbial enzymes are typically produced by controlled fermentation followed by removal of the production microorganism and purification and concentration of the enzyme. Final standardization with stabilizers, preservatives, carriers, diluents and other approved food-grade additives and ingredients is carried out after the purification and concentration steps. Enzyme preparations, depending on the application, may be produced as a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction or two or more active enzymes that catalyse different reactions during food processing.[1]

Enzyme preparations often contain organic constituents of the production organism and compounds carried over from the manufacturing process – for example, the residues of the fermentation broth. In 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), at its sixty-seventh meeting, elaborated principles and procedures for the safety assessment of enzyme preparations for use in food, whereby an enzyme preparation must comply with the *General specifications and considerations for enzyme preparations*[2] *used in food processing* (FAO, 2006; FAO/WHO, 2007a). This document addressed certain aspects of the evaluation of the safety of all enzyme preparations, including the

[1] In this section, "enzyme" refers to the enzyme and its amino acid sequence; "enzyme concentrate" refers to the enzyme concentrate used in the toxicity studies; and "enzyme preparation" refers to the enzyme preparation formulated for commercial use.

[2] Note that "enzymes" rather than "enzyme preparations" was used in the title in FAO (2006).

safety of the production organism, the enzyme components, side activities and the manufacturing process, as well as the consideration of dietary exposure.

Some of the specific safety concerns related to enzyme preparations as well as an updated classification system for enzymes used in food are outlined in the following subsections.

(b) Potential for enzymes to cause allergic reactions

Food allergies. Food allergies are adverse immunological reactions to an otherwise harmless food, such as a protein. The severity of food allergies in susceptible individuals (atopy) can range from mild to severe and, in some cases, can be life-threatening. The most common type of food allergy is mediated by allergen-specific immunoglobulin E (IgE) antibodies. Allergens are almost always proteins (e.g. Ara h2 in peanuts, papain in papaya, lactoperoxidase in cow's milk), but not all food proteins are allergens. As there is no single test that can accurately predict whether a microbially synthesized enzyme will immunologically cross-react with an established allergen, a weight-of-evidence approach should be used (FAO/WHO, 2001). One approach that has routinely been used by JECFA is to compare the amino acid sequence of an enzyme against known linear IgE-binding epitopes in allergenic proteins using in silico methods and appropriate protein databases (e.g. AllergenOnline of the Food Allergy Research and Resource Program, University of Nebraska, Lincoln, Nebraska, USA: http://www.allergenonline.org). The possibility of immunological cross-reactivity between the expressed enzyme and a known allergen is considered when there is:

- at least 35% identity in the amino acid sequence of the expressed protein (i.e. without the leader sequence, if any), using a sliding window of 80 amino acids and a suitable gap penalty (for algorithms such as FAST-All [FASTA], Basic Local Alignment Search Tool [BLAST], or equivalent; Codex Alimentarius Commission, 2003, 2009); and/or
- identification of eight contiguous amino acids common to the expressed enzyme and a known allergen (FAO/WHO, 2016).

Amino acid sequence information is not available for most enzymes – either derived from animals or plants or produced by microorganisms – that are traditionally accepted constituents of

foods. Thus, the absence of allergenicity in humans is reasoned to have been demonstrated by the presence of these enzymes in widely consumed foods for a long period of time.

Allergenic food proteins and resistance to proteolysis. The susceptibility of a dietary protein to proteolytic degradation by digestive enzymes, such as gastric pepsin, could potentially provide information on its immunological safety for human consumption. Whereas most dietary proteins are readily hydrolysed to peptides and amino acids in the gastrointestinal tract, there is evidence that many potent food allergens are resistant to proteolysis (Schmidt et al., 1995; FAO/WHO, 2001; Bannon, 2004; Moreno et al., 2005). In vitro pepsinolysis assays (Thomas et al., 2004) have been proposed as an additional piece of information as part of a weight-of-evidence approach for evaluating the safety of newly expressed proteins (Codex Alimentarius Commission, 2009). A pepsinolysis assay that is based on simulated gastric fluid and frequently used in the preclinical testing of pharmaceuticals has been described by the United States Pharmacopeia (2000). The simulated gastric fluid assay is often used to allow comparisons between different newly expressed proteins under experimental conditions (Astwood, Leach & Fuchs, 1996). To date, however, such pepsin resistance data for enzymes have rarely been submitted to JECFA for consideration within a weight-of-evidence approach. This may be because there are studies – albeit not using the same conditions (pH, purity and activity of pepsin, and pepsin-to-substrate protein ratio) – showing that the correlation with allergenic potential is not absolute and that proteins that are resistant to pepsinolysis might not be allergenic under physiological conditions of dietary exposure; in contrast, labile proteins (e.g. β-casein) or peptides formed during proteolysis may be allergenic (Vieths et al., 1999; Yagami et al., 2000; Wal, 2001; Fu, Abbott & Hatzos, 2002; Bøgh & Madsen, 2015). Consequently, data on resistance to pepsinolysis from in vitro tests are currently not considered to be strong evidence for the absence of the intrinsic allergenicity of a protein, but still may have some utility as part of a weight-of-evidence approach.

Occupational hazards: respiratory allergies, skin and eye irritation. A known safety risk linked to industrial enzyme use is respiratory allergy (Quirce et al., 1992; Green & Beezhold, 2011). For most proteases, there is also some potential for skin and eye irritation (Vanhanen, 2001; Anderson, Long & Dotson, 2017).

(c) Safety concerns pertaining to enzyme preparations produced by genetically modified microorganisms

The *General specifications and considerations for enzyme preparations used in food processing* (FAO, 2006; FAO/WHO, 2007a) provides recommendations on the safety assessment of the genetic material inserted into the genome of the production microorganism. Two new considerations that were introduced in the most recent revision of the specifications (which were first elaborated by JECFA at its twenty-sixth meeting with several revisions proposed at subsequent meetings) read as follows:

> For enzyme preparations from recombinant-DNA microorganisms, the following should also be considered:
>
> 1. The genetic material introduced into and remaining in the production microorganism should be characterized and evaluated for function and safety, including evidence that it does not contain genes encoding known virulence factors, protein toxins, and enzymes involved in the synthesis of mycotoxins or other toxic or undesirable substances.
> 2. Recombinant-DNA production microorganisms might contain genes encoding proteins that inactivate clinically useful antibiotics. Enzyme preparations derived from such microorganisms should contain neither antibiotic inactivating proteins at concentrations that would interfere with antibiotic treatment nor transformable DNA that could potentially contribute to the spread of antibiotic resistance. [FAO/WHO, 2007a:87–88]

It must be pointed out that papers identified through extensive literature searches on the safety of enzymes from microbial sources support the general assumption that industrial enzyme preparations from non-pathogenic organisms are safe (Olempska-Beer et al., 2006). Most engineered enzymes exhibit no greater amino acid sequence variability than already exists for many isozymes in the diet (Préstamo & Manzano, 1993). Also, there is no evidence to suggest that changes in amino acid sequence made through protein engineering – to confer benefits such as tolerance to heat or pH or to simply increase yield – will result in an otherwise safe enzyme being rendered toxic. That said, comparing the amino acid sequence of an enzyme with the sequence of known toxic or allergenic proteins using in silico methods is one way to exclude the possibility that the enzyme may be toxic or allergenic or have some other adverse physiological effect.

(d) Toxicological assessments of enzyme preparations

Toxicological considerations. As noted above, enzyme preparations contain either one major active enzyme that catalyses a specific reaction or two or more active enzymes that catalyse different reactions during food processing. Each enzyme in the preparation must comply with the established identity and purity specifications.

Although food enzyme preparations are considered unlikely to cause any acute toxicity, genotoxicity or repeated-dose oral toxicity, the fermentation products of microorganisms remaining from the manufacturing process are of interest due to the potential presence of secondary metabolites that may induce toxicity when ingested (e.g. aflatoxins, fumonisins and ochratoxins) (OECD, 2018). The enzyme concentrate, containing both fermentation products and the food enzyme of interest, has traditionally been used in genotoxicity tests and in repeated-dose rodent feeding studies submitted to JECFA.

The Scientific Committee on Food (SCF, 1992) elaborated the points of potential toxicological concern, noting that:

1. Different strains belonging to the same species can behave differently. For many microorganisms it is known that some of the strains in one species are harmless, while others belonging to the same species are toxic.
2. For some fungal genera, especially *Penicillium* and *Aspergillus*, there have been many misidentifications of fungal isolates. As a consequence of this, there is a risk of misclassification of fungal strains. For example in some cases it has been difficult to distinguish *A. oryzae* from *A. flavus* which has the ability to produce aflatoxin. As long as there is a risk of misidentification of microbial isolates, it is very important that the microorganism used is correctly identified and, in case of doubt, the identity should be verified by an independent, recognized laboratory.
3. The ability of a microorganism to produce toxins depends – qualitatively and quantitatively – on environmental factors such as the composition of fermentation media, pH, temperature and fermentation period. Therefore there is a risk that a microorganism which does not produce toxins under some conditions will turn out to be toxin-producing under other conditions.
4. The continuous selection processes applied to source microorganisms in order to maximize and optimize enzyme production may result in spontaneous mutations which give rise to the possibility of changing a non-toxic strain to a toxic strain.

5. There is a considerable potential to apply new techniques of genetic modification in the production of food enzymes. Along with the introduction of desirable traits, there is also the potential for introducing toxin production and therefore there is a need explicitly to characterize and evaluate the genetic construct as to host, vector and insert. [SCF, 1992:14–15]

As a result of these safety concerns, the following basic toxicological testing requirements were provided (SCF, 1992):

9.1 For enzymes derived from edible parts of animals or plants no toxicological tests are normally required. Where parts which are not generally considered as a normal part of the diet are used, some toxicological testing may be required unless other satisfactory documentation for safety in use is provided.

9.2 For enzyme preparations derived from microorganisms the following tests are normally required:

(a) 90-day oral toxicity test in a rodent species;
(b) Two short-term tests:
    1. a test for gene-mutations in bacteria,
    2. a test for chromosomal aberrations (preferably in vitro).

The toxicological tests shall, where possible, be performed on a batch from the final purified fermentation product, before addition of carriers, diluents, etc. [SCF, 1992:19]

Exemptions from the basic toxicological requirements. The exemptions from performing toxicological bioassays in the safety assessments of enzymes, as described in the original SCF (1992) guidelines, are as follows:

From a toxicological point of view it is important to perform a toxicological testing procedure on each specific enzyme preparation produced from a microbiological source.

10.1 If, however, one enzyme from a specific strain has been thoroughly tested and the manufacturing process does not differ significantly for other enzymes from the same strain, the full testing battery may be waived for these enzymes. This will be decided on a case-by-case basis.

10.2 If the microorganism used in the production

– has a long history of safety in food use, and

> – belongs to a **species** about which it has been documented that no toxins are produced, and
> – the actual **strain** used is of well documented origin,
>
> the acceptance of an enzyme preparation from this organism without specific toxicological testing may be justified. In this case a correct and confirmed identification of the organism is of extra importance. [SCF, 1992:20]

To date, very few exemptions from toxicological testing have been considered in safety assessments of enzymes by JECFA. This may be because of the uncertainty regarding compliance with the requirements of accurately identifying the microbial strain and assessing the ability of the microorganism to produce toxins. However, these requirements can more easily be met using current technologies such as analytical molecular biology techniques – for example, full genome sequencing, gene probing or RNA sequencing technologies to minimize misidentification (Yu et al., 2011) and chemometrics (Inui et al., 2012) to identify and quantify secondary metabolites in complex natural product mixtures that may result from microbial fermentation.

If the sponsor does not conduct toxicity testing, then the sponsor is obligated to provide other information to attest to the enzyme's safety. The full battery of toxicity tests may be waived for enzymes from a specific (new) strain if the manufacturing process does not differ significantly from that used for other enzymes from the same strain, a related strain or a lineage of related strains, provided other evidence is presented to support the safety of the enzyme preparation of interest (e.g. chemical assessment for known toxins, whole genome sequencing and assessment for possible toxin production).

(e) Dietary exposure and margin of exposure

Dietary exposure is calculated on the basis of the total organic solids (TOS) content in the final (commercial) enzyme preparation and is usually expressed in milligrams or micrograms of TOS per kilogram of body weight per day. TOS encompasses the enzyme component and other organic material originating from the production organism and the manufacturing process, while excluding intentionally added formulation ingredients. JECFA considers the estimated dietary exposure to an enzyme preparation based on the proposed uses and use levels in food and relates it to the no-observed-adverse-effect level (NOAEL) in its hazard assessment in order to determine a margin of exposure (MOE).

(f) Classification of enzymes

To aid in the decision-making process, in 2018, JECFA reassessed the requirements for testing the toxicity of enzyme preparations used in food and updated the classes as follows (FAO/WHO, 2019):

- *Class I: Enzymes obtained from sources that are considered safe for consumption and for which toxicological evaluations are NOT normally required*

  This class, which also includes immobilized enzymes from these sources, can be further categorized into:

  – ***Type i:*** Enzymes obtained from edible tissues of plants or animals commonly used as foods

    These enzymes are regarded as foods; consequently, their safety is considered acceptable, provided that satisfactory chemical and microbiological specifications can be established (e.g. papain, rennet). Uses and use levels should be considered.

  – ***Type ii:*** Enzymes produced by microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods

    These enzymes are regarded as foods; consequently, their safety is considered acceptable, provided that satisfactory chemical and microbiological specifications can be established (e.g. *Saccharomyces* spp.). Enzymes produced by microorganisms modified by genetic engineering are not considered to be Class I Type ii, but fall into either Class I Type iii or Class II. Uses and use levels should be considered.

– ***Type iii:*** Enzymes produced by a Safe Food Enzyme Production Strain[1] or a Presumed Safe Progeny Strain[2]

  For food enzyme preparations in this group, a detailed chemical and microbiological (genomic) narrative confirming that the enzyme is produced by an organism that meets the definition of a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain that has undergone appropriate toxicological testing (i.e. repeated-dose toxicity and genotoxicity testing) is required. Appropriate toxicological testing includes existing studies conducted on enzymes from other closely related strains derived from the same parental organism. This could be demonstrated with published or unpublished genomic sequence data of the genetically modified microorganism to exclude the possibility of the presence of genes for the production of toxic secondary metabolites. Safety assessments for these food enzymes should also include appropriate information or other experimental data to determine their potential to cause an allergic reaction when ingested.

  On completion of appropriate toxicological testing of the fermentation product from a production microorganism, this guidance anticipates that it should be possible to conclude that the microorganism can be classified as a source that is considered safe for human consumption. Such a declaration was made for *A. oryzae* at the sixty-eighth meeting of JECFA (FAO/WHO, 2007b). As of 2020, JECFA has

[1] A "Safe Food Enzyme Production Strain" is a non-pathogenic, non-toxigenic microbial strain with a demonstrated history of safe use in the production of food enzymes. Evidence supporting this history of safe use includes knowledge of taxonomy, genetic background, toxicological testing, other aspects related to the safety of the strain and commercial food use.

[2] A "Presumed Safe Progeny Strain" is developed from a Safe Food Enzyme Production Strain or from the parent of that Safe Food Enzyme Production Strain. The progeny strain is developed through specific well-characterized modifications to its genome; the modifications must be thoroughly documented, must not encode any harmful substances and must not result in adverse effects. This concept also applies to multiple generations of progeny. Evidence supporting their safety includes knowledge of taxonomy, genetic background and toxicological testing (including read-across of toxicological studies).

evaluated over 80 food enzyme preparations from a variety of microorganisms and has never recorded a positive result in any toxicity study, suggesting either that toxins were not present or that toxins were present at levels that were below the limit of detection of the bioassays. These data suggest that there are many strains of microorganisms that JECFA has previously reviewed (e.g. *Bacillus subtilis*, *B. licheniformis*, *Aspergillus niger* and *A. oryzae*) that are considered to be safe sources of food enzymes. Therefore, provided the genetic modification of the production organism, as the result of the use of either recombinant DNA or chemical mutagenesis, was well characterized, additional toxicological testing would not be required. However, as already described in the JECFA guidance (FAO, 2006; FAO/WHO, 2007a), information on other aspects of enzyme production would still be required (see Appendix in section 9.1.4.2(h) below). An acceptable daily intake (ADI) may be established.

- *Class II: Enzymes derived from sources that are NOT considered or presumed safe for consumption*

  For all enzymes that do not fall under any of the Class I subcategories listed above, chemical and microbiological specifications must be established. Similarly, enzymes from organisms that have not been previously reviewed by JECFA, although they may subsequently be considered Class I Type iii, require the submission of relevant microbiological, toxicological and chemical data. Each enzyme will be evaluated, and an ADI may be established.

  For enzymes produced by strains of microorganisms not previously evaluated by JECFA, information is required about the taxonomy, genetic background and other aspects related to the safety of the strain, and commercial use in foods (if any). Enzyme preparations produced by such microorganisms should not contain either antibiotic inactivating proteins at concentrations that would interfere with antibiotic treatment or transformable DNA that could potentially contribute to the spread of antibiotic resistance.

The absence of microorganism-derived secondary metabolites of toxicological significance in the enzyme concentrate also needs to be confirmed. This can be achieved by submitting the results of two genotoxicity (mutagenicity and clastogenicity) assays on the enzyme, as well as a short-term oral toxicity study. As an alternative to genotoxicity testing for the presence of undesirable secondary metabolites in the fermentation products, a detailed chemical characterization of the enzyme concentrate, including confirmation of the absence of toxicologically significant levels of toxic secondary metabolites (e.g. mycotoxins that are known to be generated by strains of the production microorganism or by species related to the production microorganism), can be performed using high-performance liquid chromatography or mass spectrometry. Such characterization must also be supported by detailed knowledge of the genomic sequence of the genetically modified microorganism to exclude the possible presence of genes capable of producing toxic secondary metabolites. Additional characterization of the enzyme protein would also be required, such as the inclusion of bioinformatics analyses to confirm the absence of any potential allergenic epitopes or significant amino acid sequence homology to known toxins.

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

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(h) Appendix: Information required for the safety assessment of enzyme preparations for use in foods

No.	Class(es)[a]	Information required	Details/rationale
Enzyme classification and description of active components of enzyme preparation			
1.	All	Name of enzyme(s)	e.g. Triacylglycerol lipase
2.	All	Systematic name(s) and number(s)	EC/IUBMB number; CAS number (where appropriate)
3.	All	Molecular weight(s)	As determined by SDS PAGE, gel filtration chromatography, etc.
4.	All	Amino acid sequence(s)	Predicted and determined primary amino acid sequence
5.	All	Catalytic activity	All reactions catalysed, including any secondary activities, conditions under which catalysis occurs (e.g. pH, temperature)
6.	All	Historical use(s) in food-based applications	Evidence of commercial food use, including from the parent strain or other strains in the lineage (e.g. as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in oil degumming)
7.	All	Use levels in food(s)	Express each use as TOS in mg/kg food, substrate or raw material – specify

No.	Class(es)[a]	Information required	Details/rationale
8.	All	Fate in final food(s)	Is the enzyme active, inactive or removed? How is the enzyme inactivated/removed?
9.	All	Existing safety evaluations	Include any existing health-based guidance values (e.g. ADI)
Details about the production organism			
10.	All	Identity of the production organism	Identify genus, species, strain
11.	I(iii), II	Host/recipient organism	Identify genus, species
12.	I(iii), II	Donor of genetic material	e.g. Identify origins of genetic material by genus, species (if native or modified)
13.	I(iii), II	Details of genetic modification:	
		i. To host genome	History of development of host strain (e.g. deletion of gene clusters that encode for aflatoxins, modifications that make host extracellular protease deficient or make it non-sporulating, etc.), identification of genes removed/ added

No.	Class(es)[a]	Information required	Details/rationale
		ii. Addition of rDNA (gene of interest from another microorganism) to host microorganism through mobile genetic elements	Donor of genetic material, details on how the genetic element was designed and the identity of genes on the element, stability information, copy numbers, whether it integrates or does not integrate into the host genome, etc. Evidence that genetic material does not contain genes coding for virulence factors, protein toxins or any enzymes that may be involved in the synthesis of mycotoxins
14.	I(iii), II	Genetic modification techniques	Site-directed mutagenesis, chemical mutagenesis, rDNA technology, etc.
15.	I(iii), II	Description of intended and nonspecific effects resulting from genetic modification and any changes carried out to prevent unwanted side reactions/products	e.g. An intended effect may be increased yield; a nonspecific effect may be activation of toxin production Rectification measures may include genetic modifications, specific fermentation conditions, etc.
16.	All	Deposit information (if applicable)	e.g. ATCC number

No.	Class(es)[a]	Information required	Details/rationale
Production of enzyme concentrate and preparation			
17.	All	Detailed manufacturing process	For enzymes in Class I(i) and Class I(ii), and Class II enzymes obtained from plants and animals, manufacturing details are required. For enzymes in Class I(iii) and Class II produced by microorganisms, include details describing controlled fermentation inputs and conditions, the steps taken to retain genetic modifications, and further processing, purification and concentration steps. Indicate how production strains are maintained under conditions that ensure the absence of genetic drift, and, when used in the production of enzyme preparations, indicate the methods and conditions that are applied to ensure consistency and reproducibility from batch to batch. Such conditions must ensure the absence of toxin production by the organism and prevent the introduction of microorganisms that could be the source of toxic or other undesirable substances.

No.	Class(es)[a]	Information required	Details/rationale
18.	All	Formulation ingredients	Identify the carriers, diluents, excipients, supports and other additives and ingredients (including processing aids) used in the production, stabilization and application of enzyme preparations; must be acceptable for food use In order to distinguish the proportion of the enzyme preparation arising from the source material as opposed to that contributed by diluents and other additives and ingredients, individual specifications require a statement of percentage TOS, which is defined as follows: % TOS = 100 − (A + W + D) where A = % ash, W = % water and D = % diluents and/or other additives and ingredients.
Specifications and data required for enzyme concentrates and preparations			
19.	All	Description	Physical form of the enzyme preparation – liquid, semiliquid or dried product
20.	All	Purity	Impurities, including elemental and microbiological impurities Analytical test methods, validation data, representative batch data (minimum of five batches) are required

No.	Class(es)[a]	Information required	Details/rationale
21.	All	Enzyme characterization	Enzyme activity (including method of assay, activity unit definition), molecular weight determination for the enzyme and other specific identification techniques. A universally usable test method to define enzyme activity present in the preparation should be submitted. Analytical test methods, validation data, representative batch data (minimum of five batches) are required.
22.	All	Analysis of at least five non-consecutive batches of the **enzyme concentrate** (for enzymes in Class II, at least one of which should have been used for toxicological testing)	e.g. TOS, enzyme activity, protein concentration, impurities, absence of antibiotic inactivating proteins, etc.
23.	All	Composition of at least five non-consecutive batches of the **product(s) of commerce** (enzyme preparation)	e.g. Stabilizers, pH adjustment agents, carriers, diluents, preservatives, etc.
24.	I(iii), II	Information on carryover of allergens from the fermentation media to the enzyme concentrate	Identification of major food allergens in media components and in the enzyme concentrate
25.	I(iii), II	Evidence for absence of rDNA and production organisms in the enzyme concentrate or the enzyme commercial product	This requirement applies only to enzymes produced with those production organisms that express DNA sequences of concern, e.g. antibiotic-resistant markers.

No.	Class(es)[a]	Information required	Details/rationale
Assessment of potential allergenicity of the enzyme			
26.	I(iii), II	Comparison of the amino acid sequence of the enzyme with known allergens	In silico comparison of primary amino acid structure with allergen databases to confirm the absence of sequence homology with known allergenic proteins: i. Sequence homology (35% of a sliding window of 80 amino acids) ii. Sequence identity in contiguous stretches of 8 amino acids within the enzyme sequence All the information resulting from the sequence homology comparison between an expressed enzyme and known allergens should be reported. If any of the identity scores equals or exceeds 35%, this is considered to indicate significant homology and needs to be scientifically considered in the context of a safety assessment for enzymes in food.
27.	I(iii), II	Proteolysis resistance/digestibility of the enzyme	e.g. Simulated gastric fluid studies, etc.
Toxicology			
28.	II	Results of toxicological testing of the enzyme concentrate	It is necessary to conduct toxicological studies in order to assess whether an ADI needs to be established: (a) 90-day oral toxicity test in a rodent species;

No.	Class(es)[a]	Information required	Details/rationale
			(b) Two short-term genotoxicity tests (mutagenicity and clastogenicity) 1. A test for gene mutations in bacteria 2. An in vitro micronucleus test
29.	I(iii), II	Bioinformatic analysis of the amino acid sequence for potential matches with known toxins	Explanation of the analysis and interpretation should be provided.
Dietary exposure assessment			
30.	II	Estimate of dietary exposure to the enzyme preparation calculated on the basis of the TOS. Separate dietary exposure situations may need to be considered, depending on whether they are for: (a) enzyme preparations added directly to food and not removed; (b) enzyme preparations added to food but removed from the final product according to GMP; or (c) immobilized enzyme preparations that are in contact with food only during processing.	Express the dietary exposure as mg TOS/kg body weight per day; provide an explanation of the methodology used to derive the estimated dietary exposure.

No.	Class(es)[a]	Information required	Details/rationale
31.		Additional information and comments	Additional items considered helpful in the safety assessment.

ADI: acceptable daily intake; ATCC: American Type Culture Collection; CAS: Chemical Abstracts Service; DNA: deoxyribonucleic acid; EC/IUBMB: Enzyme Commission/International Union of Biochemistry and Molecular Biology; GMP: Good Manufacturing Practice; rDNA: recombinant deoxyribonucleic acid; SDS PAGE: sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TOS: total organic solids

[a] Class I: Enzymes obtained from sources that are considered safe for consumption and for which toxicological evaluations are NOT normally required.

Type i: Enzymes obtained from edible tissues of plants or animals commonly used as foods: I(i).
Type ii: Enzymes produced by microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods: I(ii).
Type iii: Enzymes produced by a Safe Food Enzyme Production Strain or a Presumed Safe Progeny Strain: I(iii).

Class II: Enzymes derived from sources that are NOT considered safe for consumption and are not in any of the subcategories listed above.

9.1.4.3 Immobilizing agents

A number of procedures involving different chemical substances are used for immobilizing enzymes. These processes include micro-encapsulation (e.g. entrapment in gelatine to form an immobilized complex), immobilization by direct addition of glutaraldehyde, immobilization by entrapment in porous ceramic carrier and complexation

with agents such as diethylaminoethyl cellulose or polyethylenimine. Several agents may be used in the immobilizing process. Substances derived from the immobilizing material may be in the final product due to either the physical breakdown of the immobilizing system or impurities contained in the system.

The number of data necessary to establish the safety of the immobilizing agent depend on its chemical nature. The levels of residues in the final product are expected to be extremely low.

Some of the substances used in the preparation of immobilizing systems are extremely toxic. The levels of these substances or their contaminants permitted in the final product should be at the lowest levels that are technologically feasible, provided that these levels are below those of any toxicological concern. An ADI is not established, but there must be adequate safety for their approved uses.

9.2 Special considerations for nutrients and substances consumed in large amounts

9.2.1 Introduction

The safety assessment of substances that are consumed in relatively large amounts presents a number of special problems. Such materials include defined chemical substances such as the bulk sweeteners sorbitol and xylitol, modified food ingredients such as modified starches, nutrients and related substances, and non-traditional whole foods.

The safety assessment of such substances should differ from that of other food additives, such as colouring and flavouring agents and antioxidants, for the following reasons:

- Many will have a high daily intake; thus, minor constituents and processing impurities assume greater than usual significance.
- Even though they are often structurally similar or even identical to natural products used as food and thus may appear to be of low toxicity, they may require extensive toxicity testing because of their high daily intake.
- Some may be metabolized into normal body constituents.

- Some substances, particularly foods from novel sources, may replace traditional foods of nutritional importance in the diet.

- Many are complex mixtures rather than defined chemical substances.

- The difference between the maximum quantity that can be fed to laboratory animals in feeding tests without impairing the nutritional quality of the diet and the amount consumed by human beings is often relatively small on a body weight basis.

9.2.1.1 *Chemical composition, specifications and impurities*

Thorough chemical analysis should be performed on high-consumption substances to measure potential impurities and to provide information on nutritional adequacy, especially when such substances replace traditional food. It is not possible to provide a checklist of necessary chemical studies to cover all high-consumption compounds. However, the substance should be subjected to a full proximate analysis, and particular attention should be paid to the points discussed in the following paragraphs.

Because the intake of undesirable impurities concomitant with the intake of bulk ingredients is potentially high, special effort should be made to identify the impurities. Information on the production process, including the materials and procedures involved, will point to the types of contaminants for which limits may need to be specified. The specifications should be accompanied by details of product variability and of the analytical methods used to check the specifications and details of the sampling protocols. If the substance is so complex that comprehensive product specifications on chemical composition are impractical (as they might be, for example, for a microbial protein), the description of the substance in the specifications may include relevant aspects of its manufacturing process. If manufacturing data are based on production on a pilot scale, the manufacturer should demonstrate that, when produced in a large-scale plant, the substance will meet the specifications established on the basis of pilot data.

The permissible limits for impurities may in some cases correspond to the levels accepted for natural foods that have similar structure or function or that are intended to be replaced by the new

material. If the substance is prepared by a biological process, special attention should be paid to the possible occurrence of natural toxins (e.g. mycotoxins).

If the nature of the substance or manufacturing process indicates the possible presence of naturally occurring or adventitious antinutritional factors (e.g. phytate, trypsin inhibitors) or toxins (e.g. haemagglutinins, mycotoxins, nicotine), the product should be analysed for them specifically. Biological tests, either as part of the nutritional evaluation in the case of enzyme inhibitors or more specifically as part of a mycotoxin screening programme, will provide useful backup evidence concerning the presence or absence of these contaminants.

Finally, if, under the intended conditions of use, the substance may be unstable or is likely to interact chemically with other food components (e.g. degradation or rearrangement of the substance during heat processing), data should be provided on its stability and reactivity. The various tests should be conducted under conditions relevant to the use of the substance (e.g. at the acidity and temperature of the environment and in the presence of other compounds that may react).

9.2.1.2 *Nutritional studies*

With some substances, particularly novel foods, nutritional studies may be necessary to predict the likely impact of their introduction on the nutritional status of consumers. In addition to affecting the nutritional content of the diet, such substances may influence the biological availability of nutrients in the diet. The nutritional consequences of the introduction of such a substance in the diet can be judged only in the light of information about its intended use. Therefore, as much information as possible should be obtained about potential markets and uses, and the likely maximum consumption by particular subpopulations should be estimated. It is also possible to check the accuracy of premarketing predictions by use of post-marketing monitoring studies (see, for example, Allgood et al., 2001; Hlywka et al., 2003; Amanor-Boadu, 2004; Lea & Hepburn, 2006; Hepburn et al., 2008; and chapter 4, section 4.11.3).

9.2.1.3 Toxicity studies

When testing high-consumption additives, laboratory animals should generally be fed the highest levels that are consistent with palatability and nutritional status. Therefore, before beginning such studies, it is desirable to investigate the palatability of the test diet in the test animals. If a palatability problem is encountered, it may be necessary to increase the amount of the test substance to the required level gradually. Paired-feeding techniques should be used if the problem cannot be overcome. It should always be borne in mind that there are practical limits to the amounts of certain foods that can be added to animal diets without adversely affecting the animals' nutrition and health.

To ensure that the nutritional status of the test animal is not distorted, the test and control diets should have the same nutritive value in terms of both macronutrients (e.g. protein, fat, carbohydrate and total calories) and micronutrients (e.g. vitamins and minerals). When feeding substances at high levels, it is usually advisable to formulate diets from individual ingredients (rather than adding the test material to a standard laboratory diet) to provide the same nutrient levels in the control and test diets. Comprehensive nutrient analyses of the test and control diets should be performed to ensure that they are comparable. Sometimes nutritional studies are advisable before toxicological studies are performed to ensure that test diets are correctly balanced. Without due regard to nutritional balance, excessive exposure may mean that a study investigates the adverse effects of long-term dietary imbalance rather than the toxic effects of the substance.

Metabolic studies are useful and necessary for assessing the safety of high-consumption additives. With complex mixtures, studies on the metabolic fate of every constituent would be impractical. However, if contaminants or minor components are suspected as the cause of toxicity, their metabolism should be investigated. If the material, or a major component of it, consists of a new chemical compound that does not normally occur in the diet (e.g. a novel carbohydrate), studies of the metabolic fate of the new compound would be appropriate.

If biochemical and metabolic studies show that the test material is completely broken down in the food or in the gastrointestinal tract to substances that are common dietary or body constituents, then other

toxicity studies may not be necessary. The results of metabolic studies can stand on their own if it is shown that breakdown into these common constituents occurs under the conditions of normal consumption of the material, that the material contributes only a small proportion of these common constituents in the daily diet and that side reactions giving rise to toxic products do not occur.

Analysis of urine and faeces may provide important information relating to changes in normal excretory functions caused by the test substance. For example, the gut flora may be altered or preferential loss of a mineral or vitamin may occur, resulting in detrimental effects on the health of the test animals. If the substance is incompletely degraded or not degraded by the digestive enzymes of the stomach or the small intestine, appreciable concentrations may be found in the faeces or in the distal gut compartments. Such substances may also induce laxation. As a result, changes in the absorption of dietary constituents or changes in the composition and metabolic activity of the intestinal flora may be observed. Because of anatomical differences in the digestive tract and because of considerable differences in the composition of the basal diet, such effects may occur only in humans but not in rodents, or vice versa. Therefore, short-term studies should be performed in laboratory animals and humans (if possible; see chapter 4, section 4.11), in which variables likely to be affected by the test compound are examined in detail. It is especially important to investigate questions relating to whether the eventual effects are progressive or transient and whether they occur in subjects exposed to the compound for the first time or in subjects adapted to a daily intake of the substance. Clearly, no standard design for such studies can be devised. Only a thorough knowledge of the nutritional and biochemical literature can serve as a guideline.

Separate toxicological tests should be performed on toxicologically suspect impurities or minor components present in the test material. If any observed toxicity can be attributed to one of the impurities or minor components, its maximum level should be established in the specification.

Because of the relative non-toxicity of high-consumption additives, toxicity tests in animals may not show any adverse effects even at the highest dose tested. When establishing an ADI, the traditional

concept of utilizing a 100-fold safety factor is often not possible if the human consumption level is high and feeding studies do not produce adverse effects. In such cases, new approaches are indicated. It may be possible, for example, to establish a large safety margin between the highest dose tested and the expected consumption of such substances by humans. Or the ADI may be set on the basis of a smaller safety factor, which may be permissible when aspects such as similarity to traditional foods, metabolism into normal body constituents and lack of overt toxicity are considered. For a compound, such as a bulking agent, that may influence the nutritional balance or the digestive physiology by its mere bulk and that may be absorbed from the gut only incompletely or not at all, it may be more appropriate to consider the dose level in terms of the percentage inclusion in the diet. If several similar types of compounds are likely to be consumed, a group ADI (limiting the cumulative intake) should be allocated.

The results of human studies, which are discussed in relation to novel foods in section 9.2.3, may allow the use of a lower safety factor than that obtained from laboratory animal studies.

9.2.2 Nutrients and related substances

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. In turn, there has been growing interest in an international basis for determining the levels of intake that may pose a risk. JECFA has evaluated the safety of several substances that were claimed to have nutritional or health benefits. The sixty-third JECFA noted that whether such products meet appropriate definitions as nutrients or are worthy of health, nutrient or other claims was outside its remit (FAO/WHO, 2005). Therefore, JECFA reiterated that it would evaluate only the safety of these ingredients and expressed the view that its evaluation of the safety of these ingredients should not be interpreted to mean that the Committee endorses the use of these substances for their claimed nutritional or health benefits.

JECFA has assigned ADIs for several nutrients or determined "no safety concern" under the proposed conditions of use (e.g. L-5-methyltetrahydrofolic acid; FAO/WHO, 2006a).

In the risk assessment for non-nutrients, it is assumed that:

- the substance has no desirable or essential physiological roles;
- homeostatic mechanisms for the specific substance do not exist and/or detoxification pathways are not likely to be chemical specific; and
- there are no health risks if the intake is zero.

Unlike non-nutrients, nutrient substances are biologically essential or have a demonstrated favourable impact on health at specified levels of intake. This consideration influences approaches used to adjust for uncertainty associated with the data used to estimate a health-based guidance value, such as an upper level of intake (UL), and also 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.

The relationship between intake and risk for nutrient substances is illustrated in Figure 9.3. For most essential nutrients, homeostatic mechanisms that maintain the amount of nutrient substance in the body within a physiological range are associated with both low and high levels of intake. Should intakes increase or decrease, it is assumed that homeostatic responses of some type occur and that the responses may vary by age, sex or life stage. However, homeostatic adaptations have a limited capacity and can be overwhelmed by excessive intake. At the extremes, as the capacity of a homeostatic mechanism is exceeded, the incidence or impact of specific adverse health effects is likely to increase. Nutrient substances that are not established as essential may also show dual curves, with the left-hand curve reflecting the failure to optimize health. The distinctions between essentiality and a demonstrated favourable health impact require further elucidation and clarification as data evolve.

Several international working groups have provided guidance for the risk assessment of nutrients and related substances (IPCS, 2002; Renwick et al., 2003, 2004; FAO/WHO, 2006b). For the safety evaluation of nutrients and related substances, these groups recommended the use of the UL, which is defined as the maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans.

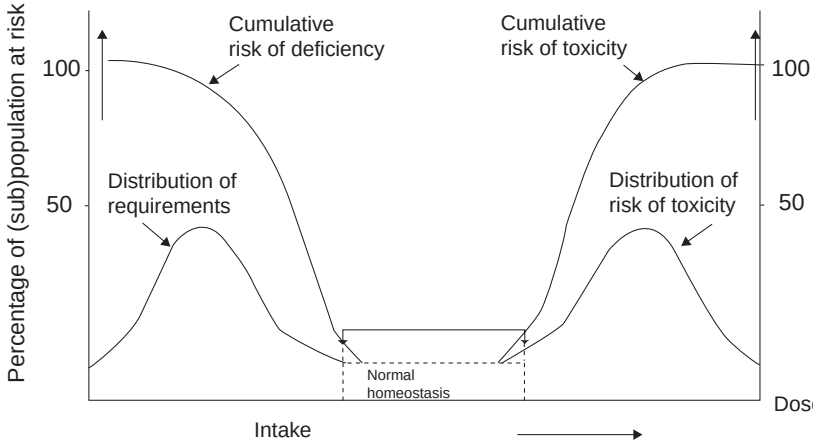

Fig. 9.3. Dual curves for risk relationship of nutrients: percentage of (sub)-population at risk of "deficiency" and then "adverse health effects" as intakes move from low to high (modified from IPCS, 2002)

The UL is not a recommended level of intake but an estimate of the highest level of regular intake that carries no appreciable risk of adverse health effects (criteria for setting a UL are discussed in section 9.2.2.2). As with all health-based guidance values, exceeding the UL is not in itself an indication of risk, but the UL does not give any indication of the magnitude of risk that may be associated with intakes in excess of the UL.

Where possible, ULs that apply to all groups of the general population, including all life stages, should be established. A generally applicable UL can be used with data from intake assessments to identify those individuals or population groups potentially at risk and the circumstances in which harm is likely to occur. However, ULs for nutrients may vary with age or for specific groups (e.g. sex and life stage, including pregnancy) because of different balances between requirements and sensitivities to adverse effects. The WHO review of the principles and methods for the assessment of risk from essential trace elements pointed out age-related factors associated with variable responses to levels of intake (IPCS, 2002). The FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) concluded that the most appropriate approach is to develop separate ULs for age, sex and life stage subpopulations. As the data allow, the ULs

can be based on different end-points as applicable to the sensitivity of the subpopulation.

The appropriateness of a UL established for adequately nourished (sub)populations cannot be assumed to transfer to inadequately nourished (sub)populations. For example, an intake well above the UL may be recommended clinically to correct a deficiency. Although the basic process of nutrient risk assessment decision-making would remain the same regardless of the nutritional status of the (sub)population of interest, it is likely that inadequately nourished (sub)populations would need a different set of ULs because of important differences in metabolism and the vulnerability that can result from these differences. However, it should be noted that too little is known about the effects of inadequate nutrition on the absorption, distribution, metabolism and elimination of nutrient substances to allow specification of considerations relevant to adjusting ULs to make them appropriate for inadequately nourished (sub)populations.

The UL is not meant to apply to individuals receiving the nutrient under medical supervision or to individuals with predisposing conditions that render them especially sensitive to one or more adverse effects of the nutrient (e.g. those with genetic predisposition or certain metabolic disorders or disease states).

For some nutrient substances, no credible evidence has demonstrated adverse health effects even at the highest intake used or observed. Vitamin B12 is an example of such a nutrient substance (IOM, 1998). In such cases, the biological threshold for an adverse health effect, if it exists, may be many times higher than the highest intake studied. Lacking data, however, this amount is not known. If no studies have revealed adverse health effects for a nutrient substance but the risk manager needs scientific advice concerning an upper intake, the FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) recommended that the highest observed intake (HOI) be used to give guidance. The HOI is defined as the highest level of intake observed or administered as reported within a study of acceptable quality. It is derived only when no adverse health effects have been identified.

There are some special considerations for the risk characterization of micronutrients and macronutrients (Renwick et al., 2003).

Micronutrients are vitamins and minerals that are essential for normal growth and physiological and biochemical functioning. It should be noted that micronutrients used in dietary or food supplements and fortified foods may be in different physical or chemical forms from those present naturally in the food or endogenously in the body. Macronutrients include dietary fats, proteins and carbohydrates, as well as their subcomponents and substitutes. In addition to those substances currently considered as macronutrients, these considerations can also be appropriate for the risk characterization of new substances, including dietary supplements and functional foods. Decision trees that could be considered for the risk characterization of micronutrients and macronutrients are given in Figures 9.4 and 9.5, respectively (Renwick et al., 2003). These are not intended to cover all eventualities, but indicate some matters of particular concern.

9.2.2.1 *Adverse health effects of nutrients and related substances—general concepts*

The general concepts concerning adverse health effects of nutrients have been described by Renwick et al. (2004). An adverse health effect has been defined as any impairment of a physiologically important function that could lead to an adverse health effect in humans (IOM, 1998) and as any change in morphology, physiology, growth, development or lifespan of an organism that results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences (IPCS, 2004). Indicators of adverse health effects, which may be used for the derivation of the UL, range from biochemical changes without adverse health effects through to irreversible pathological changes in the functioning of the organism (Figure 9.6). In practice, because of limited availability of data on adverse effects in humans, and as biochemical indicators of adverse effects are often not available, adverse effects selected for establishing ULs may cover the full range indicated in Figure 9.6, including clinical outcomes.

There is an established paradigm for determining safe intakes of foreign compounds, such as food additives, based on the dose–response relationship for adverse effects in laboratory animals or humans (see Edler et al., 2002 and chapter 5). For most types of toxicity from either

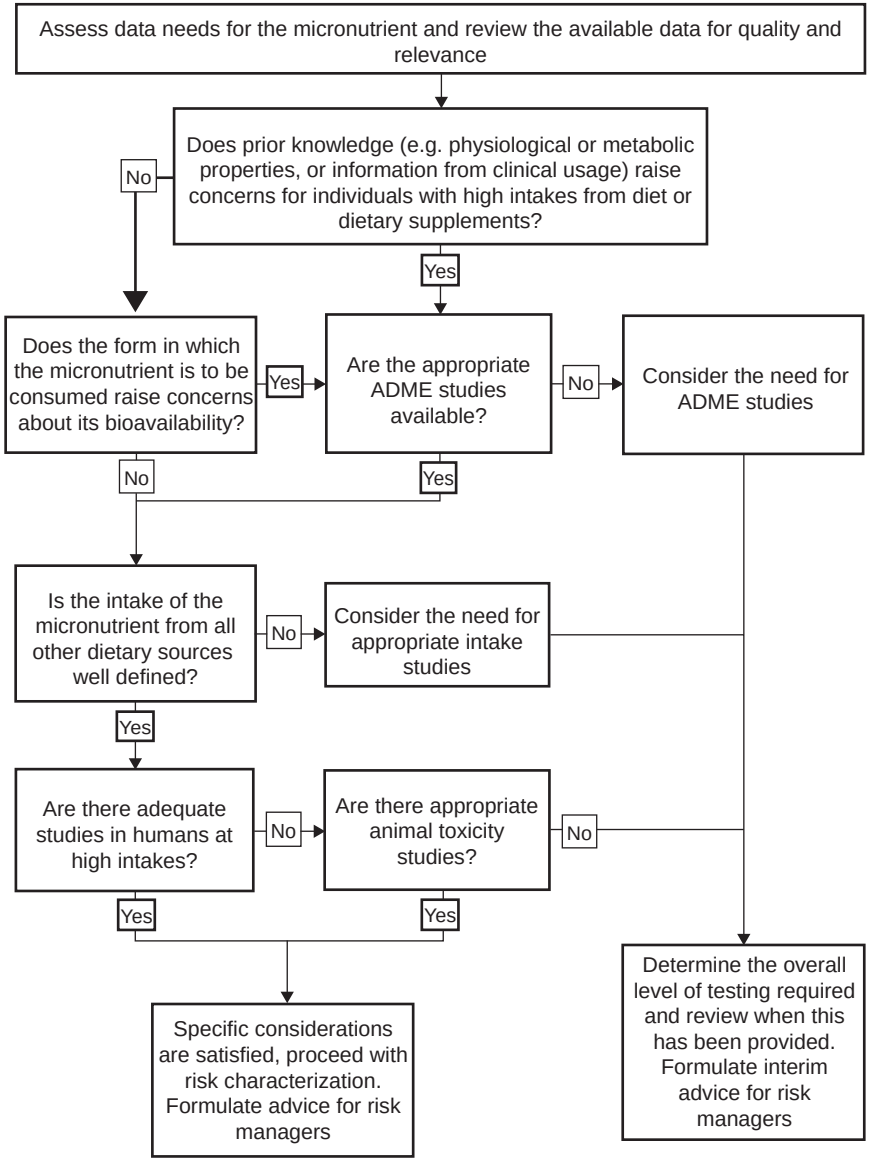

Fig. 9.4. Decision tree outlining the special considerations for the risk characterization of micronutrients (adapted from Renwick et al., 2003) [ADME, absorption, distribution, metabolism, excretion]

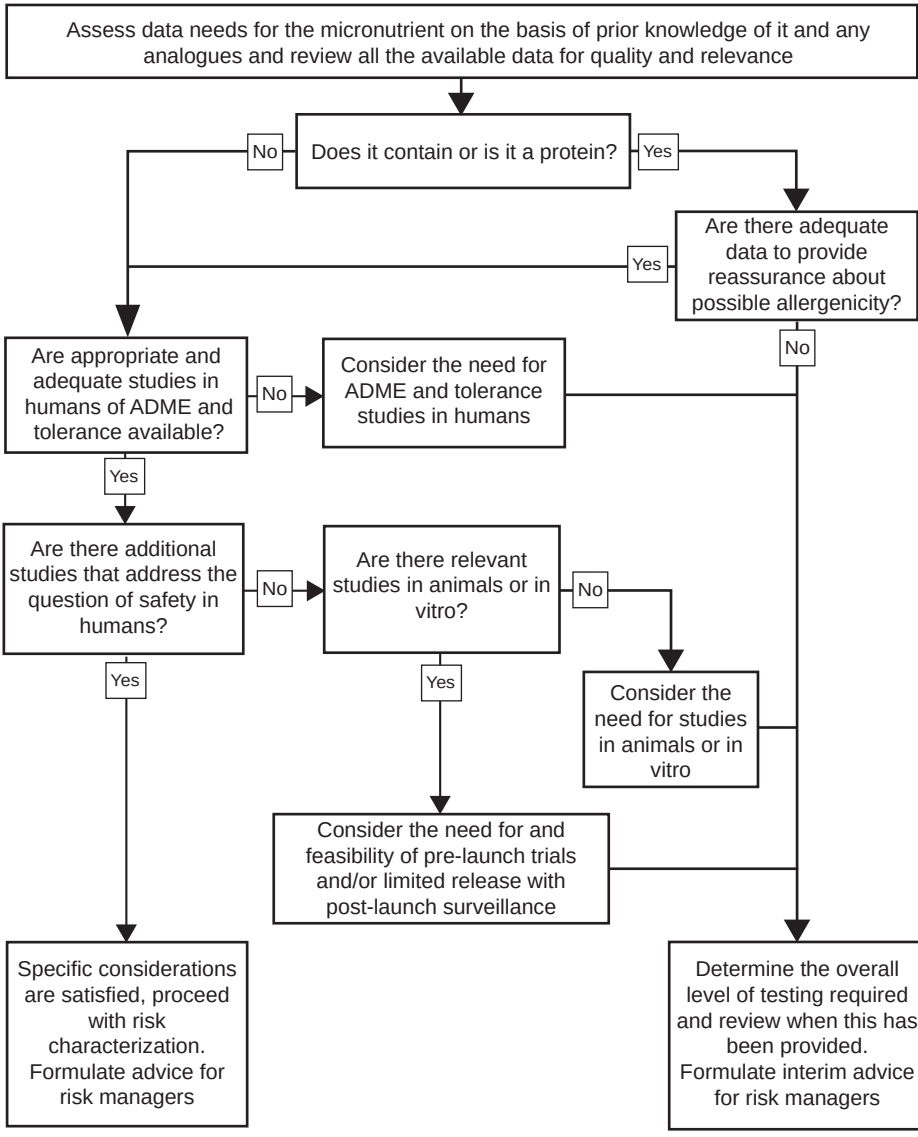

Fig. 9.5. Decision tree outlining the special considerations for the risk characterization of macronutrients (adapted from Renwick et al., 2003)

1. Biochemical changes within the homeostatic range and without indication of adverse sequelae

↓

2. Biochemical changes outside the homeostatic range without known sequelae

↓

3. Biochemical changes outside the homeostatic range that represent a biomarker of potential adverse effects due to excess

↓

4. Clinical features indicative of a minor but reversible change

↓

5. Clinical features of significant but reversible effects

↓

6. Clinical features indicative of significant but reversible organ damage

↓

7. Clinical features indicative of irreversible organ damage

Fig. 9.6. Identifying adverse health effects: sequence of "effects" in increasing order of severity (adapted from Renwick et al., 2004; "features" includes signs and symptoms)

foreign compounds or nutrients, there is believed to be a threshold dose (or intake) below which adverse health effects are not produced. Thresholds for any given adverse effect vary among members of the population. In general, there are insufficient data to establish the distribution of thresholds within the population for individual adverse effects, and uncertainty factors are used to allow for human variability (and for species differences, when necessary) (Edler et al., 2002).

Steps 4 through 7 in Figure 9.6 represent adverse health effects manifesting specific clinical features such as signs and symptoms, and for this reason they can be used readily for risk assessment in the usual manner. However, some of the effects that occur prior to step 4 could constitute appropriate "biomarkers". Because such effects can reflect "critical events", they could serve as surrogates or biomarkers for adverse health effects. However, it should be noted that biochemical effects without functional significance should not be regarded as adverse health effects (IPCS, 2002).

The following criteria have been proposed for the use of these indicators of adverse health effects (FAO/WHO, 2006b):

- The optimal end-point for use in setting a UL would be an effect at step 3 and possibly step 2, with steps 4–7 reflective of clinical features such as signs or symptoms. Step 2 may be applicable in some cases in which sufficient information is available to suggest that changes outside a homeostatic range that occur without known sequelae would be relevant as a surrogate for an adverse health effect.

- The increased use of valid, causally associated biomarkers as surrogates for adverse health effects is desirable for the purposes of nutrient risk assessment. After identifying the sequence of observable effects in the causal pathway for adverse health effects—from initial nonspecific biochemical changes to clear clinical outcomes—if the biomarker meets other relevant criteria, including causal association, biochemical changes outside the homeostatic range can be relevant surrogates for adverse health effects associated with nutrient substances.

9.2.2.2 *Deriving the UL*

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. A pivotal point in the assessment process is the selection of the critical adverse health effect. This is the effect upon which the UL is based—or, more specifically, the effect upon which a set of ULs for the various age, sex and life stage subpopulations is based. The critical adverse health effect is usually the effect that occurs at the lowest level of excessive intake within the (sub)population of interest or at the lowest experimental dose if only laboratory animal data are available. For a given nutrient substance, different critical adverse health effects may be selected for the different age, sex and life stage subpopulations, because metabolic and physiological differences among these subpopulations mean that adverse health effects may manifest differently. Issues related to the physiological severity of the adverse health effect are considered separately rather than as a component of selecting the critical adverse health effect (FAO/WHO, 2006b).

Once the critical adverse health effect is identified, the process moves to deriving the UL. Again, iterations may occur between this activity and those conducted under hazard identification. The first step is to analyse and describe clearly the relationship between the intake of the nutrient substance and the onset of the adverse health effect for those age, sex and life stage subpopulations for which data are available. The analysis (see also chapter 5) is called the intake–response assessment, and its outcome is the determination of one or more of the following three values, depending upon the nature of the existing evidence:

1) a benchmark dose (BMD) (or benchmark intake [BI]): the intake of a substance that is expected to result in a prespecified level of effect (the benchmark response [BMR]; see chapter 5);
2) a NOAEL: the greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism under defined conditions of exposure (IPCS, 1994); or
3) a lowest-observed-adverse-effect level (LOAEL): the lowest concentration or amount of a substance, found by experiment or observation, that causes a detectable adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism under defined conditions of exposure (IPCS, 1994).

The NOAEL and LOAEL are based on observed intake levels that are set as part of the study design. Neither takes into account the shape of the intake–response curve that would be seen at other levels of intake. If data allow, the specification of a BMD (BI) permits the derivation of the ULs to be carried out with greater certainty. In any case, any of the three values can serve as the starting point for deriving the UL. The BMD (BI) approach can be particularly useful when the adverse health effect is seen within the range of the current levels of human intake and a NOAEL cannot be identified. This would apply to sodium, for example. Under such circumstances, the BMD (BI or lower confidence limit of the BI, the BIL) is useful, because it defines a point on the intake–response curve that is reliable and relevant to the minimization of the risk of adverse health effects that result from high intake.

Overall, the data sets available for nutrient substances usually are not designed to assess intake–response for adverse health effects. Therefore, not only is the estimation of a BMD (BI) problematic, there are challenges associated with establishing the NOAEL or LOAEL. In addition, the uncertainties and limitations of the usual data sets could, in most cases, result in a value for the lower confidence limit of the BMD (BMDL) (see chapter 5) that was so low that it might lead to nutritional inadequacy. Study quality and design for both human and laboratory animal data are notable issues for the NOAEL (or LOAEL), and they should be considered carefully. Several "study-dependent" factors that influence the magnitude of the value observed include the group size, the sensitivity of the methods used to measure the response, the duration of intake and the selection of intake levels. For laboratory animal studies, important factors include species, strain, sex, age and developmental status.

The NOAEL or LOAEL cannot be used as the final value for the UL—except in the unlikely situation that the value was derived from a large study that is truly representative of the exposed population and contains no uncertainties and negligible errors. Given that available data will usually contain uncertainties, risk assessment principles stipulate that the risk assessor must take these into account. Therefore, an allowance is made for these uncertainties by establishing a UL at some value less than the NOAEL or LOAEL. A similar allowance would need to be made if a BMD (BI) were to be used, but only the NOAEL and LOAEL were discussed at the FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b).

Following the identification of a NOAEL, LOAEL or BMD (BI), allowances for uncertainty must be made in order to establish a UL. If needed, this is followed by scaling or extrapolating the data to derive ULs for those age, sex and life stage subpopulations for which no data are available. If available data allow, a quantitative allowance for uncertainties may be applied to the NOAEL, LOAEL or BMD (BI) value derived from the intake–response assessment. The first consideration is whether there are sufficient data to make a quantitative allowance for uncertainty: that is, do the data allow the magnitude of uncertainty or variability to be defined? This consideration is equivalent to the determination of a chemical-specific adjustment factor (CSAF) for a non-nutrient substance (see chapter 5, section 5.2.3).

Quantitative allowances are data-derived factors that can be applied to the NOAEL or LOAEL to derive a lower (or sometimes higher) health-based guidance value (a UL), based on information relevant to the target population but not addressed in the data used to derive the values. These adjustments are objective and based on specific data, and they can relate to either kinetic or dynamic aspects of the nutrient substance in different species (IPCS, 1994). While quantitative allowances are theoretically possible for all uncertainties, in practice available data usually allow relatively few quantitative allowances to be made when setting the ULs for nutrient substances. One example of the use of quantitative allowances is the process used to address differences in body size between test animals and humans. Bioavailability is another uncertainty for which quantitative allowances may be used, particularly when data are available for different forms of the same nutrient substance. This allowance could, in principle, lead to setting different ULs for different forms of the nutrient substance—for example, the nicotinic acid and nicotinamide forms of niacin.

Generally, however, allowances for uncertainty must make use of uncertainty factors. Application of the default uncertainty factors that are used for non-nutrient substances poses a potential problem for nutrient substances: the resulting UL could be a value that is below the intake required to ensure nutritional adequacy. This issue arises primarily for those nutrient substances that have recommended intakes that are relatively close to intake levels that may pose a risk; examples commonly quoted include iron, zinc, copper and sometimes calcium. It is now widely recognized that the use of large generic default factors is not usually applicable to nutrient risk assessment. Instead, uncertainty factors used in nutrient risk assessment require consideration on a case-by-case basis and must be placed within the context of established intake requirements.

The FAO/WHO Technical Workshop on Nutrient Risk Assessment (FAO/WHO, 2006b) concluded that it is preferable to develop a composite uncertainty factor case by case rather than apply separate uncertainty factors for different issues. The substance-specific composite factor for uncertainty is applied to the NOAEL or LOAEL after any available quantitative allowances have been made. Because the risk assessment of nutrient substances has to consider both toxicity and essentiality, the use of a composite factor increases the likelihood that

the final value will not be so large as to result in a UL that is lower than the required intake of the nutrient substance. The impact of uncertainty considerations related to the toxicity data must be checked against the level of recommended intake for biological essentiality or for normal health. After uncertainties are taken into account, the resulting value is the UL for the specified subpopulation. When data are insufficient for setting a UL for one or more age, sex and life stage subpopulations (as often is the case), the gap is filled by adjusting a UL that has been established for another subpopulation. Therefore, although it is desirable to establish ULs based on data and end-points, such as differences in the metabolism, homeostatic mechanisms and toxicokinetics between children and adults, in the absence of such data, appropriate scaling is needed. Adjusting or scaling an adult UL into a UL relevant to children may be undertaken by correction using:

- the quantified reference body weight established for the age group;
- body surface area, which is calculated using the reference body weight taken to the power of 0.66 (i.e. $BW^{0.66}$); or
- energy requirement, which is sometimes referred to as metabolic body weight and is calculated using the reference body weight taken to the power of 0.75 (i.e. $BW^{0.75}$).

Because nutrient substances usually are components of normal intermediary metabolism, scaling on the basis of either surface area (i.e. $BW^{0.66}$) or energy requirement (i.e. $BW^{0.75}$) is likely to be more appropriate.

Quantitative data on the dietary intake of a nutrient substance by the (sub)population of interest are required to estimate the proportion of the (sub)population that is likely to exceed the UL. Data on the basis for derivation of the UL and other information gleaned from hazard identification and characterization are essential for describing the risk associated with intake above the UL.

There are several special considerations for the intake assessment for nutrients and related substances. The exposure or intake assessment is population relevant rather than globally relevant. That is, it is dependent on the types of foods and supplements consumed and on dietary patterns within a region or nation-state. This means that risk characterizations can be inherently different depending upon the

target population. This difference holds true even when the derivation of the UL is conducted in a consistent manner using internationally applicable guiding principles. There are wide variations in data types used for dietary intake assessment and in the methods of analysis and presentation of the findings. The FAO/WHO Technical Workshop on Nutrient Risk Assessment reviewed in detail the approaches to nutrient intake assessment and proposed harmonized protocols to improve these data (FAO/WHO, 2006b).

9.2.3 Foods from novel sources

Developments have made possible the production of foods from unconventional sources (e.g. fungal mycelia and yeast cells). In addition, so-called "exotic" fruits and vegetables are being introduced from their region of origin to other regions. Foods that are well known and traditional in one country or region may be unknown and thereby novel in another country or region.

These foods are intended for consumption, either directly or after simple physical modification to provide a more acceptable product. They may be consumed in large amounts, even by infants and children, particularly if they are permitted for use as protein supplements in otherwise protein-deficient diets.

Although the definition of what constitutes a novel food is basically a risk management decision, the following working definitions have been proposed (adapted in part from IPCS, 1987 and Knudsen et al., 2005):

- *History of safe use for a food*: Term used for the qualified presumption of safety. There is evidence for the safety of the food from compositional data and from experience since the food has been an ongoing part of the diet for a number of generations in a large, genetically diverse population. This presumption is for a certain context of use (conditions of use, defined part of the plant used and required processing) and allows for minor population predispositions, such as intolerance and allergenicity.

- *Traditional foods*: Foods that have a history of significant human consumption by the broad community for several generations as

part of the ordinary diet at the global, regional or local level or as a part of an ethnic diet.

- *Non-traditional foods*: Foods that do not have a history of significant human consumption by the broad community for several generations as part of the ordinary diet.

- *Novel foods*: Non-traditional foods for which there is insufficient knowledge in the broad community to ensure safe use or that have characteristics that raise safety concerns due to composition, levels of undesirable substances, potential for adverse effects, traditional preparation and cooking, and patterns and levels of consumption. These include food or food ingredients produced from raw materials not normally used for human consumption or food that is severely modified by the introduction of new processes not previously used in the production of food.

- *Foods for special dietary uses*: Those foods that are specially processed or formulated to satisfy particular dietary requirements that exist because of a particular physical or physiological condition or specific diseases and disorders and that are presented as such. These include foods for infants and young children. The composition of these foodstuffs must differ significantly from the composition of ordinary foods of comparable nature, if such ordinary foods exist.

A decision tree for points that could be considered in the evaluation of whole foods has been proposed by Renwick et al. (2003) and is shown in Figure 9.7.

9.2.3.1 *Chemical composition*

Complete chemical identification of whole foods may not be feasible, but specifications are necessary to ensure that levels of potentially hazardous contaminants, such as mycotoxins and heavy metals or other substances of concern, are kept to a minimum. Toxicological evaluations must be closely related to well-defined materials, and evaluations may not be valid for all preparations from the same source material, if different processing methods are used.

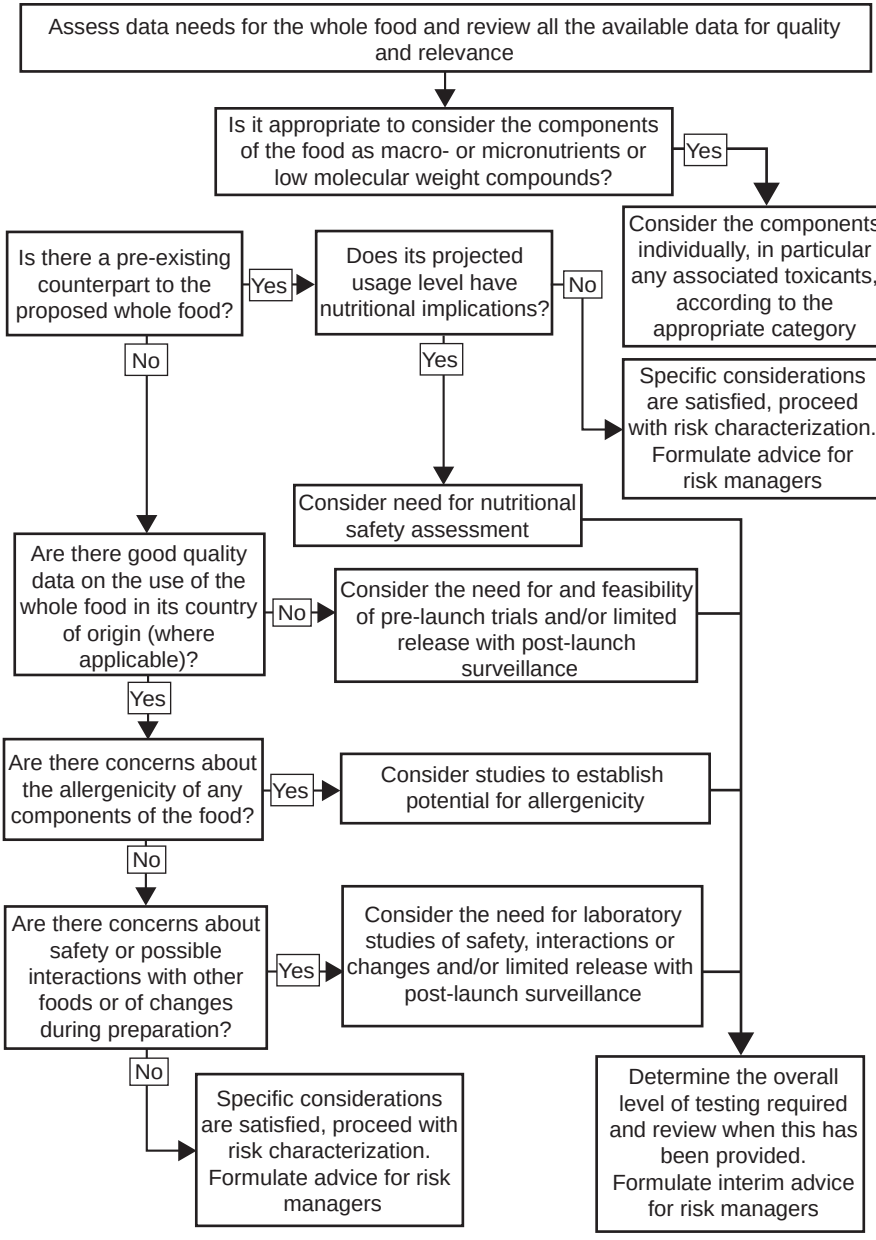

Fig. 9.7. Decision tree outlining the special considerations for the risk characterization of whole foods (adapted from Renwick et al., 2003)

9.2.3.2 Nutritional considerations

When a novel food is intended to replace a significant portion of traditional food in the diet, its likely impact on the nutritional status of consumers requires special consideration.

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). In order not to adversely affect the nutritional quality of the diet, it may be necessary to fortify the substance with vitamins, minerals or other nutrients.

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. The possible influence of components of the novel food, such as antinutritional factors (e.g. inhibitors of enzyme activity or mineral metabolism), on the nutritional value or keeping quality of the remainder of the diet should also be established.

9.2.3.3 Toxicological evaluations

Depending on the nature and intended uses of the novel food, studies in laboratory animals may be needed to supplement the chemical studies. If the novel food is intended to be an alternative significant supply of protein, tests on its protein quality will be necessary. In vivo studies will also be needed when it is appropriate to determine 1) the availability of vitamins and minerals in the novel food in comparison with the food it would replace and 2) any interaction the novel food might have with other items of the diet that would reduce the whole diet's nutritional value. If the novel food is expected to play an important role in the diet, it may be necessary to verify that the results of laboratory animal studies can be extrapolated to humans by measuring the availability of nutrients to human subjects.

In most cases, novel foods constitute a large percentage of the daily diet in laboratory animal studies because they are of a non-toxic nature. Therefore, the considerations discussed in section 9.2.1.3 apply to the toxicological testing and evaluation of foods from novel sources.

9.2.3.4 Human data

The general principles of studies in humans have been set out in section 4.11 of chapter 4. Human studies on novel foods need to be designed on a case-by-case basis. Human studies should not be embarked upon until there has been a full appraisal of the safety of the novel food using all available data (e.g. history of safe use, data on chemical and microbiological impurities, composition and toxicology). After the launch of a novel food on the market, post-marketing surveillance studies may also be helpful in providing confirmation of anticipated usage patterns and exposure levels. It may be necessary to conduct allergenicity studies on the novel food because of its composition (e.g. if it is highly proteinaceous) or because the results of laboratory animal or human feeding studies suggest that the food might produce hypersensitivity in some people. Important information can be gained by monitoring the health of workers, such as laboratory staff and employees in the manufacturing plant, coming into contact with the novel food. It is not realistic to strive for absolute absence of risk for allergenicity, and the aim of any study should be to ensure that a novel food is at least as safe as its traditional counterpart (i.e. the food that it will replace in the diet).

9.2.3.5 History of use

Human experience, but normally not formal human scientific studies, is an essential part of the data collection in the history of use. The human experience with respect to the consumption of a certain food in a region different from the one that has deemed the food to be novel is normally just an empirical observation that the food in question has been eaten for generations in that region. It will normally be coupled with information on how it is prepared, how it is eaten, how much is eaten and whether the food in question has had any special claims linked to it. This kind of information is often anecdotal and not scientifically well documented and is a history of "use"; however, owing to the absence of health measurements, it is not a history of "safe use".

The following information can be considered for the evaluation of a history of use (adapted from Health Canada, 2006):

- Historical evidence indicating ongoing, frequent consumption by a cross-section of the population where it has been used over several generations. This evidence may be derived from various

sources, including, but not limited to, scientific publications and patents, non-scientific publications and books, cookbooks, books on the history of food culture or affidavits from two or more independent, reputable authorities that include well-documented accounts of the way in which the food is used and how they know it has the history it does. Limited usage or short-term exposure would not be adequate to demonstrate a history of safe use.

- A declaration of any possible adverse effects linked to the food documented in its country of origin or a country where there is a high degree of consumption.

- A description of the standard methods of commercial or domestic processing and preparation for consumption.

- A description of how the food is cultivated or (if from wild sources) harvested.

- Amounts of the food that people are likely to consume, including typical serving sizes and expected frequency of consumption, at both average and high consumption levels.

- Analysis of the composition of the food based on randomly selected, statistically valid samples. This analysis should include proximate data as well as amino acid profile, fatty acid profile, mineral and trace mineral composition and vitamin composition, as well as any nutrients, antinutrients or bioactive phytochemicals in the product that are known to be of particular interest. The analysis should pay special attention to the presence of compounds in the food that may have implications for the health of any subgroups of the population (e.g. possible toxicants or allergens or unusually high levels of nutrients in the food source or final food product).

- Metabolism or gastrointestinal effects in humans.

9.2.3.6 *Exposure assessment*

For novel foods, exposure will need to be estimated from proposed uses. For many novel foods, accurate prediction of the likely commercial success, and therefore intakes, is particularly difficult. Therefore, post-launch monitoring can be essential to verify that the

risk characterization was appropriate to the exposure. Information on the intended or anticipated uses of the novel food is essential for the assessment of whether the uses will be safe or will constitute a risk. For exotic fruits and vegetables, experience from the region from which they originate can provide helpful information; consumption patterns must be considered in the local context of the novel use proposed. A food traditionally consumed only occasionally or exclusively in combination with another material may cause problems when consumed in larger quantities or in a different combination.

The exposure assessment should also consider the appropriate ways of preparing and cooking the novel plant food. Some are to be eaten raw; some are to be milled to flour and go through baking processes; some are to be peeled and cooked; some are to be extracted, treated with acids or bases, dried and fried. All these processes greatly influence the contents and digestive availability of inherent toxicants, macronutrients and micronutrients of the individual novel food as assessed in the hazard characterization.

9.2.3.7 Risk characterization

For the risk characterization of novel foods, the margin of exposure (MOE) approach may be suitable. The MOE is calculated from the estimated daily safe intake divided by the likely human daily exposure. This value can then be used by the risk managers to guide further decisions on the use of the novel plant food in the general food supply and—if properly indicated on the food—by the individual consumer to guide his or her choice for proper food that meets individual expectations and needs.

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