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EVALUATION OF CERTAIN FOOD ADDITIVES

Fifty-first report of the
Joint FAO/WHO Expert Committee on
Food Additives

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 42, 1999.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 6. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 9 to 18 June 1998. The meeting was opened by Dr M. Younes, Chief, Assessment of Risks and Methodology, International Programme on Chemical Safety, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr Younes noted the increasing importance in the work of the Committee of intake assessments, which are carried out in response to requests for advice by the Codex Committee on Food Additives and Contaminants in the course of its development of the General Standard for Food Additives and the General Standard for Contaminants and Toxins in Food.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been 50 previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the forty-ninth meeting (Annex 1, reference *131*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
— to undertake toxicological evaluations of certain food additives and flavouring agents (sections 3 and 4 and Annex 2);
— to assess the intake of certain food additives (section 5 and Annex 2); and
— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 6 and Annex 2).

2.1 Modification of the agenda

Montanic acid esters were removed from the agenda because data were not submitted. Methylmercury was removed because some of the relevant studies needed for the evaluation had not yet been completed. Furfural was added to the agenda at the request of an international organization. (–)-Menthol ethylene glycol carbonate, a mixture of (–)-menthol 1- and 2-propylene glycol carbonate, (–)-menthone 1,2-glycerol ketal, (±)-menthone 1,2-glycerol ketal, mono-menthyl succinate and 1-ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenoate) were also added to the agenda at the present meeting.

On the basis of comments received at the Thirtieth Session of the Codex Committee on Food Additives and Contaminants (*2*), 11 substances were added to the agenda for review of the specifications only: citric acid, carthamus yellow, calcium propionate, sucrose esters of fatty acids, potassium sorbate, sodium sorbate, calcium sorbate, aluminium powder, talc, microcrystalline cellulose and gum arabic.

Six substances were added to the agenda for review of the specifications only at the initiative of FAO, by the Committee itself, or in response to requests of other interested parties: ferrous sulfate, furfuryl alcohol, polydextroses, sorbitan monolaurate, xanthan gum, and tartaric, acetic and fatty acid esters of glycerol, mixed (DATEM).

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as the principles elaborated subsequently at meetings of the Committee (Annex 1, references *77, 83, 88, 94, 101, 107, 116, 122* and *131*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) embraces the major observations, comments and recommendations on the safety assessment of food additives and contaminants contained, up to the time of its publication, in the reports of the Committee and other associated bodies. The Committee noted that the document reaffirms the validity of recommendations that are still appropriate, and points out the problems associated with those that are no longer valid in the light of modern technical advances.

2.2.1 *Prediction of metabolism of flavouring agents into innocuous products*

Comprehensive metabolism data were not available for most of the flavouring agents evaluated at the present meeting. However, data were available for at least one member of each group of flavouring agents, and the response to question 2 of the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) "Can the substance be predicted to be metabolized to innocuous products?" was based on knowledge of the substrate specificities of the enzymes involved in the different metabolic pathways, and on the fates and toxicological profiles of structural analogues. The Committee recognized that the Procedure for the Safety Evaluation of Flavouring Agents requires scientific judgement of the predicted metabolic fates of the

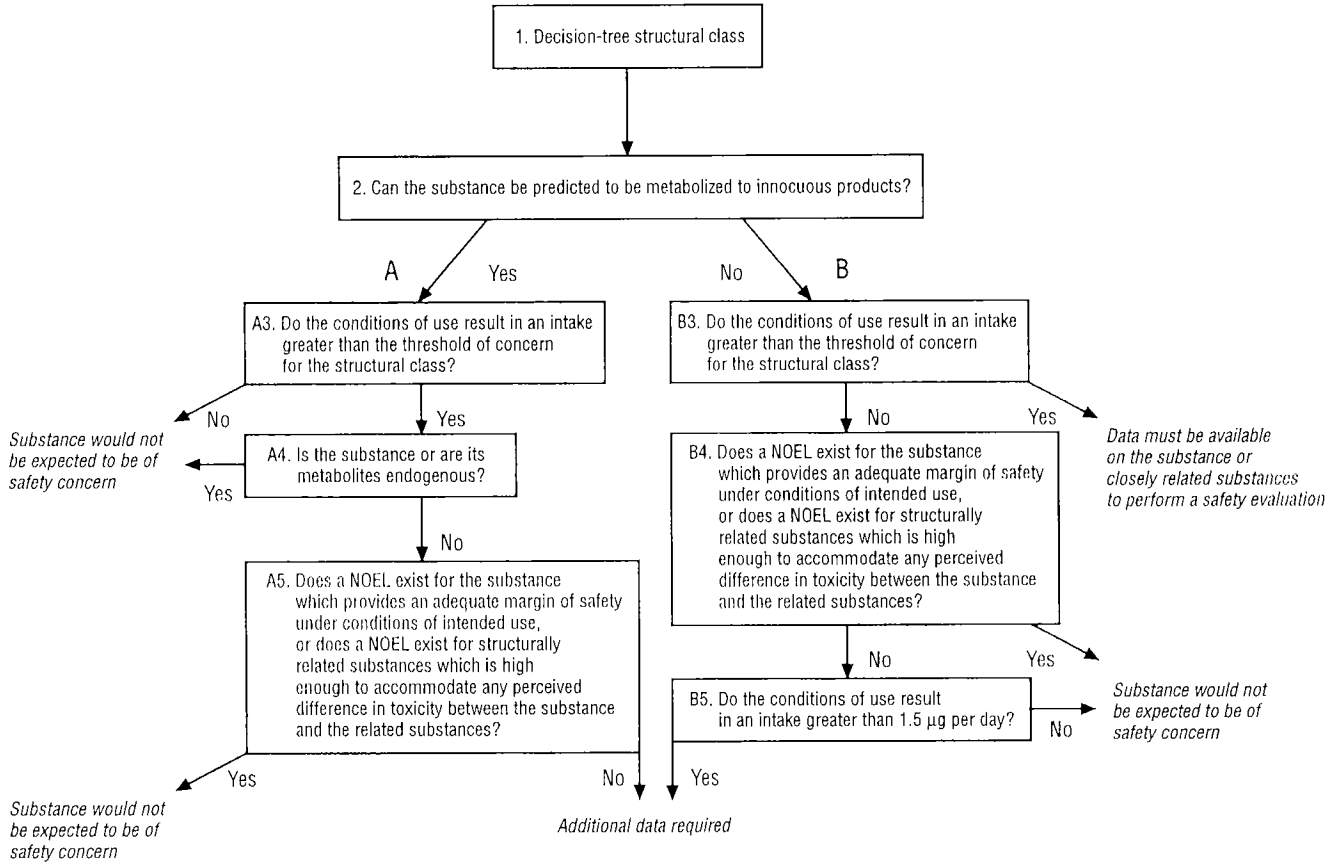

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents

compounds under evaluation and the possible toxicological consequences. Such judgements are inherent in the adoption of the Procedure, and are considered acceptable only because of the low intakes of the flavouring agents, and the conservative assumptions made in defining the thresholds of concern for the different structural classes. In many cases, the prediction of the metabolic fates for individual members of the groups of flavouring agents considered at the present meeting was based on extrapolation from data on compounds with analogous structures, rather than from data on the individual flavouring agent *per se*. Accordingly, the Committee recognized that there are limitations in the extent to which reliable predictions of metabolic fate can be made. For instance, it is unlikely that the metabolism of pulegone into a menthofuran capable of oxidation to a hepatotoxic epoxide (*3*) would have been predicted. In consequence, steps B3, B4 and B5 of the Procedure should be used when the metabolic fate of a flavouring agent and its toxicological consequences cannot be predicted with confidence. Whenever flavouring agents are predicted to be metabolized to innocuous products, the validity of this conclusion should be checked by comparison with available toxicological data on the agent and/or its structural analogues.

All safety evaluations are based on the information available at the time. New data, including information on novel pathways of metabolic bioactivation, should be the stimulus for a re-evaluation of flavouring agents.

2.2.2 *Literature surveys*

The Committee expects sponsors of food additives to include in their dossiers relevant published papers that they have identified in literature surveys. However, the extent to which the literature has been surveyed is not always clear from the information submitted. Sponsors should perform comprehensive literature searches and indicate their origin and extent. They should specify the databases scanned, the years covered and the search strategy employed, including the key words used. This will permit authors of working papers and those doing independent literature surveys for the Committee to focus their own searches more productively.

2.3 Principles governing intake assessments

Important considerations underlie the procedures for evaluating assessments of intake of (or dietary exposure to) food additives and contaminants. Intake assessments may be part of an assessment of total exposure that includes water and other non-dietary sources of chemicals, where relevant. Exposure assessments are an integral part

of the risk assessment process which includes four stages: hazard identification, hazard characterization, exposure assessment and risk characterization (*4*).

Intake assessments can be requested by the Expert Committee, Member States, the Codex Committee on Food Additives and Contaminants, Codex commodity committees or international organizations. When substances are evaluated toxicologically by the Expert Committee, intake should also be assessed. All relevant information should be submitted and the purpose and scope of the intake assessment should be described.

To ensure that the submitted data are uniform, the Committee requests that data be collected using standardized methods as detailed in Table 1. Information about assessments of national intake is generally based on five methods: "poundage" (disappearance) data; food balance sheet data; household economic surveys or retail sales surveys; model diets; and individual dietary records. Countries are requested to submit information on dietary intake obtained using one or more of these methods, chosen on the basis of the availability of data.

General considerations

- Whenever a substance undergoes toxicological evaluation, the Committee should assess its intake so that the risk can be characterized.
- Intake assessments should be undertaken in a transparent and consistent manner.
- The budget method should be used at the international level as a screening tool to identify food additives requiring detailed intake assessments.
- Intake assessments should integrate data on food consumption with data on the level of chemicals in food to enable the intake of the chemical to be estimated.
- Estimates of chronic (long-term) intake should generally be derived for the population group to which the Acceptable Daily Intake (ADI) for a food additive or the Provisional Tolerable Weekly Intake (PTWI) for a contaminant applies.
- The estimate of chronic intake is adjusted for body weight and then compared with the ADI for a food additive or PTWI for a contaminant, where established by the Committee (*5*).
- Estimates of acute (short-term) intake can be derived when regarded as necessary because of potential acute adverse effects on health.

Table 1
Methods for screening for and assessing dietary intake of food additives

Method	Description	Disadvantages
For screening		
Budget method	Estimates a theoretical maximum additive level in the proportion of the food and/or beverage supply likely to contain the additive such that the ADI for the additive cannot be exceeded by the population (*6*). In cases where the permitted level of use of the additive exceeds the calculated maximum theoretical level, further intake assessments are required.	Used to screen for additives that require detailed intake assessment at an international level. Not intended for the assessment of intake.
For assessing dietary intake		
Population-based methods		
"Poundage" (disappearance) data	Estimates the amount of food additive available per capita for use in food manufacturing in a country during a given time period. Where appropriate, estimates can take into account the import or export of foods containing the additive, and non-food uses. Estimates may be adjusted for the proportion of the population likely to consume the additive.	Household wastage of the food containing the additive not usually accounted for. Will underestimate the intake of an additive for individuals with high intakes if the number of consumers of foods containing the additive is not considered.
Food balance sheet (FBS) data	Estimates the amount of food available for use per capita per day based on food (raw and certain semi-processed commodities) available for consumption (food available = food production + imports – exports – non-food use).	Household wastage of the food containing the food chemical not usually accounted for. May overestimate per capita intakes, but underestimate the intake of a food chemical for individuals with high intakes.
	For use in estimating food additive intake, the amount of commodity likely to contain the additive must be predicted by estimating the percentage of the commodity that is processed and the percentage of this processed food that contains the additive.	Difficult to obtain accurate estimates of the percentage of food that is processed and of the percentage of processed food containing the additive.

Household-based methods		
Household economic survey	Estimates the daily per capita intake of a food chemical based on household surveys of food bought by households over a given time period.	Household wastage of the food containing the food chemical not usually accounted for. May underestimate the intake of a food chemical for individuals with high intakes.
Retail sales survey	Estimates the daily per capita intake of a food chemical based on sales of food items in shops (food sold at retail level over a given time period).	Household wastage of the food containing the food chemical not usually accounted for. May underestimate the intake of a food chemical for individuals with high intakes.
Individual-based methods		
Model diets	Estimates are based on model diets constructed from available information on food consumption to represent a typical diet of a specified population subgroup or for individuals with high intakes of foods containing the food chemical. Interpretation of intake estimates made using model diets requires that the assumptions made in constructing each model diet are stated.	Model diets can be extremely useful in estimating intakes of food chemicals, but are only as good as the underlying data and assumptions.
Individual dietary records	Estimates are based on data on food consumption by individuals. Individual intakes of additives or contaminants can be adjusted according to individual body weights, if available, before deriving population statistics from the distribution of additive or contaminant intakes. Interpretation of intake estimates requires that the assumptions made are stated.	Duration of the survey can influence intake estimates, especially for assessments of chronic intake.

- Additional assessments of intake for a specific population subgroup can be undertaken when the Committee has identified the groups that are potentially susceptible or at risk for toxicological reasons.
- When a number of compounds have a group ADI, the intake assessment should cover the whole group of compounds.
- A body weight of 60kg for entire populations is used for intake assessments unless alternative body weights are provided by countries for use with national data.

Data used in evaluations

- The Committee uses the data on intake assessments submitted by countries to undertake additional calculations, where appropriate.
- Data on food consumption are collected at the national, household or individual level (Table 1).
- Data on food consumption should be representative of the population group to which the ADI or PTWI refers.
- Intake assessments are based on three types of data: maximum levels of use from the proposed draft General Standard for Food Additives or the proposed draft General Standard for Contaminants and Toxins in Food, national food standards, and measured levels, where available.
- Intake assessments based on national data on food consumption and maximum levels of use specified in the draft General Standard for Food Additives should state whether all the food categories proposed in the General Standard have been considered or only those food categories permitted in national standards.
- Food intakes reported on an "as consumed" basis should be grouped for assessments of intake of a food additive according to the food classification system used in the draft General Standard for Food Additives (Annex 4), and for assessments of intake of contaminants according to the General Standard for Contaminants and Toxins in Food.

Evaluations

- Evaluations of national estimates of intake should include comments on the quality, uncertainty and variability of the data. All data sources should be documented.
- Assessments of chronic intake should be presented as means, percentiles or distributions for the entire population studied and for actual consumers of foods containing the food chemical of

interest. Assessments of chronic intake should be expressed as a percentage of the ADI or PTWI.

- Intake estimates represent the intake of the additive or contaminant from the total diet. They include both average and high intakes of the additive or contaminant.
- Food groups that account for a high proportion of the total estimated mean intake of a food chemical by consumers should be identified in the evaluation, where possible.

Variability and uncertainty

It is important to distinguish between the relative contributions of variability and uncertainty to the accuracy and precision of intake estimates (*5*). In evaluating intake estimates based on "poundage" data, food balance sheet data, household economic surveys or retail sales surveys, model diets or individual dietary records, it is important to note that the data may not be strictly comparable even among countries using any one approach to estimate intakes due to differences in the following:

— duration of the survey;
— number of individuals surveyed;
— population groups selected, such as age/sex groupings;
— average body weights;
— definition of "high-intake consumers";
— chemical levels (depending on whether the maximum levels specified in the draft General Standard for Food Additives, the draft General Standard for Contaminants and Toxins in Food, or national food standards are used, or levels are measured); and
— assumptions made about how to assign foods reported on an "as consumed" basis in the food classification systems used in the draft General Standard for Food Additives, the draft General Standard for Contaminants and Toxins in Food or national standards.

In addition, the use of certain methods of data collection, data sets and assumptions in estimating consumption of food and/or food chemicals may result in different estimates of the intake of additives or contaminants. Potential sources of uncertainty and variability are detailed in Table 2.

Recommendations

In making recommendations based on the evaluation of national assessments of intake, it is essential that information on the reliability, variability and uncertainty of data used in the assessments be provided. In cases where data are insufficient for the purpose of the

Table 2
Uncertainty and variability associated with methods for estimating food consumption and intakes of food additives and contaminants

Method	Uncertainty and variability
For estimating food consumption	
"Poundage" data and household-based methods	May underestimate intakes of food additives and contaminants.
Short-term surveys of food consumption	The assumption that data on short-term food consumption (1–14 days) across several age groups (cross-sectional data) represents a lifetime of consumption will tend to overestimate average intakes of food chemicals over a lifetime, especially for foods that are not staples.
	Short-term surveys of food consumption tend to underestimate the number of consumers of a selected food over a longer time period.
One-day dietary surveys versus surveys of consumption averaged over several days	One-day surveys of food consumption tend to overestimate the consumption of specific foods or food groups and result in higher levels of estimated consumption for consumers with high intakes compared with surveys in which average food consumption over several days is recorded.
Dietary recall versus methods in which the weight of food consumed is recorded	Dietary recall methods may result in over- or underestimates of consumption compared with methods in which the weight of food consumed is recorded. The extent of uncertainty may depend on the selected food item and the sex and body weight of respondents.
Data on mean food consumption in a population versus individual dietary records	Data on mean food consumption in a population tend to overestimate daily food consumption when compared with data from individual dietary records (in most cases mean food consumption is higher than median food consumption).
Surveys of chronic food consumption (food frequency surveys)	Data on the frequency of consumption of a particular food is combined with data on portion size derived from short-term surveys. Such estimates of food consumption will generally be lower than estimates derived from short-term surveys, resulting in lower estimates of intake of food chemicals.

For estimating food additive intake	
Assumption that the maximum permitted additive level is present in relevant food groups	Will overestimate food additive intakes compared with estimates based on the mean or median level of an additive derived from data on reported use.
Assumption that all foods in any one food group contain a permitted additive	Will generally overestimate food additive intakes because not all manufacturers of foods in a group will use the additive.
Assigning the maximum permitted additive level to a wide food group	Will tend to overestimate food additive intakes when the maximum level applies to a minor component of the food group.
Combining two or more food categories into one and assigning the maximum permitted additive level to all foods in the new single category	Will tend to overestimate food additive intakes.
Inclusion of foods from two or more categories (prepared foods) in intake estimates	Will tend to improve intake estimates when the weighted average of food additive levels in each food category is used, based on standard recipes.
Inclusion of corrections for concentrates, dried mixes or dehydrated foods or fat content for fat-soluble chemicals	Will tend to improve intake estimates compared with the assignment of a maximum additive level for concentrates or fat to the food "as consumed".
For estimating contaminant intake	
Assumption that the maximum permitted level of a contaminant is present in relevant food groups	Will overestimate contaminant intakes.
Assumption that all foods in any one food group contain a contaminant at the maximum permitted level	Will generally overestimate contaminant intakes because not all foods in the food group may contain the contaminant at the same level.
Assigning a contaminant level to a wide food group	Will tend to overestimate contaminant intakes when the contaminant level applies to a minor component of the food group.
Combining two or more food categories into one and assigning the highest level of a contaminant to all foods in the new single category	Will tend to overestimate contaminant intakes.
Inclusion of foods from two or more categories (prepared foods) in intake estimates	Will tend to improve intake estimates when the weighted average of contaminant levels in each food category is used, based on standard recipes.

evaluation, recommendations will be made for the submission of additional data.

Recommendations will be accompanied by relevant supporting information that may be important for Codex committees when considering various options for risk management. For example, information may be included on alternative food additives that serve the same technological function. For contaminants, information may be included on the feasibility of reducing contaminant levels in specific foods, monitoring levels in foods and the availability of suitable methods of analysis.

2.4 Principles governing the establishment and revision of specifications

2.4.1 *Specifications and the ADI*

For easy reference the Committee decided to indicate in all specifications the status of the ADI of the substance.

2.4.2 *Microbiological criteria in specifications monographs for food additives*

In some instances, particularly for products of natural origin, the Committee has included microbiological criteria in the specifications monograph. At its present meeting, the Committee agreed on the appropriateness of establishing a policy for setting microbiological criteria. The Committee therefore established the following policy.

Policy on microbiological criteria

In general, manufacturers of food additives are expected to use good manufacturing practices and to establish microbiological controls in production processes as necessary. This is to ensure that food additives are not contaminated with pathogenic or other undesirable organisms or with microbial metabolites and that the food additive is suitable for its intended use. Such a requirement will be included in the specifications monograph when the Committee recognizes the need for microbiological criteria for an individual substance.

The Committee will consider, on a case-by-case basis, the following factors when developing microbiological criteria for food additives:

— the origin of the food additive (plant, animal or natural mineral source or microbially derived via fermentation);
— evidence of a health hazard or potential health hazard based on epidemiological data, hazard analysis or specific populations that may be at risk;

— the nature of the natural and commonly acquired microorganisms that contaminate the food additive and the ability of the food additive to support their growth;
— the effect of further processing on the microorganisms that contaminate the food additive;
— the potential for microbial contamination and/or growth in a food additive during its procurement, processing, handling, storage and distribution;
— the state in which the food additive is packaged, stored and distributed (e.g. frozen, refrigerated, heat-processed); and
— the potential for direct use by consumers.

Any one of these factors may signal the need to consider establishing microbiological criteria for a food additive.

2.4.3 *Specifications for flavouring agents*

The Committee considered the specifications for 232 flavouring agents at the present meeting. These included 224 substances that were on the original agenda (nos 139 and 219–442) and a further eight that were added to the agenda at the meeting (nos 443–448, 450 and 451). No information was provided on substance no. 436 (3-(L-menthoxy)propane-1,2-diol), which was not considered further. The specifications considered included a number of substances that were previously described in separate monographs. The new specifications replace these monographs for flavouring uses of the substances.

Sixty-eight specifications were classified as tentative because not all the necessary relevant information had been provided. Many of the flavouring agents examined at the present meeting were more complex than those considered previously, and the sponsors were asked to examine specifications carefully in the future to ensure that all relevant information is available and to correct any information that does not accurately represent flavouring agents that are on the market.

As at previous meetings, identification of most of the flavouring agents was based on infrared spectra. However, this may not always be the method of choice because the infrared spectra of closely related substances may be indistinguishable, even in the fingerprint region. The Committee would welcome further comments on this point, in particular on the possible merits of using more sophisticated methods such as mass spectrometry and/or nuclear magnetic resonance.

2.4.4 *Specifications for vitamins and minerals*

At its present meeting, the Committee was requested by FAO to develop food-grade specifications for ferrous sulfate for use in food fortification. Vitamins and minerals do not fall within the scope of the Committee's traditional definition of the term "food additive";[1] nonetheless, FAO receives repeated requests for food-grade specifications for substances used in food fortification.

Although the Committee has prepared specifications for approximately 40 traditional food additives that also have incidental uses as vitamins and minerals, more than 60 substances remain for which internationally recognized food-grade specifications are lacking (*7*). It was agreed that such substances would normally be on the agenda for the development of specifications only. In certain cases, however, it may be necessary to undertake toxicological evaluations as well, for example when novel forms of nutrients are involved.[2]

2.4.5 *Enzyme preparations derived from genetically modified microorganisms*

At the thirty-seventh meeting of the Committee (Annex 1, reference *94*), an addendum to the "General specifications for enzymes used in food processing" (Annex 1, reference *96*) was prepared. This addendum addressed issues relating to enzyme preparations derived from genetically modified microorganisms. The text is published in Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*).

At its present meeting, the Committee concluded that the first part of Appendix B (General considerations) should be revised as follows.

For the proper evaluation of enzyme preparations derived from genetically modified microorganisms, information should be provided on the host microorganism, the genetic material introduced into the

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[1] At its first meeting in 1956, the Committee defined the term *food additives* as "non-nutritive substances added intentionally to food, generally in small quantities, to improve its appearance, flavour, texture or storage properties" (Annex 1, reference *1*). The Committee later expanded its scope to include food contaminants as well as certain nutritive substances consumed in high amounts (Annex 1, reference *76*).

[2] Such a case occurred at the forty-first meeting when the Committee evaluated the safety of the use of sodium iron EDTA (ethylenediamine tetraacetate or edetate) in supervised food fortification programmes in populations in which iron-deficiency anaemia is endemic (Annex 1, reference *107*). The Committee expressed concern about the potential for over-fortification of food because of the enhanced bioavailability of iron in this form.

host microorganism and the recombinant production microorganism. Annex 1 of the *Compendium of food additive specifications* (Annex 1, reference *96*) addresses factors relevant to all microbial sources (conventional and recombinant) used in the production of enzyme preparations and to fermentation and recovery procedures. The following points need emphasis when the production of enzyme preparations derived from genetically modified microorganisms is considered:

- The host microorganism should be taxonomically and genetically characterized.
- Documentation demonstrating that the host microorganism is non-pathogenic and non-toxicogenic should be provided.
- The genetic material (i.e. the expression vector or plasmid) intended for introduction into the host microorganism should be characterized and a description of its construction provided. As appropriate, it should be demonstrated that the genetic material does not contain genes coding for virulence factors, protein toxins or enzymes that may be involved in the synthesis of mycotoxins or any other toxic or undesirable substances. The source of the DNA encoding the enzyme of interest should be identified where possible.
- The production microorganism should be characterized with respect to the introduced DNA, its genetic stability and its growth properties.
- If the production microorganism is capable of producing proteins that inactivate clinically useful antibiotics, documentation should be provided that the finished enzyme preparation contains neither antibiotic-inactivating proteins at concentrations that would interfere with antibiotic treatment nor DNA that is capable of transforming microorganisms, which potentially could lead to the spread of antibiotic resistance.
- All enzyme preparations should be evaluated for their potential to elicit allergic reactions. As a general rule, if a food is known to cause an allergic reaction in humans, its use as a source of DNA encoding the enzyme of interest should be avoided. In exceptional cases, where there is a demonstrated need to use an allergenic source of DNA, documentation should be provided indicating that the enzyme is not associated with the allergic reaction. Worldwide the most common allergenic foods are fish, crustaceans, peanuts, tree nuts, soybeans, milk, eggs and wheat.

The above points cover the major issues pertinent to the development of enzyme preparations derived from genetically modified microorganisms. These points emphasize and supplement the issues that must be considered in the safety evaluation of such enzyme preparations which in general relate to the avoidance of undesirable impurities.

The following properties might be useful in characterizing enzymes from recombinant microorganisms: relative molecular mass, isoelectric point, substrate specificity, reaction kinetics, activity as a function of pH and temperature, amino acid composition, amino acid sequence, a peptide map and the sequence of DNA bases coding for the enzyme.

In revising the second part of Appendix B (Specifications for enzymes from genetically manipulated microorganisms), the Committee removed the tentative designation. The revised section on specifications to be published in the specifications monograph focuses on the section of the monographs that deals with the host microorganism for enzymes from genetically modified microorganisms. It notes that any microbial strain that meets the general considerations for enzymes specified in Annex 1 of the *Compendium of food additive specifications* (Annex 1, reference *96*) should be a safe and suitable host for the introduced DNA. Citation of the genus and species of the host and donor organisms is usually adequate for microorganisms that have been determined to be safe and suitable. The citation of the strain is appropriate where a non-pathogenic and non-toxicogenic strain belongs to a species that also encompasses pathogenic and toxicogenic strains. Citation of the specific expression plasmids is generally unnecessary where the plasmid vector is well characterized and documentation on the production microorganism, including the introduced DNA, can be used to verify the appropriateness of the expression plasmid selected.

The Committee requested comments on the revised text of Appendix B, which will be published as an Annex in the specifications monograph.

The Committee also amended the "General notices applying to the standards, tests and assays of the specifications prepared by the Joint FAO/WHO Expert Committee on Food Additives" in the *Compendium of food additive specifications* (Annex 1, reference *96*) to align the text on the source of enzyme preparations with the revision of Appendix B to Annex 1, as described above.

2.4.6 *Limit test for heavy metals*

The Committee reaffirmed the decision taken at its forty-ninth meeting (Annex 1, reference *131*) to replace, as appropriate, the general test for heavy metals in the specifications with tests for specific metals, particularly for lead, cadmium, mercury and arsenic.

In order to confirm the limits for heavy metals, the Committee would require confirmation of the actual levels determined in the food additives in question from sponsors.

The Committee will endeavour to establish guidelines for setting limits for specific heavy metals. At its present meeting, the Committee confirmed that it would aim to set limits that were as low as practicable for those elements of concern.

The Committee adopted a general limit for lead of 2 mg/kg. When an additive was known to be used in substantial amounts, a limit of 1 mg/kg was chosen. In a few cases where there was evidence that the lead content of the product could not be reduced to these levels, a limit of 5 mg/kg was specified.

3. Specific food additives

The Committee evaluated four food additives for the first time and re-evaluated 12 food additives considered at previous meetings. In addition, the Committee evaluated a large number of flavouring agents using the Procedure for the Safety Assessment of Flavouring Agents (see section 4). Information on the evaluations and on specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Enzyme preparations

3.1.1 α-*Acetolactate decarboxylase*

α-Acetolactate decarboxylase derived from a genetically modified organism was previously reviewed by the Committee at its forty-ninth meeting (Annex 1, reference *131*), when a temporary ADI "not specified" was established. The ADI was designated as "temporary" because the specifications were tentative.

At its present meeting, the Committee prepared specifications that are consistent with the specifications for the product that was tested toxicologically. Therefore, the Committee removed the temporary qualification and established an ADI "not specified".

A toxicological monograph was not prepared. The existing specifications were revised in accordance with the revised Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) as described in section 2.4.5 and the "tentative" designation was deleted.

3.1.2 *Maltogenic amylase*

Maltogenic amylase derived from a genetically modified organism was previously reviewed by the Committee at its forty-ninth meeting (Annex 1, reference *131*), when a temporary ADI "not specified" was established. The ADI was designated as "temporary" because the specifications were tentative.

At its present meeting, the Committee prepared specifications that are consistent with the specifications for the product that was tested toxicologically. Therefore, the Committee removed the temporary qualification and established an ADI "not specified".

A toxicological monograph was not prepared. The existing specifications were revised in accordance with the revised Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms) to Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference *96*) as described in section 2.4.5 and the "tentative" designation was deleted.

3.2 Flavouring agents

3.2.1 *trans-Anethole*

trans-Anethole was first evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*) and re-evaluated at its twenty-third meeting (Annex 1, reference *50*), when a temporary ADI of 0–2.5 mg/kg of body weight was allocated. At the thirty-third meeting (Annex 1, reference *83*), the temporary ADI was reduced to 0–1.2 mg/kg of body weight on account of the tumorigenic effects observed in the liver of female rats in a 27-month study. At that time, the Committee requested further details of the long-term study in rats and the results of metabolic studies, and further noted that a long-term study in mice and an epidemiological study might be required. At the thirty-seventh meeting (Annex 1, reference *94*), the temporary ADI for *trans*-anethole was reduced to 0–0.6 mg/kg of body weight after consideration of the results of three independent reviews of the histological changes in the liver of rats in the long-term study. At that time, a

recommendation was made for further metabolic and pharmacokinetic studies, chromosomal aberration studies and *in vitro* tests for gene mutations in mammalian cells. At its thirty-ninth and forty-ninth meetings (Annex 1, references *101* and *131*), the Committee further extended the temporary ADI of 0–0.6 mg/kg of body weight, pending completion of the recommended studies.

At its present meeting, the Committee evaluated the results of new 90-day studies in rats and mice, together with those of studies on the metabolism of *trans*-anethole in mice and rats, studies on the effects on hepatic enzyme induction in these species of short-term administration of *trans*-anethole in the diet, *in vitro* cytotoxicity and genotoxicity studies and some epidemiological data which had become available since the thirty-seventh meeting. These studies provided information on the significance to humans of the results of the long-term study in rats. In addition, studies on reproductive toxicity and immunotoxicity were reviewed. A long-term study in mice, which had been requested at the thirty-ninth meeting, was not available.

The no-observed-effect level (NOEL) from the previously reviewed long-term study in rats was 2.5 g/kg (0.25%) in the diet (equivalent to 125 mg/kg of body weight per day), based on an increased incidence of hepatic focal nodular hyperplasia in animals of both sexes at higher doses. Administration of *trans*-anethole to 30 female CD-1 mice for 12 months at 4.6 g/kg (0.46%) in the diet (equivalent to 700 mg/kg of body weight per day) did not result in the induction of hepatomas in any of the animals up to 18 months after the feeding of *trans*-anethole had been initiated; the related alkenylbenzenes, safrole and estragole, induced hepatomas in the same assay.

Most of the evidence on genotoxicity and DNA interactions of *trans*-anethole reviewed at the present meeting suggested that it is not genotoxic. Under standard assay conditions, *trans*-anethole was not mutagenic in *Salmonella typhimurium*, and negative results were obtained in tests for induction of chromosomal aberrations in Chinese hamster ovary cells *in vitro*, induction of micronuclei in mice *in vivo* and unscheduled DNA synthesis *in vitro* and *in vivo*. The only positive results were obtained for mutation at the *tk* locus in mouse lymphoma cells in both studies performed and in three assays in *S. typhimurium* when a microsomal activation system with enhanced protein content or when the co-factor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) was added. The rate of formation of adducts in hepatic DNA from adult and weanling mice exposed to *trans*-anethole was very low in comparison with the rate after exposure to

the hepatocarcinogenic alkylbenzenes, safrole, estragole and methyleugenol.

The potential of metabolites of *trans*-anethole to induce unscheduled DNA synthesis in rat hepatocytes *in vitro* was also investigated. Neither the initial ω-oxidation product, 3′-hydroxy anethole, nor the epoxide products, anethole 1,2-oxide and its metabolite, anethole 1,2-diol, induced unscheduled DNA synthesis. Anethole 1,2-oxide was cytotoxic, while anethole 1,2-diol exhibited little cytotoxicity. Agents that prevented further metabolism of the epoxide markedly enhanced the cytotoxicity of *trans*-anethole.

The metabolism of *trans*-anethole proceeds via *O*-demethylation, side-chain ω-oxidation and epoxidation of the side chain. These pathways of metabolism occur in rats, mice and humans, but the proportion of the dose metabolized by different routes is species- and dose-dependent. *O*-Demethylation and ω-oxidation are the major pathways in rats and mice receiving single doses of 200–300 mg/kg of body weight (similar to those administered in the toxicity studies), and also in humans receiving a single dose of 1 mg (equivalent to 0.015 mg/kg of body weight). The formation of the cytotoxic epoxide metabolite has been deduced from the urinary excretion of the isomeric diols and of the *N*-acetylcysteine metabolite derived from conjugation with glutathione. The total amounts of these metabolites excreted by rats (about 15–20%) were higher than those excreted by mice (about 5–10%) or humans (about 3%). However, in this respect, the human data have been described adequately for only two subjects given [^{14}C] *trans*-anethole. The available data indicate that rats given single doses of 200–300 mg/kg of body weight metabolize proportionally more *trans*-anethole to the cytotoxic metabolite than do humans given a single dose of 1 mg. The metabolic data from humans were considered by the Committee to be too limited to permit accurate quantitative comparisons of formation of anethole epoxide in rats and humans.

Recently conducted 90-day feeding studies in CD-1 mice and Sprague–Dawley rats were reviewed, together with preliminary 28-day range-finding studies. Most of the observed effects were related to inanition resulting from decreased food consumption by the treated animals. The effects of diet rejection were much more apparent in mice and were reflected in the much lower dietary levels tolerated. In contrast, in studies in which single doses were given by gavage, mice appeared to tolerate higher doses of *trans*-anethole than did rats. The doses used in the 90-day studies were 30–240 mg/kg of body weight per day for mice and 150–900 mg/kg of body weight per

day for rats. In both species, the major indications of inanition were large decrements in body weight, depletion of hepatic glycogen and decreased organ weights, while enlarged livers and hypertrophy of the centrilobular hepatocytes were suggestive of hepatic enzyme induction. The NOEL in the study in mice was 120 mg/kg of body weight per day, based on decrements in body weight of greater than 10% in males at the highest dose.

Toxicologically significant effects were apparent from the results of the 28-day and 90-day studies in rats. In both male and female rats a hepatotoxic effect of *trans*-anethole was suggested by elevated serum activity of γ-glutamyl transferase in the 90-day study at doses of 600 or 900 mg/kg of body weight per day, and by a reduction of serum protein and albumin concentrations at a dose of 900 mg/kg of body weight per day. However, no proliferative or other toxicologically significant lesions were apparent on histopathological examination of the livers. There was no evidence of a similar effect in mice over the lower dose range necessitated by their lower tolerance to *trans*-anethole in the diet. The NOEL was 300 mg/kg of body weight per day, based on the elevated γ-glutamyl transferase activity in both male and female rats and decrements in body weight exceeding 10% in the males at higher doses.

Studies of reproductive toxicity in rats given *trans*-anethole were conducted over one or four generations. In the 1-generation study, litter effects (reduced viability of pups, pup survival and pup body weights) were noted only at doses that were toxic to the dams. The NOEL for maternal toxicity was 175 mg/kg of body weight per day, based on reduced food consumption and body-weight gain and overall poor condition in the dams. In the 4-generation study, in which treated groups received *trans*-anethole at a dose of 10 g/kg (1%) in the diet (equal to 700–1400 mg/kg of body weight per day), the only effect seen in the pups was a reduction in body-weight gain. A cross-fostering experiment conducted as part of the 4-generation dietary study indicated that the reduced body-weight gain observed in pups in the treated groups was related to exposure during lactation, rather than during the gestation period.

trans-Anethole was not immunotoxic when tested in the sheep erythrocyte plaque-forming assay or a challenge assay with *Listeria monocytogenes*.

The data reviewed at the present meeting indicated that *trans*-anethole and its metabolites are unlikely to be genotoxic *in vivo* and suggested that a cytotoxic metabolite, anethole epoxide, is the possible causative agent of the hepatotoxic effects noted in the liver of

rats. Since *trans*-anethole is metabolized along the same three major pathways in mice, rats and humans, hepatotoxicity in rats was considered an appropriate end-point on which to base the ADI. Because the 90-day study in rats was well conducted by current standards and the hepatic effects observed were consistent with the hepatotoxicity observed in the long-term study, it was used to derive the ADI. The NOEL was 300 mg/kg of body weight per day on the basis of alterations in serum parameters considered to be indicators of hepatotoxicity. Because of the low tolerance of mice for *trans*-anethole administered in the diet, they could not be fed doses comparable to those that were toxic to rats. It is therefore unlikely that a new long-term study in mice could include sufficiently high doses to induce effects in the liver uncomplicated by inanition.

Because of limitations in the long-term studies in rats and mice, the Committee concluded that the recent 90-day study in rats provided the most reliable data for the determination of the NOEL for adverse effects in the liver. Application of the usual safety factor of 100 to the NOEL from the 90-day study was considered inappropriate because of the limitations in the long-term studies. An overall safety factor of 200 was considered adequate to allow for deficiencies in the long-term studies and to provide a suitable safety margin for the maternal toxicity observed in reproductive toxicity studies as well as for the effects seen in the 90-day study in mice.

The Committee allocated an ADI of 0–2 mg/kg of body weight on the basis of the NOEL of 300 mg/kg of body weight per day in the 90-day study in rats, to which a safety factor of 200 was applied. The ADI was rounded to one significant figure, in accordance with usual practice.

An addendum to the toxicological monograph was prepared. The existing specifications included in the list of flavouring agents in the *Compendium of food additive specifications, addendum 5* (Annex 1, reference *133*) were maintained.

3.2.2 *Furfural*

Furfural was previously evaluated at the thirty-ninth meeting of the Committee (Annex 1, reference *101*). An ADI was not established because of evidence available to the Committee regarding the genotoxicity and carcinogenicity of furfural. At that time, the Committee considered that the direct addition of furfural as a flavour was not appropriate, and that its use as a solvent should be restricted to situations in which alternatives were not available. The Committee also considered that transfer of furfural into food should be reduced

to the lowest extent technically feasible. At its present meeting, the Committee considered furfural as a flavouring agent.

Furfural is used as a flavouring agent in a variety of food products and alcoholic and non-alcoholic beverages. Furfural and many of its derivatives occur widely as natural constituents of food.

Since the last evaluation additional data, including those from studies of the metabolism of furfural, its potential genotoxicity and hepatotoxicity, have become available.

In both humans and rodents, furfural is efficiently metabolized by oxidation of the aldehyde functional group to furoic acid, most of which is conjugated with glycine and excreted. A minor proportion (<5%) of furoic acid is condensed with acetyl coenzyme A to form 2-furanacryloyl coenzyme A, which is conjugated with glycine and excreted in the urine. About 5% of [^{14}COOH]furfural is eliminated by rats and mice as radiolabelled carbon dioxide. The metabolic pathway, which could involve either direct decarboxylation or epoxidation and opening of the carbon ring, has not been defined.

In short-term studies, furfural was clearly hepatotoxic at doses at and above 90 mg/kg of body weight per day and 150 mg/kg of body weight per day in male rats and male mice, respectively. Minor changes were reported in the liver of male rats given furfural at lower doses in corn oil by gavage for 13 weeks and were also present to a lesser extent in controls given corn oil by gavage. In a study of developmental toxicity furfural was not toxic in rats at doses of up to 150 mg/kg of body weight per day, the highest dose tolerated.

Furfural, like other aldehydes such as endogenous acetaldehyde, is a reactive aldehyde; it is reported to bind to soluble proteins and protein components of cell membranes. Various metabolic processes (i.e. oxidation, conjugation and condensation) will effectively eliminate the reactive aldehyde functional group when these metabolic pathways are not saturated by high, non-physiological doses.

Furfural was not genotoxic in most tests *in vitro*. Positive results were reported at relatively high concentrations in only three of 16 reverse mutation assays in *Salmonella typhimurium* and in one of three *rec* assays in *Bacillus subtilis*. A few chromosomal aberrations were seen in Chinese hamster ovary cells exposed to relatively high concentrations. Sister chromatid exchanges and forward mutations were induced in mouse lymphoma cells. This weak activity of furfural *in vitro* in some tests for genotoxicity might be explained by the reactivity of its aldehyde group. Recent studies of unscheduled DNA

synthesis in hepatocytes of rats treated *in vivo* and in human liver treated *in vitro* gave negative results. Negative results were obtained in tests for genotoxicity *in vivo*, except in *Drosophila melanogaster* injected with furfural.

In a 2-year study of carcinogenicity in rats given furfural in corn oil by gavage at doses of 30 or 60 mg/kg of body weight per day, hyperplasia of the bile duct and cholangiofibrosis were seen in essentially all rats in all groups (treatment and control); the incidence was highest in the control groups. Mild hepatocellular necrosis was seen in all groups; however, the rates in males were higher in all treated groups. Cholangiocarcinomas were observed in two of 50 males at the highest dose, but the incidence was not statistically significant.

In mice, the combined incidence of adenomas and carcinomas of the liver (64%) was significantly increased ($p < 0.01$) in males given the highest dose (175 mg/kg of body weight per day), but not in females. Hepatocellular adenomas and carcinomas also occurred in the controls and in animals that received the lowest dose (50 mg/kg of body weight per day) and in those given the intermediate dose (100 mg/kg of body weight per day), at incidences of 34–44% in males and 6–14% in females. The incidences in historical controls were 38% (14–50%) in males and 6.2% (0–30%) in females. Hepatotoxicity, manifested by features such as focal and multifocal necrosis and chronic inflammation, was seen in all groups, including the controls, but was considerably more frequent in mice given the highest dose.

Studies of oncogene activation in samples of liver tumours in this study revealed some differences in the pattern of mutations in the oncogenes observed in treated mice and controls. As it was not possible to identify the animals from which the tumours originated and since hepatotoxicity was seen at the intermediate and highest doses, it was not possible to determine whether a direct genotoxic event or a secondary genotoxic pathway was involved.

Because of concern about the tumours observed in male mice given furfural and the fact that no NOEL was identified for hepatotoxicity in rats, the Committee was unable to allocate an ADI. Before reviewing this substance again, the Committee would wish to review the results of studies of DNA binding or adduct formation *in vivo* to clarify whether furfural interacts with DNA in the liver of mice. The Committee also requested the results of a 90-day study of toxicity in rats to identify a NOEL for hepatotoxicity.

A toxicological monograph was prepared. The existing specifications for furfural were revised and, as the only direct food-related use of

furfural is as a flavouring agent, they were transferred to the list of flavouring agents in the specifications monograph.

3.2.3 *Menthol*

Menthol was first evaluated at the eleventh meeting of the Committee (Annex 1, reference *14*), when it was allocated an unconditional ADI of 0–0.2 mg/kg of body weight and a conditional ADI of 0.2–2 mg/kg of body weight. At the eighteenth meeting, an ADI of 0–0.2 mg/kg of body weight was established (Annex 1, reference *35*). Menthol was re-evaluated at the twentieth meeting of the Committee (Annex 1, reference *41*), when the previous ADI was maintained. At that meeting, the Committee considered it desirable to evaluate the results of long-term studies of toxicity and carcinogenicity in rats, information on the average and likely maximum levels of intake of menthol, clinical observations on subjects with a higher than average intake of menthol and the results of studies on metabolism (Annex 1, reference *42*). Since that time, new studies have become available, principally, 2-year studies of carcinogenicity in mice and rats.

Menthol exists as two optical isomers: (+)-menthol, (−)-menthol and the racemic mixture ((±)-menthol). The (−)-and (±)-forms are used as flavouring agents.

Menthol is readily absorbed. Up to 100% of an ingested dose appears to be absorbed as measured by the elimination of menthol metabolites in the faeces and urine. Absorbed menthol is known to be eliminated largely as glucuronides; 70–80% of an oral dose is eliminated in the urine and faeces within 48 hours of ingestion. Metabolic studies indicate that oral doses of menthol are metabolized mainly in the liver and excreted via the kidneys and in the bile. Menthol is efficiently metabolized by normal metabolic pathways. The metabolites are simple glucuronic acid conjugates and oxidation products. Mammals can efficiently metabolize menthol by pathways that do not create hazardous metabolites.

In 13-week toxicity studies in which (±)-menthol was given in the diet, the NOEL was 560 mg/kg of body weight per day in mice and 750 mg/kg of body weight per day in rats on the basis of slightly increased incidences of interstitial nephritis at higher doses.

In a 2-year study of toxicity and carcinogenicity in mice, (±)-menthol was administered in the diet at levels equivalent to 300 or 600 mg/kg of body weight per day. The incidences of hepatocellular tumours in males and lung tumours in females at the higher dose level were not significantly different from those in concurrent or historical controls.

The survival rate was decreased in female mice but remained within the range of historical controls. The NOEL was 600 mg/kg of body weight per day. In a 2-year toxicity/carcinogenicity study in rats given (±)-menthol in the diet at concentrations equivalent to 190 or 380 mg/kg of body weight per day, the NOEL was 380 mg/kg of body weight per day.

Neither menthol nor its metabolites were genotoxic in *in vitro* or *in vivo* studies.

While no reproductive toxicity studies were available for (±)-menthol, (–)-menthol was tested at doses of up to 190–430 mg/kg of body weight per day for teratogenicity in mice, rats, hamsters and rabbits; no teratogenic effects were observed.

The limited data available on metabolism and toxicity provided no indication of a difference in the toxicity of (–)-menthol and (±)-menthol. Therefore, the Committee concluded that an ADI could be established for the two optical isomers of menthol.

The Committee noted that the highest dose of (±)-menthol used in the long-term studies in mice and rats caused no specific toxic effects. As survival was reduced in mice given a dose of 600 mg/kg of body weight per day, the Committee allocated an ADI for menthol of 0–4 mg/kg of body weight, based on the NOEL of 380 mg/kg of body weight per day in the long-term study in rats and a safety factor of 100. The ADI was rounded to one significant figure, in accordance with usual practice.

A toxicological monograph was prepared. The existing specifications for (+)-menthol and (–)-menthol were revised and replaced by a single set of specifications for menthol, which was added to the list of flavouring agents in the specifications monograph.

3.3 Food colours

3.3.1 *Curcumin*

Turmeric oleoresin and curcumin (the main colouring component of turmeric oleoresin) were evaluated previously by the Committee at its thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth, thirty-ninth and forty-fourth meetings (Annex 1, references *19*, *35*, *47*, *53*, *59*, *73*, *88*, *101* and *116*). At the eighteenth meeting, a temporary ADI of 0–0.1 mg/kg of body weight was established for curcumin, based on the ADI for turmeric oleoresin (0–2.5 mg/kg of body weight per day) and an assumed average concentration of 3% curcumin in turmeric. The temporary ADI for curcumin was extended at the twenty-second, twenty-fourth, twenty-

sixth, thirtieth, thirty-fifth and thirty-ninth meetings. At the thirty-ninth meeting, the Committee requested the results of carcinogenicity studies in mice and rats fed turmeric oleoresin (which were known to have been completed) and the results of a study of reproductive toxicity and teratogenicity with curcumin.

At its forty-fourth meeting, the Committee evaluated the results of the carcinogenicity studies on turmeric oleoresin in rats and mice, together with new biochemical and genotoxicity data. The Committee concluded that teratogenicity data on curcumin were no longer required but reiterated the request for a reproductive toxicity study. On the basis of a NOEL of 220 mg/kg of body weight per day in the carcinogenicity study in mice and a safety factor of 200, the Committee increased the temporary ADI to 0–1 mg/kg of body weight and extended it, pending the submission of the results of a reproductive toxicity study with curcumin for review in 1998.

At its present meeting, the Committee evaluated the results of fertility studies with turmeric oleoresin given in the diet at levels equal to 0, 120, 650 or 3300 mg/kg of body weight per day in female rats; 0, 80, 430 or 2200 mg/kg of body weight per day in male rats; 0, 220, 1100 or 5500 mg/kg of body weight per day in female mice; and 0, 210, 1100 or 5500 mg/kg of body weight per day in male mice. The curcumin content of the turmeric oleoresin was 68–76.5%. In mice low rates of survival of pups (44% or less) were seen in all treated groups, the lowest rate occurring at the highest dose. The highest-dose group also showed a decrease in mean litter size. In rats there were no effects on the survival of parents or their F_1 offspring, but pregnancy rates were decreased in all groups, and were lower in treated than in control groups. Decreased body-weight gain of pups was observed at the intermediate and highest doses on days 0–14 postpartum. Because of the low survival rate of pups in the study in mice, and the low rates of pregnancy observed in rats, the Committee was unable to evaluate with confidence the significance of the reproductive effects observed in the two studies.

The Committee concluded that the studies of fertility in rats and mice provided very limited data on the effects of curcumin on this endpoint and that these studies did not provide assurance that the potential reproductive effects of curcumin have been adequately investigated. The Committee again requested the results of a reproductive toxicity study on a substance complying with the specifications for curcumin.

The temporary ADI of 0–1 mg/kg of body weight per day for curcumin was extended until 2001, pending the submission of data from a reproductive toxicity study.

A toxicological monograph was not prepared. The existing specifications were revised and designated as "tentative", pending the receipt of information on the need and technological justification for alternative solvents for use in the current manufacturing processes of curcumin, which was required for evaluation in 2001.

3.3.2 *Riboflavin from genetically modified Bacillus subtilis*

Riboflavin derived from a fermentation process using a genetically modified strain of *Bacillus subtilis* has not been previously evaluated by the Committee. Synthetic riboflavin was evaluated previously by the Committee at its thirteenth meeting (Annex 1, reference *19*), when an ADI of 0–0.5 mg/kg of body weight was allocated on the basis of a limited database. It was re-evaluated at the twenty-fifth meeting (Annex 1, reference *56*), when a group ADI of 0–0.5 mg/kg of body weight was allocated to riboflavin and riboflavin-5′-phosphate, expressed as riboflavin. The data reviewed at that time included information on reproductive toxicity.

The strain of *B. subtilis* used in riboflavin production has been genetically modified to overproduce riboflavin by amplification of the chromosomal region of the riboflavin operon containing suitable promoters and flanked by pUC19 and antibiotic resistance marker genes. The lack of pathogenicity and toxicity of the strain of *B. subtilis* from which the genetic information encoding riboflavin was cloned is extensively and well documented, and the methods for genetic modification have been well described. The production strain of *B. subtilis* was shown to be genetically stable during fermentation. It was convincingly established that the final product is riboflavin of a purity comparable to, or greater than, that produced by conventional methods and is free of DNA from the production organism. Thus, antibiotic resistance marker genes are not present in the final product.

In a 90-day study of toxicity, rats were fed diets containing either 98% pure food-grade riboflavin produced by fermentation from genetically modified *B. subtilis* or 98% pure riboflavin derived by chemical synthesis. The doses tested were 0, 20, 50 or 200 mg/kg of body weight per day. Some animals in the highest-dose groups had reduced weight gain, but the reduction was generally less than 10%, and the efficiency of food conversion was not affected. In the group fed the highest dose of fermentation-derived riboflavin some minor changes in red blood cell parameters were observed, but they were not considered relevant. The NOEL for 98% pure riboflavin produced by fermentation was 200 mg/kg of body weight per day, which was the same as that for chemically synthesized riboflavin.

Fermentation-derived riboflavin and the degradation product 8α-hydroxyriboflavin did not induce gene mutations in bacteria *in vitro* in either the presence or absence of metabolic activation.

The Committee concluded that the recombinant DNA techniques used to derive the production strain of *B. subtilis* were well characterized, providing assurance that no DNA is present in the end-product. On the basis of molecular biological data and chemical analytical research, it can be concluded that fermentation-derived riboflavin from genetically modified *B. subtilis* is substantially equivalent to synthetic riboflavin.

For 98% pure riboflavin produced by fermentation for use in food, the NOEL in the 90-day toxicity study in rats was 200 mg/kg of body weight per day, the highest dose tested. Riboflavin produced by fermentation was evaluated on the basis of its substantial equivalence to synthetic riboflavin. Therefore, the Committee included riboflavin derived from a production strain of genetically modified *B. subtilis* with the previously established group ADI of 0–0.5 mg/kg of body weight for synthetic riboflavin and riboflavin-5′-phosphate.

A toxicological monograph and new specifications were prepared.

3.4 Glazing agents: mineral oils (medium- and low-viscosity)

Mineral oils[1] were previously considered by the Committee at its thirty-seventh and forty-fourth meetings (Annex 1, references *94* and *116*). At the latter meeting, the Committee allocated a temporary group ADI of 0–0.01 mg/kg of body weight to class II mineral oils (including the medium-viscosity oils N70(H) and N70(A)) and to class III mineral oils (including the low-viscosity oils P15(H), N15(H) and N10(A)) and a temporary ADI of 0–1 mg/kg of body weight to class I mineral oils (including the medium-viscosity oil P70(H)), based on the results of 90-day toxicity studies in Fischer 344 (F344) rats.

At its forty-fourth meeting, the Committee requested more information about the compositional factors in mineral oils that influence their absorption and toxicity for review in 1998. It also requested a study of at least 1 year's duration of one of these materials in F344

[1] Oils may be obtained from crude oil sources of naphthenic (N) or paraffinic (P) origin and by either the conventional acid- (oleum) treatment process (A) or the hydrogenation or hydrotreatment process (H). Their viscosity ranges from 10 to 100 centistokes (cSt) (10–100 mm^2/s). Thus a P100(H) oil refers to a paraffinic oil with a viscosity of 100 cSt produced by the hydrogenation process and an N10(A) oil to a naphthenic oil with a viscosity of 10 cSt produced by the acid-treatment process.

rats, including an assessment of immune function at appropriate time periods (with a reversal period of 1 year), and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached.

Only some of the requested information was available at the present meeting. The new information indicated that Fischer rats are more sensitive to mineral oils with respect to granuloma formation than other strains and species. An adequate long-term bioassay in Fischer rats given mineral oils at concentrations of 25 or 50 g/kg (2.5 or 5%) in the diet (equal to 1000 or 2000 mg/kg of body weight per day) did not show any carcinogenic potential for the materials studied, which were comparable to the medium-viscosity oils P70(H) and N70(H). The Committee understood that research on the pharmacokinetics of white mineral oils and their potential effects on immune function was still in progress, and that the requested long-term feeding study with a 1-year reversal period would not be available before January 2001.

The additional data did not provide a basis for changing the previously established temporary ADIs, which were extended to 2002, pending the review of reports of the requested studies.

A toxicological monograph was not prepared. The existing specifications were revised, with minor changes.

3.5 Preservatives: sulfur dioxide and sulfites

Sulfur dioxide and sulfites were evaluated at the sixth, eighth, ninth and seventeenth meetings of the Committee (Annex 1, references *6*, *8*, *11* and *32*). An ADI of 0–0.7 mg/kg of body weight was allocated at the seventeenth meeting to sulfur dioxide and to sulfur dioxide equivalents arising from sodium and potassium metabisulfite, sodium sulfite and sodium hydrogen sulfite. At subsequent meetings, potassium and calcium hydrogen sulfite and sodium thiosulfate were included in the group ADI (Annex 1, references *41*, *47* and *62*).

At its thirtieth meeting, the Committee retained the ADI of 0–0.7 mg/kg of body weight allocated to this group of compounds (Annex 1, reference *73*). The ADI was based on long-term studies in rats, including a 3-generation study of reproductive toxicity, with a NOEL of 2.5 g/kg (0.25%) sodium metabisulfite in the diet (supplemented with thiamine, as treatment of foods with sulfites reduces their thiamine content), equivalent to 70 mg/kg of body weight per day of sulfur dioxide equivalents. At doses at or above 10 g/kg (1%) in the diet, local irritation of the stomach was observed, with inflammatory changes and hyperplasia, and occult blood was detected in the faeces.

The histopathological changes were limited to the stomach; the incidence of neoplasms was not increased at any site or at any dose. A safety factor of 100 was used. Similar local changes in the stomach were observed in pigs fed thiamine-supplemented diets to which sodium metabisulfite was added. The Committee also reviewed case studies and challenge tests for idiosyncratic sensitivity to sulfiting agents, and noted the life-threatening nature of the adverse effects in some cases. It recommended that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications in which the use of sulfites may lead to high levels of acute intake. The Committee also reiterated the view expressed at its twenty-seventh meeting (Annex 1, reference *62*, section 2.4) that appropriate labelling was the only feasible means of protecting individuals who cannot tolerate certain food additives. At its thirtieth meeting, the Committee recommended that the situation with regard to the frequency of idiosyncratic adverse reactions and the relative toxic effects of free and bound sulfur dioxide should be kept under review. It also requested information on the chemical forms of sulfur dioxide in food.

At its present meeting, the Committee identified two issues pertaining to the toxicological evaluation of sulfites: general toxicity and idiosyncratic intolerance. Idiosyncratic intolerance did not appear to be related to deficiency of sulfite oxidase in humans. Since idiosyncratic intolerance and general toxicity appeared to be unrelated they were considered separately.

General toxicity

Studies performed since the thirtieth meeting of the Committee have demonstrated the quantitative conversion of sulfite ions to sulfate ions. The release of reduced glutathione by both isolated hepatocytes and perfused liver is probably due to sulfitolysis of oxidized glutathione by sulfite ions. The other product of sulfitolysis, glutathione *S*-sulfonate, was found to be a strong competitive inhibitor of both microsomal and cytosolic glutathione *S*-transferase in rat lung and liver cells and tumour cells from human lungs. These results suggested that sulfite ions could interfere with the glutathione pathway through their reaction with oxidized glutathione. In a study in rats, administration of 25 g/kg (2.5%) metabisulfite in the diet for 5 weeks increased the level of three disaccharidases involved in carbohydrate metabolism and of alkaline phosphatase in the brush-border membrane of cells of the small intestine.

The toxicity of bound and free forms of sulfite (acetaldehyde hydroxysulfonate and sodium metabisulfite, respectively) administered

in drinking-water was assessed in normal rats and rats made deficient in sulfite oxidase by administration of tungstate ions in drinking-water for 8 weeks. The rats deficient in sulfite oxidase were considered to be a better model for humans than the conventional rat model, which has an estimated 10–20 times higher concentration of this enzyme in the liver. Acetaldehyde hydroxysulfonate is a major bound form of sulfite in wines and other fermented foods and beverages and is considered to be a very stable form of bound sulfite. The doses of bound and free sulfite chosen were calculated to deliver the same dose in terms of sulfur dioxide equivalents (7, 70 or 350 mg/kg of body weight per day, the highest dose being reduced to 175 mg/kg of body weight per day after 3 weeks). The effects on body weight, food consumption and water intake were most marked in rats that received free rather than bound sulfite and in enzyme-deficient rats as compared with normal rats. The concentrations of sulfite and thiosulfate in urine and of *S*-sulfonates in plasma were elevated in untreated rats deficient in sulfite oxidase. Administration of bound sulfite to these rats resulted in increased excretion of sulfite in the urine, while administration of free sulfite was associated with increases in the concentrations of urinary thiosulfate and plasma *S*-sulfonates. The measured concentrations were variable, and were not clearly related to the dose. Histopathological lesions of the forestomach and glandular stomach were detected in all groups of rats treated at the highest dose. The severity and frequency of the gastric lesions were greater in the rats deficient in sulfite oxidase. Hepatic lesions were observed in animals receiving acetaldehyde hydroxysulfonate, and the NOEL was lower in the rats deficient in sulfite oxidase than in normal rats (7 mg and 70 mg of sulfur dioxide equivalents/kg of body weight per day, respectively). The effect may be due to the acetaldehyde that was formed after dissociation. The results showed that the gastric toxicity of bound sulfites, in the form of acetaldehyde hydroxysulfonate, was equivalent to that of free sulfite. Although the enzyme-deficient rats were more sensitive at doses above the NOEL, the NOEL was the same as that in normal rats.

Three assays of *in vitro* genotoxicity were reviewed; the results confirmed the previously noted observation that sulfites are genotoxic *in vitro* but not *in vivo*. This conclusion is consistent with the high reactivity of sulfite and its rapid inactivation in mammals. Two studies of teratogenicity in which sulfite was fed in the diet were available: neither was conducted in a completely satisfactory manner, as treatment did not cover the entire period of organogenesis. Maternal toxicity was demonstrated in both studies at doses equivalent to 840 mg of sulfur dioxide equivalents/kg of body weight per day and

above. No teratogenic effects were noted at intakes below this concentration.

The results of a study of the effects of ingested sulfite on the rat kidney demonstrated that administration of sodium metabisulfite by gavage at a dose of 5 mg/kg of body weight per day (3.4 mg of sulfur dioxide equivalents/kg of body weight per day) for up to 2 weeks reduced alkaline phosphatase activity in kidney tissue and concomitantly increased the concentrations of the enzyme in serum and urine; these effects occurred after the first dose. The activity of lactate dehydrogenase was likewise decreased in kidney tissue and increased in urine but not in serum. Urinary excretion of protein was increased 10-fold by the end of 2 weeks. These effects appear not to be related to treatment, as sulfite is quickly and quantitatively metabolized to sulfate and no sulfite was detected in urine nor were lesions of the kidney detected in normal rats after 8 weeks of treatment with a 20–50 times higher dose of sulfite in drinking-water.

Thus, the previous NOEL in studies of animals that ingested sulfite was confirmed in an 8-week study in rats in which the NOEL for gastric lesions was 70 mg of sulfur dioxide equivalents/kg of body weight per day in both normal rats and those deficient in sulfite oxidase, whether they were fed free sulfite or acetaldehyde hydroxysulfonate, which is a stable and prevalent form of bound sulfite. These gastric effects of sulfite, which were reported in rats and pigs, arise from local irritant effects. These effects would therefore be dependent on concentration rather than dose and a numerical ADI might not be appropriate. The Committee considered that the role of acetaldehyde in the reported effects of acetaldehyde hydroxysulfonate on the liver needed to be resolved before a re-evaluation of the safety of sulfur dioxide and sulfites could be completed. In view of these reservations, the previously established ADI of 0–0.7 mg/kg of body weight was maintained.

Idiosyncratic reactions in humans

A number of case studies in humans were available in which suspected hypersensitivity to sulfites based on adverse reactions to foods was confirmed by administration of single- or double-blind oral challenges with sulfites in solution or capsule form. While many of the cases involved individuals with chronic asthma whose response was primarily respiratory, a number of reports described adverse allergic-type responses in people without asthma, which did not involve respiratory symptoms. Some of these individuals had chronic allergic conditions. These responses were consistent with self-reported reactions documented by the Adverse Reaction Monitoring System

of the United States Food and Drug Administration. Evidence for an allergic basis for the adverse reactions to free and bound sulfites was provided in a number of the case reports. In studies in which only skin-prick testing had been attempted, allergy could not be ruled out, as further testing had not been done. Challenges with various food commodities containing sulfites also resulted in positive responses. In a double-blind study, lettuce treated with sulfites elicited the most consistent responses; sulfites in sulfite-treated lettuce are considered to be present mostly as free sulfite. While the mechanism by which sulfite ingestion precipitates idiosyncratic adverse reactions has not been established, there was some evidence of a mechanism involving the action of nitric oxide on parasympathetic receptors.

The prevalence of sulfite sensitivity was determined in groups of asthmatic adults and children who were either dependent or not dependent on steroids. In adults, the prevalence among those dependent on steroids was 4–8% and appeared to be less than 1% in those who were not dependent on steroids. The prevalence in the total population of asthmatic adults has been estimated to be 4%. The prevalence in asthmatic children was higher, approximately 20–30%, following double-blind challenges to children who were either dependent or not dependent on steroids.

The Committee reiterated the recommendation made at its thirtieth meeting (Annex 1, reference *73*) that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications (e.g. control of enzymic browning in fresh salad vegetables) in which the use of sulfites may lead to high levels of acute intake and which have most commonly been associated with life-threatening adverse reactions. The Committee considered that appropriate labelling would help to alert individuals who cannot tolerate sulfites.

The intake of sulfites is assessed in section 5.4.

An addendum to the toxicological monograph was prepared. The existing specifications for potassium metabisulfite, potassium sulfite, sodium hydrogen sulfite, sodium metabisulfite, sodium sulfite and sodium thiosulfate were revised and designated as "tentative", pending the receipt of information regarding the test for selenium. The existing specifications include a limit for selenium for which the test method is no longer feasible because of a lack of availability of the required reagents. The Committee also questioned the need for a limit for selenium because new processes by which sulfur is produced as a by-product of the oil industry have replaced extraction methods from sulfur mines. The Committee noted that the limit for

selenium may no longer be necessary and requested information in this regard.

The existing specifications for calcium hydrogen sulfite were maintained and designated as "tentative". Information was requested regarding a modified name for calcium hydrogen sulfite that would be more reflective of its physical properties, the extent and functionality of its use as a food additive, and the need for limits for selenium and arsenic and associated analytical methods.

The existing specifications for sulfur dioxide were revised.

No information was made available to the Committee regarding calcium metabisulfite, calcium sulfite and potassium hydrogen sulfite. No specifications were prepared for these substances.

3.6 Sweetening agent: stevioside

Stevioside is a sweet glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-18-oic acid). It is a natural constituent of the plant *Stevia rebaudiana* Bertoni, belonging to the Compositae family. Stevioside has not been previously evaluated by the Committee.

After oral administration to rats, stevioside is not readily absorbed from the upper small intestine but is hydrolysed to the aglycone, steviol, before absorption from the gut. Steviol is completely absorbed and is excreted in the bile as conjugates; only a very small fraction is detectable in urine. After biliary excretion, the conjugates are hydrolysed, and steviol undergoes enterohepatic circulation; its elimination half-life is 24 hours. Steviol is the only faecal metabolite of stevioside that has been identified, and excretion in the faeces is the major route. After intravenous administration, stevioside is rapidly distributed throughout the body, partially secreted by the renal tubular epithelium, and excreted in the urine.

At high concentrations, stevioside affected a variety of biochemical parameters in rat tissues *in vitro*. It weakly inhibited oxidative phosphorylation; steviol was about 30 times more potent in this respect. The most likely mechanism for this effect is inhibition of the mitochondrial translocation of adenine nucleotides. Steviol also inhibited glucose absorption from rat gut by reducing the ATP content of the intestinal mucosa. Stevioside may also act as a calcium antagonist, showing a hypotensive effect and inducing diuresis, natriuresis and a fall in reabsorption of glucose in the renal tubules. Stevioside may not, however, be able to penetrate cell membranes. Although most of these studies were performed after intravenous

injection of stevioside, oral administration of extracts of *S. rebaudiana* to rats caused similar effects (hypotension and diuresis).

Stevioside has very low acute oral toxicity. Oral administration of stevioside at a concentration of 25 g/kg (2.5%) in the diet to rats for 2 years, equal to 970 and 1100 mg/kg of body weight per day in males and females, respectively, had no significant effect. Reduced body-weight gain and survival rate were observed at a level of 50 g/kg (5%) in the diet. There was no indication of carcinogenic potential in the long-term study and no evidence of potential to promote tumours of the urinary bladder in a separate bioassay.

In reproductive toxicity studies, administration of stevioside at doses of up to 2500 mg/kg of body weight per day to hamsters and 3000 mg/kg of body weight per day to rats had no effect. Although an aqueous infusion of *S. rebaudiana* administered orally to female rats was reported to cause a severe, long-lasting reduction in fertility, the contraceptive effect was probably not due to stevioside. Neither teratogenic nor embryotoxic effects were observed in rats given stevioside at doses of up to 1000 mg/kg of body weight per day by gavage.

The results of genotoxicity tests with stevioside in various *in vitro* systems were uniformly negative.

The aglycone, steviol, exhibited greater acute toxicity than stevioside in hamsters but not in rats. Steviol was clearly genotoxic after metabolic activation, inducing forward mutations in bacteria and gene mutations and chromosomal aberrations in lung fibroblasts of Chinese hamsters. Several mechanistic studies indicated that the structural features necessary for the expression of mutagenic activity include a hydroxyl group at position C13 and an unsaturated bond joining the C16 and C17 carbon atoms of steviol. The fact that stevioside is glycosylated at position C13 could explain the absence of mutagenicity. The active metabolite of steviol responsible for its mutagenic activity is not known. While some data suggest that epoxidation may be involved in the metabolic activation of steviol, other data indicate that the active metabolite is not an epoxide. Preliminary data indicate that steviol may be activated to a mutagenic metabolite by human liver microsomes.

The Committee noted a number of shortcomings in the information available on stevioside. In several studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of metabolism of stevioside and steviol in humans were available. In addition, data on long-term toxicity and

carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

In view of the absence of information for the elaboration of specifications for stevioside and since the evaluation of the available toxicological data revealed several limitations, the Committee was unable to relate the results of the toxicological investigations to the commercial product and could not allocate an ADI to stevioside.

Before reviewing stevioside again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

A toxicological monograph was prepared. No specifications were prepared as no information was forthcoming.

3.7 Thickening agents

3.7.1 *Carrageenan*

Carrageenan is a sulfated polygalactan with an average relative molecular mass well above 100000 derived from a number of seaweeds of the class Rhodophyceae. It has no nutritive value and is used in food preparation for its gelling, thickening and emulsifying properties. Three main types of carrageenan, known as ι-, κ- and λ-carrageenan, are used commercially in the food industry. These names do not reflect definitive chemical structures but only generalized differences in the composition and degree of sulfation at specific locations in the polymer.

Carrageenan was previously reviewed by the Committee at its thirteenth, seventeenth and twenty-eighth meetings (Annex 1, references *19*, *32* and *66*). At the twenty-eighth meeting, an ADI "not specified" was allocated on the basis of the results of a number of toxicological studies on carrageenans obtained from various species of seaweed. The studies included a 3-generation study of reproductive toxicity, short-term and long-term toxicity studies in rats at levels of up to 50 g/kg (5%) in the diet, and short- and long-term toxicity studies in hamsters, guinea-pigs and monkeys. In general, the only effect observed was soft stools or diarrhoea at high doses, except in two studies in which material, identified as being ι-carrageenan, administered at 10 g/l (1%) in the drinking-water or 50 g/kg (5%) in

the diet, produced ulceration in the gastrointestinal tract of guinea-pigs. Degraded carrageenans can produce this effect; they are not, however, used as food additives. At its twenty-eighth meeting, the Committee pointed out that degraded carrageenans and "semi-refined carrageenan" (or "processed *Eucheuma* seaweed") were not included in the specifications for the food-grade material. At its forty-fourth meeting, in reviewing the data on processed *Eucheuma* seaweed obtained from *E. cottonii*, the Committee concluded that a complete review of data on carrageenan should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested (Annex 1, reference *116*).

At its present meeting, the Committee considered studies published since the review at the twenty-eighth meeting and, for earlier studies, indicated the identity of the source material and the type of carrageenan, when these could be identified.

Most of the toxicological studies of the carrageenans in which an identifiable type of carrageenan and an identifiable seaweed species were used were undertaken with κ- or κ/λ-carrageenan from *Chondrus crispus*. There have been a few parallel studies, the results of which do not suggest that there are large differences between the effects of various forms of carrageenan or between the effects of carrageenans prepared from different species of seaweed.

The carrageenans are dietary fibres, generally of a high relative molecular mass, which are not broken down to very small molecules in the gastrointestinal tract. At high levels of intake, these properties can cause adverse effects through their physical action on the gastrointestinal tract. As mentioned above, ulceration has been observed in the gastrointestinal tract of guinea-pigs given high levels of ι-carrageenan. These effects were not observed in a recent well conducted 90-day study in which rats were fed diets containing 50 g/kg (5.0%) of conventionally processed ι-carrageenan from *E. spinosum* or κ-carrageenan from *E. cottonii*. The changes that occurred during the study, most notably an increase in the relative weight of the full and empty caecum, were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and to be without toxicological significance. The partial reversal of the caecal weight changes during the 28-day recovery phase of the study and the absence of histopathological changes support this conclusion.

Studies of the carcinogenicity of carrageenan in rats have failed to demonstrate any effect. In addition, assays of the genotoxicity of

carrageenan have been negative. A proliferative response of the mucosa of the gastrointestinal tract of rats fed two forms of carrageenan at 26 g/kg (2.6%) or 50 g/kg (5.0%) in the diet has been reported; the response was reversible in the study in which carrageenan was given at 50 g/kg in the diet. This response might explain the promotion of the action of known experimental colon carcinogens administered to rats given carrageenans at 25 g/kg (2.5%) or 60 g/kg (6%) in the diet. The Committee was aware of an abstract of a conference report which indicated that tumour promotion does not occur in rats in which the intestinal microflora are derived from human donors who have been "adapted" to the presence of carrageenan in the gastrointestinal tract. This would suggest that promotion of colon carcinogenesis in the rat is dependent on the presence of the normal microflora of the gastrointestinal tract.

Early reports of the detection of carrageenan in parenteral tissues after dietary intake are probably unreliable. The presence of carrageenans in the macrophages found in the walls of the caecum and colon may reflect the relative molecular mass distribution of the preparation used in the study. Maintenance of a restriction on the relative molecular mass distribution in the specifications for carrageenan for food use provides protection against the adverse effects of carageenans of low relative molecular mass.

Some data suggest that carrageenan affects the immune response of the gastrointestinal tract; however, no validated tests for assessing the nature and potential consequences of such an effect were available. A short communication relating to an epidemiological study did not indicate that carrageenan was immunotoxic in neonates receiving milk preparations containing carrageenan. The Committee reiterated its previous statement that the ADI should not be considered applicable to neonates and young infants below the age of 12 weeks.

The Committee extended the previous ADI "not specified" for carrageenan to include processed *Eucheuma* seaweed in a group ADI "not specified". It expressed concern about the potential promotion of colon carcinogenesis by carrageenans and processed *Eucheuma* seaweed and therefore made the group ADI "not specified" temporary, pending clarification of the significance of the promotion of colon cancer observed in studies in rats. The Committee requires this information for evaluation in 2001.

An addendum to the toxicological monograph was prepared. The existing specifications were revised in order to improve both the description and the definition of the materials covered. The test methods were modified by introduction of the methods initially

prepared for processed *Eucheuma* seaweed. Microbiological criteria were also added.

3.7.2 *Processed Eucheuma seaweed*

Processed *Eucheuma* seaweed was previously considered by the Committee at its thirtieth, thirty-ninth, forty-first and forty-fourth meetings (Annex 1, references *73*, *101*, *107* and *116*). At its thirtieth and thirty-ninth meetings, the Committee was unable to evaluate the use of processed *Eucheuma* seaweed in food because no relevant toxicological data were available. At its forty-first meeting, the Committee considered a 90-day feeding study in rats, for which complete details were not available, and a series of genotoxicity studies on processed *Eucheuma* seaweed from *E. cottonii*. The Committee allocated a temporary ADI of 0–20 mg/kg of body weight to processed *Eucheuma* seaweed from *E. cottonii*, pending submission of the complete details from the 90-day study, including histopathological data for individual animals. At its forty-fourth meeting, the Committee reviewed these data and the results of new genotoxicity and cytotoxicity assays. Because of the chemical relationship between processed *Eucheuma* seaweed and traditionally refined carrageenan, the Committee considered that toxicological data on the latter were relevant to the safety assessment of the carrageenan polysaccharide constituents of processed *Eucheuma* seaweed, but could not replace adequate toxicological studies on processed *Eucheuma* seaweed itself. Although the data for individual animals in the 90-day feeding study in rats confirmed the accuracy of the summary data and the conclusions derived from them, the Committee expressed reservations about the design, conduct and documentation of the study. Additionally, none of the available genotoxicity studies was considered to be adequate because of deficiencies in conduct or reporting. The Committee therefore extended the temporary ADI, pending submission of the results of a new 90-day feeding study in rats and an appropriate battery of genotoxicity studies, all meeting present-day standards, for processed *Eucheuma* seaweed derived from *E. cottonii*.

At the forty-fourth meeting, a request was made to amend the specifications to include *E. spinosum* as a source material for processed *Eucheuma* seaweed. In recognition of the fact that ulcerative lesions in the caecum had been noted in toxicity studies in guinea-pigs given ι-carrageenan, the major component of *E. spinosum*, the Committee concluded that a separate 90-day feeding study in rodents and a separate battery of genotoxicity studies were required for processed *Eucheuma* seaweed derived from *E. spinosum*,

if the specifications were to be expanded to include processed *Eucheuma* seaweed derived from this species. The Committee also concluded that a complete review of all data on carrageenan should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested.

At its present meeting, the Committee reviewed the results of a new 90-day toxicity study in rats fed processed *Eucheuma* seaweed from two sources, *E. cottonii* and *E. spinosum*, at concentrations of 0 g/kg, 5 g/kg (0.5%), 15 g/kg (1.5%) or 50 g/kg (5%) in the diet, or conventionally processed carrageenan from these two sources at 50 g/kg in the diet. At the highest concentrations, the intake of the processed *Eucheuma* seaweeds was equal to 4300 and 5000 mg/kg of body weight per day, respectively, for male and female rats fed the material derived from *E. cottonii* and to 4500 and 5100 mg/kg of body weight per day, respectively, for male and female rats fed the material from *E. spinosum*. No adverse effects were noted in the study. The changes observed in rats receiving the highest concentrations of processed *Eucheuma* seaweed from these two sources, most notably an increase in the relative weight of the full and empty caecum, were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and to be without toxicological significance. There was no indication that the effects of the processed *Eucheuma* seaweeds differ from those of conventionally prepared carrageenans from the same seaweed species.

Processed *Eucheuma* seaweed derived from either *E. cottonii* or *E. spinosum* was not mutagenic in well conducted assays for reverse mutation in *Salmonella typhimurium* strains. The Committee considered that no further studies of genotoxicity were required.

In view of the lack of toxicity of processed *Eucheuma* seaweeds derived from either *E. cottonii* or *E. spinosum*, the Committee determined that both these species could be included in the specifications for processed *Eucheuma* seaweeds. Additionally, because of the similarities in the nature of the processed *Eucheuma* seaweeds and the conventionally processed carrageenans and in the effects that they caused in the recent 90-day toxicity study in rats, the Committee included carrageenans and processed *Eucheuma* seaweed in a temporary group ADI "not specified", to be reviewed in 2001 (see section 3.7.1).

An addendum to the toxicological monograph was prepared. The existing specifications were revised and *E. spinosum* was included as a source material.

3.7.3 *Sodium carboxymethyl cellulose, enzymatically hydrolysed*

Sodium carboxymethyl cellulose, enzymatically hydrolysed, is a water-soluble dietary fibre with a relative molecular mass in the range of about 800–10000. It acts mainly as a stabilizer with fat-extending properties. It can be used in formulating low-fat and reduced-fat foods and soft drinks.

Sodium carboxymethyl cellulose, enzymatically hydrolysed, is prepared from regular, food-grade sodium carboxymethyl cellulose by partial enzymatic hydrolysis under mildly acidic conditions using a food-grade cellulase enzyme from *Trichoderma longibrachiatum*.

Sodium carboxymethyl cellulose was allocated an ADI "not specified" at the thirty-fifth meeting of the Committee (Annex 1, reference *88*). The cellulase enzyme was allocated an ADI "not specified" at the thirty-ninth meeting (Annex 1, reference *101*). Sodium carboxymethyl cellulose, enzymatically hydrolysed, has not been previously evaluated by the Committee.

At its present meeting, the Committee reviewed two studies in which the properties of carboxymethyl cellulose, enzymatically hydrolysed, were compared with those of the parent material, carboxymethyl cellulose. The first was a 90-day study of toxicity in which male and female rats received diets containing 0 g/kg, 25 g/kg (2.5%), 50 g/kg (5.0%) or 100 g/kg (10%) of either sodium carboxymethyl cellulose, enzymatically hydrolysed, or sodium carboxymethyl cellulose. The second was a comparative study of the absorption, distribution and excretion of these two materials in rats.

In the 90-day study in rats receiving either sodium carboxymethyl cellulose, enzymatically hydrolysed, or carboxymethyl cellulose histopathological changes in the kidney and urinary bladder were reported. These were associated with changes in kidney weight, increased urine volume and increased excretion of some ionic substances in the urine. Caecal enlargement was also reported. For comparison, the Committee considered the toxicity studies on sodium carboxymethyl cellulose that had been reviewed at its thirty-fifth meeting (Annex 1, reference *88*). It noted that, in early studies that appeared to be comparable with regard to species, dietary concentration of sodium carboxymethyl cellulose and duration of exposure, no changes of organ weight or histopathological appearance were found that were comparable to those reported in the 90-day study reviewed at the present meeting.

Since the morphological changes in the kidney occurred only in males receiving the highest dose of sodium carboxymethyl cellulose, enzymatically hydrolysed, and the lowest dose of carboxymethyl

cellulose, the Committee was not convinced that the response was of toxicological concern. The observed epithelial hyperplasia of the urinary bladder was generally graded as slight or very slight and occurred in male rats given either sodium carboxymethyl cellulose, enzymatically hydrolysed, or carboxymethyl cellulose at 100 g/kg (10%) in the diet. The Committee considered that this represented a response to a high intake of sodium ions by rats of the strain used in the new study. Administration in the diet of additional sodium (approximately four times that available in the diet of the control group) could have led to the observed histopathological changes and changes in urinary excretion. Also, the caecal enlargement, diarrhoea and other secondary changes were considered to be the consequence of the accumulation of poorly absorbed, water-soluble material in the caecum and colon and to be without toxicological significance. The NOELs for sodium carboxymethyl cellulose, enzymatically hydrolysed, were equal to 6000 and 6600 mg/kg of body weight per day in male and female rats, respectively.

The metabolic study showed that passage of carboxymethyl cellulose through the gastrointestinal tract results in partial breakdown of the bonds between monomeric units to result in a material with a relative molecular mass equivalent to that of carboxymethyl cellulose, enzymatically hydrolysed. The amounts of radioactivity excreted in the faeces, urine and expired air after administration of radiolabelled carboxymethyl cellulose and sodium carboxymethyl cellulose, enzymatically hydrolysed, were quantitatively almost identical, providing additional evidence that in rats, the end-products of digestion of the two products are similar.

The Committee concluded that these similarities are consistent with the absence of any toxicologically significant difference between carboxymethyl cellulose and carboxymethyl cellulose, enzymatically hydrolysed. Therefore, the Committee included carboxymethyl cellulose, enzymatically hydrolysed, in the group ADI "not specified" with ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, methyl ethyl cellulose and sodium carboxymethyl cellulose.

A toxicological monograph was prepared. The existing tentative specifications were revised and the "tentative" designation was deleted.

3.8 Miscellaneous substances

3.8.1 *γ-Cyclodextrin*

γ-Cyclodextrin is a ring-shaped molecule made up of eight glucose units linked by α-1,4 bonds. It is produced by the action of the enzyme

cyclomaltodextrin glucanotransferase (cyclodextrin-glycosyl transferase; EC 2.4.1.19) on hydrolysed starch syrups. The circular structure of γ-cyclodextrin provides a hydrophobic cavity that allows complexes to be formed with a variety of organic molecules, while the hydrophilic outer surface makes γ-cyclodextrin water-soluble. Because of these properties, γ-cyclodextrin can be used in a variety of ways in food with potential intakes of the order of grams per person per day.

γ-Cyclodextrin has not been previously evaluated by the Committee; however, the structurally related β-cyclodextrin (seven glucose units) was evaluated at the forty-first and forty-fourth meetings of the Committee (Annex 1, references *107* and *116*). The Committee noted that the close structural similarity between γ-cyclodextrin and β-cyclodextrin allows some comparisons to be made of the metabolism and the toxicity of these two compounds.

The metabolism of γ-cyclodextrin is different from that of β-cyclodextrin, as demonstrated in both *in vitro* and *in vivo* studies. In *in vivo* studies in rats, γ-cyclodextrin (at single doses of up to 1000 mg/kg of body weight) was rapidly metabolized to glucose, presumably by the luminal and/or epithelial enzymes of the gastrointestinal tract. In contrast to the metabolism of β-cyclodextrin, there was little involvement of the gastrointestinal microflora. Similarly, in contrast to β-cyclodextrin, much less γ-cyclodextrin was absorbed and only very low levels could be detected in the urine. After intravenous injection, γ-cyclodextrin (at single doses of up to 600 mg/kg of body weight) was rapidly cleared from the blood and was excreted, largely unchanged, in the urine. Although no studies of metabolism in humans *in vivo* were available, γ-cyclodextrin, unlike β-cyclodextrin, can be readily hydrolysed by human salivary and pancreatic amylases *in vitro*.

Short-term (28- and 90-day) toxicity studies indicate that γ-cyclodextrin has little toxicity when given intravenously or orally to rats or orally to dogs. After administration of a very high concentration (200 g/kg or 20%) in the diet, caecal enlargement and associated changes were seen in both species. This effect is likely to result from the presence of a high concentration of an osmotically active substance in the large intestine. This result suggests that the metabolism of γ-cyclodextrin is less efficient at doses higher than those used in the studies of metabolism. The results of the studies in rats treated intravenously indicate that γ-cyclodextrin is well tolerated when given systemically, and may be less toxic than β-cyclodextrin.

Studies conducted in rats and rabbits with γ-cyclodextrin at doses of up to 200 g/kg (20%) in the diet did not indicate any teratogenic effects. Similarly, the results of a battery of genotoxicity studies were

negative. No long-term studies of toxicity, carcinogenicity or reproductive toxicity have been conducted, but, given the rapid metabolism of γ-cyclodextrin to glucose and its lack of genotoxicity, the Committee concluded that such studies were not required for an evaluation of this substance.

In *in vitro* studies, γ-cyclodextrin, like β-cyclodextrin, sequestered components of the membranes of erythrocytes, causing haemolysis. The threshold concentration for this effect was, however, higher than that of β-cyclodextrin. Furthermore, γ-cyclodextrin was not detected in blood after dietary administration of high doses to animals.

It was considered unlikely that interaction of γ-cyclodextrin with lipophilic vitamins would impair their bioavailability because of the rapid metabolism of γ-cyclodextrin *in vivo*. Also, there was no evidence that vitamin deficiency was induced in experimental animals given high doses of γ-cyclodextrin.

The Committee considered studies on the short-term toxicity and genotoxicity of the enzyme, cyclomaltodextrin glucanotransferase, used in the production of cyclodextrins, and of the complexant, γ-cyclohexadecen-1-one, used to optimize formation of γ-cyclodextrin. The toxicological data indicated that these substances are unlikely to be of toxicological concern in the final preparation of γ-cyclodextrin complying with the specifications. The Committee also reviewed information on the genetic modification of the organism used to produce the enzyme, which did not raise any concerns.

Although no study of human tolerance to γ-cyclodextrin was submitted before the meeting, the Committee was aware that such a study was available. Although it was unable to review the data, the Committee noted that the toxicity of this compound is relatively low in animals and that it is less toxic than β-cyclodextrin, for which studies of human tolerance were available. Also, its rapid metabolism to maltose, maltotriose and glucose *in vitro* by human salivary and pancreatic amylases supports the conclusion that, as in laboratory animals, it would be metabolized to innocuous metabolites before absorption.

The Committee concluded that there were sufficient data to allocate a temporary ADI "not specified", but that the study of human tolerance known to have been conducted should be reviewed in order to confirm the absence of adverse effects on the gastrointestinal tract at normal levels of intake. These data should be submitted for consideration by the Committee by 1999.

A toxicological monograph and new specifications for γ-cyclodextrin were prepared.

3.8.2 *Glucono-δ-lactone, and the calcium, magnesium, potassium and sodium salts of gluconic acid*

Glucono-δ-lactone was previously evaluated by the Committee at its tenth, eighteenth and thirtieth meetings (Annex 1, references *13*, *35* and *73*). At its eighteenth meeting, the Committee established an ADI of 0–50 mg/kg of body weight for glucono-δ-lactone. At the thirtieth meeting, the Committee changed the ADI for glucono-δ-lactone to an ADI "not specified" on the basis of biochemical and metabolic data on glucono-δ-lactone and gluconic acid, noting that in an aqueous medium glucono-δ-lactone exists in equilibrium with D-gluconic acid. These compounds are intermediates in the oxidation of glucose through the pentose phosphate cycle. Data from studies that were evaluated previously by the Committee showed no evidence of carcinogenicity, teratogenicity or genotoxicity caused by glucono-δ-lactone.

Since the last toxicological evaluation of glucono-δ-lactone, a new acute toxicity study and two new 28-day oral toxicity studies on sodium gluconate in rats have become available. These studies were evaluated in order to determine whether the current ADI "not specified" for glucono-δ-lactone could be extended to a group ADI "not specified" for glucono-δ-lactone and the calcium, magnesium, potassium and sodium salts of gluconic acid.

Calcium, magnesium, potassium and sodium gluconate were previously evaluated by the Committee as individual compounds or in other group categories as inorganic salts and salts of organic acids (Annex 1, references *32*, *50* and *70*). The Committee concluded that they were freely ionizable and that it was appropriate to allocate ADIs on the basis of data on their corresponding anion (gluconic acid).

The results of the new acute toxicity study showed no evidence of toxicity in rats that were given single doses of 500, 1000 or 2000 mg of sodium gluconate/kg of body weight by gavage.

In the two new 28-day studies in rats, sodium gluconate was administered orally either by gavage at doses of 0, 500, 1000 or 2000 mg/kg of body weight per day or in the feed at doses of 0 g/kg, 12.5 g/kg (1.25%), 25 g/kg (2.5%) or 50 g/kg (5.0%) (equal to 0, 1000, 2000 and 4100 mg/kg of body weight per day, respectively). A control group received sodium chloride at 13.5 g/kg (1.35%) in the feed (equal to 1100 mg/kg of body weight per day), which was equivalent to the concentration of sodium in the sodium gluconate given to the highest-dose group.

In the gavage study, there was a significant increase in the relative weight of the kidneys (unilateral) in the males that received 1000 and 2000 mg of sodium gluconate/kg of body weight per day. No treatment-related or dose-related effects were observed on any of the other parameters examined in this study.

The effects observed in the feeding study, i.e. increased water intake, prothrombin time and relative kidney weights, were not dose-related.

Qualitative analysis of urine revealed effects in both 28-day studies that were considered by the Committee to be related to the high sodium intake arising from the sodium gluconate.

On the basis of a re-evaluation of the data previously considered by the Committee and the new short-term toxicity data on sodium gluconate, the Committee extended the previous ADI "not specified" for glucono-δ-lactone to a group ADI for glucono-δ-lactone and the calcium, magnesium, potassium and sodium salts of gluconic acid.

An addendum to the toxicological monograph was prepared. The existing specifications for glucono-δ-lactone, calcium gluconate, potassium gluconate and sodium gluconate were revised. The specifications for magnesium gluconate were revised and designated as "tentative", with a request for information on the need for maintaining the microbiological criteria included in the specifications, and for introducing maximum limits for sulfate and chloride.

3.8.3 *Polyglycitol syrup*

Polyglycitol syrup has not previously been evaluated by the Committee. Since its components are the same as those of maltitol syrup, differing only in the relative proportions of sorbitol, maltitol and higher-order polyols, the evaluation conducted for maltitol syrup at the forty-ninth meeting (Annex 1, reference *131*) in order to accommodate a wide range of starch hydrogenation products would also apply to polyglycitol syrup.

At the forty-ninth meeting, toxicological data in support of new specifications for maltitol syrup were reviewed. Since an ADI "not specified" had been established for both sorbitol and maltitol, the toxicological review concentrated only on the consequences of the higher-order hydrogenated saccharides. The Committee concluded at its forty-sixth meeting (Annex 1, reference *122*, section 2.2.4) that the occurrence of proliferative lesions of the adrenal medulla in rats fed polyols (including sorbitol and maltitol) and lactose was a species-specific response and was not relevant to the toxicological evaluation of these substances for humans. Several short-term studies in which

rats and dogs were given materials with a higher-order polyol content exceeding 80% of the syrup were reviewed at the forty-ninth meeting, as were data on the metabolic fate of higher-order polyols.

At its present meeting, the Committee reviewed new data relating to digestibility *in vitro*, a short-term toxicity study in rats given material with a higher-order polyol content of 78%, and a study on the effects of hydrogenated starch hydrolysates on the glycaemic response in diabetic and non-diabetic individuals.

Hydrolysis of a polyglycitol syrup composed of 14% sorbitol, 8% maltitol and 78% higher-order polyols resulted in the production of more glucose and less sorbitol than did hydrolysis of products conforming to the specifications for maltitol syrup. This is consistent with the relative proportion of glucose released on hydrolysis of each component. The greater digestibility of the hydrogenated starch hydrolysates with the highest content of highly polymerized saccharides was considered to be the consequence of a greater activity of digestive enzymes or a higher affinity for glucose–glucose bonds than for glucose–sorbitol bonds. Data on the disposition of maltitol syrup reviewed at the forty-ninth meeting indicated that the higher-order polyols would be completely hydrolysed to glucose and maltitol, with a considerable portion undergoing fermentation in the lower gut. Bacterial fermentation was demonstrated by detection of an increased amount of hydrogen in the air expired by human subjects in studies reviewed at the present meeting.

Inclusion of a polyglycitol syrup (composed of 14% sorbitol, 8% maltitol and 78% higher-order polyols) and two maltitol syrups (one containing 7% sorbitol, 60% maltitol and 33% higher-order polyols and the other 7% sorbitol, 52% maltitol and 41% higher-order polyols) in the diet of rats at doses of up to 200 g/kg (20%), equal to 13 g/kg of body weight per day, for 13 weeks, was not associated with adverse effects. The only effects observed — increased weight of the empty caecum and increased excretion of calcium in the urine in the absence of elevated levels of serum calcium — were considered to be the consequence of the accumulation of poorly absorbed material in the caecum and were without toxicological significance.

In studies of diabetic and non-diabetic human subjects, ingestion of maltitol syrup resulted in a lower glycaemic response than with polyglycitol syrup, which in turn was lower than the response observed with glucose. These results reflect the relative proportion of glucose released following hydrolysis of each material.

On the basis of the data on hydrogenated oligo- and polysaccharides reviewed at the forty-ninth meeting and the present one, the

Committee allocated a group ADI "not specified" to materials conforming to the specifications for polyglycitol syrup and maltitol syrup.

A toxicological monograph and new specifications were prepared.

4. Substances evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

Seven groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Fig. 1 (see section 2.2.1; Annex 1, references *116*, *122* and *131*).

The Committee noted that in applying the Procedure, the substance is first assigned to a structural class as identified at the forty-sixth meeting of the Committee (Annex 1, reference *122*). The structural classes are as follows:

- Class I. Substances that have simple chemical structures and efficient modes of metabolism which would suggest a low order of oral toxicity.
- Class II. Substances that have structural features that are less innocuous than those of substances in Class I, but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Substances that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting:

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

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 intake of 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.

Intake data

Intake estimates were derived from surveys in Europe and the USA. Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. In the USA, a series of surveys was conducted between 1970 and 1987 by the National Academy of Sciences National Research Council (under contract to the Food and Drug Administration) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added in a number of broad food categories. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in the European Union during the previous year. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In using these survey data to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported and that the total amount used in food is consumed by only 10% of the population.

$$\text{Intake } (\mu\text{g/person per day}) = \frac{\text{Annual volume of production (kg)} \times 10^9\ (\mu\text{g/kg})}{\text{Population of consumers} \times 0.6 \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 24×10^6 in the USA.

4.1 General aspects of metabolism

The flavouring agents evaluated at the present meeting share a number of functional groups, e.g. linear, branched, alicyclic and unsaturated alkyl chains, and alcohol, ester and ketone groups. Therefore, they have a number of common and interrelated metabolic features, and data on one or more members of a group under evaluation could be used to predict the metabolic fate of analogues for which there were no data. Metabolic pathways common to the groups of flavouring agents evaluated at the present meeting are:

— hydrolysis of esters;
— oxidation of alcohols and aldehydes;
— conjugation of alcohols;
— reduction of ketones;
— reduction of double bonds;
— oxidation of side-chains;

— oxidation of alicyclics; and
— conjugation with glutathione.

4.1.1 *Hydrolysis of esters*

The hydrolysis of ethyl, isoamyl and allyl esters was considered at the forty-sixth meeting of the Committee (Annex 1, reference *122*). Most esters are hydrolysed rapidly by enzymes present in the gut lumen, intestinal wall and liver to yield the corresponding acids and alcohols.

A wide range of enzymes (carboxyesterases and carboxylic acid hydrolases) is capable of hydrolysing esters into their constituent alcohols and acids. Carboxyesterases are found in many tissues, and there is high esterase activity in the intestinal tract, blood, liver, kidney, lung, brain and pancreas. The multiplicity of esterases and their wide distribution in the tissues result in the rapid hydrolysis of esters *in vivo*. This extensive metabolic activity is the basis for the development by the pharmaceutical industry of prodrugs, which are carboxylic acid esters of therapeutically active agents. The esters considered at the present meeting contain larger and more complex alkyl constituents in both the alcohol and acid moieties, and there were only limited data on their *in vitro* hydrolysis, but the data were comparable to those considered at the forty-sixth meeting of the Committee. Isopropyl phenylacetate and isopropyl butyrate (isopropyl butanoate) underwent 40% hydrolysis after 2 hours' incubation with pancreatin, a value similar to that for some of the esters considered at the forty-sixth meeting. Essentially complete hydrolysis of isopropyl palmitate (isopropyl hexadecanoate), isopropyl oleate or isopropyl stearate (isopropyl octadecanoate) radiolabelled in the fatty acid moiety was demonstrated *in vivo* in rats by the detection of over 95% of the radioactivity in the triglyceride fraction of blood. *n*-Octyl esters would be expected to undergo very rapid hydrolysis on the basis of the relationship between an increase in chain length (in the range C1–C8) and an increase in V_{max} (maximal rate of reaction) and a decrease in K_m (Michaelis constant), as discussed at the forty-sixth meeting. The hydrolysis of branched-chain octyl esters and esters of longer chain length would probably be slower than that of the corresponding *n*-alkyl analogues, and absorption of the intact ester is possible.

The hydrolysis of esters with larger alcohol substituents, such as are present in the linalyl, terpinyl, carvyl and menthyl esters evaluated at the present meeting, has been the subject of only limited study. Linalyl acetate was hydrolysed rapidly in water and in artificial gastric and pancreatic fluids, and the mean half-lives for hydrolysis were

5.5 minutes in artificial gastric fluid and 52.5 minutes in artificial pancreatic fluid, values comparable to those considered at the forty-sixth meeting. Allyl tiglate and benzyl tiglate were not hydrolysed by artificial gastric or pancreatic fluid, but were hydrolysed extensively on incubation with intestinal tissue and would be predicted to be completely hydrolysed by the liver, both *in vitro* and *in vivo*. (–)-Menthol ethylene glycol carbonate and (–)-menthol propylene glycol carbonate were hydrolysed slowly on incubation with rat liver homogenate for 4 hours, with 85% hydrolysis to menthol for (–)-menthol ethylene glycol carbonate and 75% for (–)-menthol propylene glycol carbonate. The structurally related flavouring agent bornyl acetate was excreted largely as the glucuronic acid conjugate of borneol after oral administration to rabbits, which is consistent with extensive hydrolysis *in vivo*. More than 80% of radiolabelled cyclandelate, a trimethyl cyclohexyl ester, was hydrolysed after 20 minutes of incubation with rat hepatic microsomes. In an *in vivo* study in which methyl cinnamate was administered orally to rats, it was hydrolysed only slowly in the intestinal lumen, but underwent essentially complete first-pass hydrolysis, because none of the parent compound could be detected in the systemic circulation. In contrast, propyl anthranilate was not completely hydrolysed during absorption, and the intact ester was detected in the peripheral blood and urine. These findings are similar to the hydrolysis data considered at the forty-sixth meeting.

These results indicate that hydrolysis would be the major pathway for all of the esters evaluated at the present meeting; however, first-pass metabolism may be incomplete for a few of the esters in some groups. In general, esters that reach the systemic circulation intact would be expected to be hydrolysed extensively by tissue esterases into their constituent acids and alcohols.

4.1.2 *Oxidation of alcohols and aldehydes*

Primary alcohols and aldehydes

Primary aliphatic alcohols (attached to linear, branched or unsaturated alkyl chains) and their corresponding aldehydes are efficiently oxidized to the corresponding carboxylic acids by high-capacity enzyme pathways. NAD^+/NADH-dependent alcohol dehydrogenase catalyses the oxidation of primary aliphatic saturated and unsaturated alcohols to their corresponding aldehydes. A comparison of the alcohol structure with the enzyme-binding affinity of alcohol dehydrogenase indicates that increased binding (lower K_m) occurs with increasing chain length (C1–C6) of the substrate and the presence of unsaturation. Maximum rates of oxidation were

essentially constant regardless of the alcohol structure, suggesting that alcohol–enzyme binding is not the rate-limiting step for oxidation; the activity of the enzyme appears to be dependent on the lipophilic character of the alcohol substrate. The three classes of alcohol dehydrogenase present in human liver show a decrease in K_m with an increase in chain length from C2 to C8; the alcohol groups of 12-hydroxydodecanoic acid and 16-hydroxyhexadecanoic acid show K_m values similar to that of hexanol, indicating rapid oxidation of alcohols arising from ω-oxidation reactions (see p. 57, section 4.1.6).

Similarly, aldehyde dehydrogenase, which is present predominantly in liver cytosol, has a broad substrate specificity for the oxidation of aldehydes to carboxylic acids. Aldehyde dehydrogenase is more active for aldehydes of high relative molecular mass. Three isoenzymes of aldehyde dehydrogenase, with overlapping substrate specificities, are present in human liver. They oxidize a range of naturally occurring aldehydes, such as γ-butyraldehyde, 3,4-dihydroxyphenylacetaldehyde, 5-hydroxyindoleacetaldehyde and acrolein. Alkyl aldehydes (C1–C6) have K_m values which are similar to those for these naturally occurring aldehydes, and the K_m decreases with an increase in chain length. Xanthine oxidase and aldehyde oxidase also catalyse oxidation of a wide range of aldehydes to their corresponding unsaturated acids. Before oxidation to the corresponding acid, the aldehyde may be conjugated with sulfhydryl groups such as glutathione to yield thiohemiacetals. Oxidation of aldehydes with low relative molecular masses requires glutathione, which implies that the substrate for aldehyde dehydrogenase-mediated oxidation may be the thiohemiacetal.

Branched-chain aliphatic primary alcohols and aldehydes are substrates for alcohol dehydrogenase and aldehyde dehydrogenase, although the rates of oxidation are lower than those for linear primary alcohols and aldehydes. Unsaturated, linear and branched-chain primary alcohols are better substrates than the saturated analogues, but the reverse is true of aldehydes. Citral, a mixture of two unsaturated branched-chain aldehydes (neral and geranial), was not oxidized to the corresponding acid by aldehyde dehydrogenase in rat liver preparations *in vitro* but was reduced by alcohol dehydrogenase in the presence of NADH; oxidation is a major metabolic pathway *in vivo,* producing acid metabolites.

Secondary and tertiary alcohols

Secondary alcohol groups (such as those present in carveol and β-ionol) undergo reversible oxidation to the corresponding ketone (see

section 4.1.4); the alcohol is the more important form *in vivo* because of its removal from the equilibrium by conjugation with glucuronic acid. Tertiary alcohol groups (such as those present in linalool and terpineol) do not undergo oxidative metabolism, and tertiary alcohols are eliminated by conjugation.

4.1.3 *Conjugation of alcohols*

Conjugation represents the major pathway of metabolism for both secondary and tertiary alcohols. Secondary and tertiary alcohols are metabolized extensively by conjugation with glucuronic acid followed by excretion primarily in the urine or the bile, depending on the relative molecular mass and the animal species. Both the liver and the intestinal wall have a high capacity for glucuronic acid conjugation with a wide range of substrates. Sulfate conjugation of alcohols occurs in many tissues, especially the liver, and results in the formation of highly polar, water-soluble excretory products. The urine is the main route of elimination of sulfate conjugates of alcohols resulting from the metabolism of the flavouring agents evaluated at the present meeting.

Conjugation with glucuronic acid followed by excretion in the urine and faeces represents the primary route of elimination of linalool. Conjugation with glucuronic acid represents the major pathway of metabolism of menthol. In rodents, the glucuronic acid conjugates of linalool and menthol are excreted primarily via the bile into the intestine, where they may be hydrolysed to yield the free alcohol, which undergoes reabsorption and subsequent oxidative metabolism. The metabolites of menthol are eliminated in the urine and faeces either unchanged or conjugated with glucuronic acid. Bicyclic tertiary alcohols are relatively stable *in vivo*, and in rabbits thujyl alcohol and β-santenol (2,3,7-trimethyl bicyclo[2.2.1]-heptan-2-ol) are extensively conjugated with glucuronic acid.

4.1.4 *Reduction of ketones*

Aliphatic ketones are metabolized primarily via reduction to the corresponding secondary alcohol. The reduction of aliphatic ketones is mediated by alcohol dehydrogenases and NADH/NADPH-dependent cytosolic carbonyl reductases. The reaction catalysed by carbonyl reductase is stereoselective and favours formation of the *S*-enantiomer of the alcohol. The reaction is reversible under physiological conditions, and oxidation of the secondary alcohol may lead to the formation of the corresponding ketone *in vivo*. Ketones have been shown to undergo reduction to alcohols in human hepatic

microsomes. Three reductases have been purified from human liver cytosol: two aldehyde reductases, which can reduce aliphatic aldehydes, alicyclic ketones and α-diketones, and a carbonyl reductase that is active with a broad range of aldehyde and ketone substrates. The activity of carbonyl reductase in human liver showed limited variability between individuals, and the enzyme reduced both 4-nitrobenzaldehyde and 4-nitroacetophenone.

Alicyclic monoketones, such as cyclohexanone, are reduced to their corresponding alcohols or undergo α-hydroxylation and reduction to yield diols, which are excreted as the glucuronic acid conjugates.

Unsaturated ketones, such as menthone, dihydrocarvone and carvone, undergo extensive reduction of the ketone functional group to yield the corresponding secondary alcohols (pulegone, dihydrocarveol and carveol), which are conjugated mainly with glucuronic acid and then excreted in the urine or bile. In rodents, but probably not in humans, the conjugate is excreted primarily in the bile, following which it may be hydrolysed to yield the free alcohol, which may then undergo enterohepatic recirculation and finally be excreted by the kidney. Conjugates with relative molecular masses below about 500, such as the glucuronides of menthan-2-ol, dihydrocarveol and carveol, would be eliminated by humans in the urine; the absence of significant biliary excretion would reduce the extent of secondary oxidative metabolism.

In general, alicyclic α-diketones are metabolized via the reduction pathway. Methyl-substituted diketones may be reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. α-Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates. Excretion as glucuronic acid conjugates is favoured at elevated concentrations of α-hydroxyketones and diols *in vivo*, especially for ketones of long chain length. If the carbonyl functional group is located elsewhere on the chain, reduction is the predominant pathway of detoxification.

4.1.5 *Reduction of double bonds*

Reduction of endocyclic and exocyclic double bonds occurs with some of the flavouring agents evaluated at the present meeting. For example, the endocyclic double bond of carvone is reduced to form dihydrocarvone and dihydrocarveol and the exocyclic double bond in β-ionone is reduced to give dihydro-β-ionol. The enzymes involved in these reactions have not been characterized, and the intestinal microflora may be involved either prior to absorption or following biliary excretion.

4.1.6 *Oxidation of side-chains*

The position and size of substituents in carboxylic acids influences the route of metabolism. As chain length and lipophilicity increase, ω-oxidation becomes more important than β-oxidative cleavage.

β-Oxidation

Linear and branched-chain saturated carboxylic acids are substrates for β-oxidative cleavage to yield acetyl coenzyme A (acetyl CoA) and a new CoA thioester of the carboxylic acid, which has been reduced by two carbon atoms. This process continues to give complete oxidation or until a branch point is reached. Acids with an even number of carbon atoms continue to be cleaved to yield acetyl CoA, while acids with an odd number of carbon atoms yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly, whereas propionyl CoA is transformed into succinyl CoA which then enters the citric acid cycle. Acids with a methyl substituent located at an even-numbered carbon (e.g. 2-methylpentanoic acid and 4-methyldecanoic acid) are metabolized to carbon dioxide via β-oxidative cleavage in the fatty acid pathway. If the methyl group is located at the C3 position, β-oxidation is inhibited and ω-oxidation predominates, leading primarily to polar diacid metabolites that are excreted in the urine. Ethyl or propyl substituents located at the α- or β-position in relation to the carboxylic acid group inhibit metabolism to carbon dioxide, in which case there is direct conjugation of the acid with glucuronic acid or ω-oxidation followed by conjugation.

Linear unsaturated carboxylic acids participate in normal fatty acid metabolism. In this pathway, the saturated fatty acid is condensed with CoA, which then undergoes catalytic dehydrogenation mediated by acyl-CoA dehydrogenase. The resulting *trans*-2,3-unsaturated ester (*trans*-Δ^2-enoyl CoA) is converted to the 3-ketothioester, which undergoes β-oxidative cleavage to yield an acetyl CoA fragment and a new thioester reduced by two carbons. Cleavage of acetyl CoA units continues along the carbon chain until the position of unsaturation is reached. If unsaturation begins at an even-numbered carbon, fragmentation of acetyl CoA units yields a Δ^2-enoyl CoA product which is a substrate for further fatty acid oxidation. If unsaturation begins at an odd-numbered carbon, fragmentation of acetyl CoA units will eventually yield Δ^3-enoyl CoA, which cannot enter the fatty acid cycle until it is isomerized to the *trans*-Δ^2-enoyl CoA by enoyl CoA isomerase. If the stereochemistry of the double bond is *cis*, it is isomerized to the *trans* double bond by the action of 3-hydroxyacyl CoA epimerase before entering the fatty acid oxidation pathway. Short-chain acids containing terminal unsaturation may be

metabolized via desaturation to yield a substrate which may participate in the fatty acid pathway. For example, 4-pentenoic acid is converted into the CoA thioester, which is dehydrogenated to yield *trans*-2,4-pentadienoyl CoA, which undergoes NADPH-dependent enzyme-catalysed reduction of the Δ^2-alkene to *trans*-2-pentenoic acid, which then participates in normal fatty acid oxidation. A second but minor pathway involves β-oxidation to yield 3-keto-4-pentenoyl CoA.

ω-Oxidation and (ω-1)-oxidation

ω-Oxidation and (ω-1)-oxidation are important in the elimination of compounds with long and/or complex alkyl chains, because they eventually yield polar acid metabolites that are eliminated in the urine. ω-Oxidation results in the formation of a primary alcohol, which may undergo further oxidation to the corresponding carboxylic acid, which may then be excreted in the urine. For example, linalool undergoes cytochrome P450-mediated oxidation to form 8-hydroxylinalool and 8-carboxylinalool, whereas geraniol is oxidized to 8-hydroxygeraniol, 8-carboxygeraniol and the dibasic acid, Hilderandt's acid (3,7-dimethyl-2,6-octadienedioic acid). Citral, the aldehyde analogue of geraniol, undergoes oxidation of the aldehyde group *in vivo* followed by ω-oxidation to yield a mixture of diacids and hydroxy acids. Menthol undergoes ω-oxidation of the side-chain substituents to yield various polyols and hydroxyacids; the (−)-isomer undergoes more extensive oxidation than the (+)-isomer.

Short-chain (containing four or fewer carbon atoms) ketones that contain a carbonyl functional group at the C2-position (e.g. butanone) and α-diketones, such as acetoin, may undergo ω-oxidation of the terminal methyl group to give a diketocarboxylic acid, which would undergo decarboxylation to yield an α-ketocarboxylic acid. As intermediary metabolites, α-ketoacids undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolized via the fatty acid pathway and citric acid cycle. Almost one-half of a dose of radiolabelled acetoin, administered by intravenous injection to rats, was eliminated as carbon dioxide in the expired air and about 20% was eliminated as unidentified urinary metabolites. *trans*-Methyl styryl ketone undergoes essentially complete metabolism of the methyl group after oral administration to rats; the glycine conjugates of phenylacetic and benzoic acid were the major metabolites in the urine.

ω-Oxidation of ketones yields hydroxyketones that may be further reduced to diols and excreted in the urine as glucuronic acid conjugates. Participation in these pathways depends on chain length,

the position of the carbonyl functional group, and the dose. Longer-chain aliphatic ketones (containing five or more carbon atoms) are metabolized primarily via reduction, but ω-oxidation and/or (ω-1)-oxidation are competing pathways at high aliphatic ketone concentrations, which yield ketoacids and diketones, respectively.

A number of the flavouring agents evaluated at the present meeting are alicyclic ketones containing an alkyl or alkenyl side-chain; these undergo oxidation of the side-chain (probably by cytochrome P450) to form polar metabolites that are excreted as the glucuronic acid or sulfate conjugates in the urine and, to a lesser extent, in the faeces.

Oxidation of double bonds

Oxidation of double bonds, via an epoxide intermediate, to form diols is probably only a minor pathway of metabolism for the flavouring agents considered at the present meeting. For example, α-terpineol is oxidized by cytochrome P450 in rats, via an epoxide intermediate, to a 1,2-dihydroxy metabolite; this diol metabolite has been detected in humans following ingestion of α-terpineol.

4.1.7 *Oxidation of alicyclics*

Cyclohexane and cyclohexene rings, which are present in the carvone and ionone groups of flavouring agents considered at the present meeting, are rapidly oxidized *in vivo* by ring hydroxylation, even when other pathways of elimination are present. For example, the main pathways of elimination of cyclohexane are in the expired air (as the unchanged substance) and in the urine as glucuronic acid conjugates of cyclohexanol and cyclohexane-1,2-diol. Cyclohexylamine is highly water-soluble, and is excreted in the urine as both the parent compound and as the glucuronic acid conjugates formed via ring hydroxylation, together with small amounts of cyclohexane-1,2-diol formed via deamination and ring hydroxylation. Ring oxidation, which is catalysed by cytochrome P450 (isoenzyme CYP3A4), is a major pathway of metabolism for the substituted cyclohexene ring moieties that are present in vitamin A and its metabolites such as 13-*cis*- and all-*trans*-retinoic acid, as well as the ionone group of flavouring agents. Oxidation of the substituted cyclohexene rings present in retinoids and ionones occurs on the carbon atoms adjacent to the double bond.

4.1.8 *Conjugation with glutathione*

The vast majority of the flavouring agents evaluated at the present meeting are metabolized by the pathways described in sections 4.1.1–4.1.7. The possible exceptions are carvone, carveol (and its esters) and

some members of the ionone group in which a ketone group, or a precursor secondary alcohol group, are adjacent to a double bond or conjugated double bonds (α,β-unsaturated ketone). Glutathione conjugation is an important pathway for detoxification, especially for reactive compounds. The products of conjugation with glutathione are usually eliminated in the bile as the glutathione conjugate *per se*, and in the urine as the *N*-acetylcysteine metabolite produced from the glutathione conjugate. Glutathione conjugates may also be split by C–S (carbon–sulfur) lyases (β-lyases) in the kidney and intestinal microflora to give a thiol compound: this reaction represents a bioactivation process for some chemicals. Only limited data on metabolism were available on the ionone group of flavouring agents, and glutathione conjugation products have not been reported as urinary metabolites of ionones or of carvone. Oral administration of carvone by gavage in corn oil to mice at a dose of 20 mg per animal for 3 successive days caused a reduction in the concentration of glutathione in the liver, and an increase in glutathione transferase activity. However, these effects may not have been due to the α,β-unsaturated ketone moiety, because the decrease in glutathione was not found with α,β-unsaturated carvone analogues lacking the exocyclic allyl group, while the increase in glutathione transferase was detected with compounds such as *p*-menthan-2-one, which contain neither an α,β-unsaturated ketone moiety nor an exocyclic allyl group.

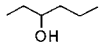
The potential for non-enzymatic interaction with glutathione has been shown by *in vitro* studies. The greatest reactivity with glutathione was found with the simplest α,β-unsaturated ketone (butenone), which showed a rate of reaction about 1000 times that of oct-2-ene-4-one. Damascenone, the 1,3-endocyclic diene analogue of damascone and carvone, which were evaluated at the present meeting, showed rates of reaction that were 17 000, 11 000 and 320 000 times slower than butenone, which indicates a low reactivity.

Overall, the flavouring agents with an α,β-unsaturated ketone moiety which were evaluated at the present meeting showed a low reactivity, and conjugation with glutathione would not be a major route of metabolism. The low reactivity predicted is supported by the toxicological data on the ionones and carvone.

4.2 Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters

The Committee evaluated 39 saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters (Table 3) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Table 3
Summary of the results of safety evaluations of 39 saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters

Substance	No.	CAS no. and structure	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
Structural class I							
Acetone	139	67-64-1 	Yes	Yes Europe: 7100 USA: 36000	Yes	NA	No safety concern
Isopropyl alcohol	277	67-63-0 	Yes	Yes Europe: 99000 USA: 9900	Yes	NA	
2-Butanone	278	78-93-3 	Yes	No Europe: 110 USA: 36	NA	NA	
2-Pentanone	279	107-87-9 	Yes	No Europe: 140 USA: 42	NA	NA	
2-Pentanol	280	6032-29-7 	Yes	No Europe: 6 USA: 0.04	NA	NA	
3-Hexanone	281	589-38-8 	Yes	No Europe: 0.4 USA: 1	NA	NA	
3-Hexanol	282	623-37-0 	Yes	No Europe: 13 USA: 11	NA	NA	

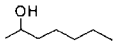
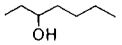
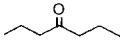
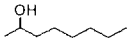
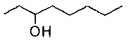
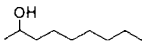
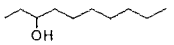
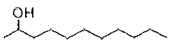
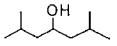
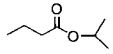
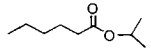
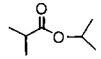
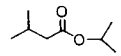
2-Heptanol	284	543-49-7 	Yes	No Europe: 8 USA: 1	NA	NA	No safety concern
3-Heptanol	286	589-82-2 	No	No Europe: 0.2 USA: 0.6	NA	Yes	
4-Heptanone	287	123-19-3 	Yes	No Europe: 2 USA: 2	NA	NA	
2-Octanol	289	123-96-6 	Yes	No Europe: 13 USA: 4	NA	NA	
3-Octanol	291	589-98-0 	Yes	No Europe: 6 USA: 320	NA	NA	
2-Nonanol	293	628-99-9 	Yes	No Europe: 1 USA: 1	NA	NA	
3-Decanol	295	1565-81-7 	Yes	No Europe: ND USA: 16	NA	NA	
2-Undecanol	297	1653-30-1 	Yes	No Europe: 0.2 USA: 0.04	NA	NA	
3-Methyl-2-butanol	300	598-75-4 	Yes	No Europe: 1 USA: 0.2	NA	NA	
2,6-Dimethyl-4-heptanol	303	108-82-7 	Yes	No Europe: ND USA: 5	NA	NA	

Table 3 *(continued)*

Substance	No.	CAS no. and structure	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
Isopropyl formate	304	625-55-8 	Yes	No Europe: 0.5 USA: 0.02	NA	NA	No safety concern
Isopropyl acetate	305	108-21-4 	Yes	No Europe: 41 USA: 9	NA	NA	
Isopropyl propionate	306	637-78-5 	Yes	No Europe: 0.01 USA: 0.02	NA	NA	
Isopropyl butyrate	307	638-11-9 	Yes	No Europe: 7 USA: 0.08	NA	NA	
Isopropyl hexanoate	308	2311-46-8 	Yes	No Europe: 4 USA: 0.02	NA	NA	
Isopropyl isobutyrate	309	617-50-5 	Yes	No Europe: 0.6 USA: 0.06	NA	NA	
Isopropyl isovalerate	310	32665-23-9 	Yes	No Europe: 0.3 USA: 0.2	NA	NA	

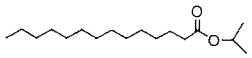
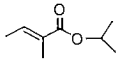
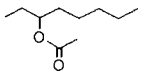
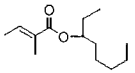
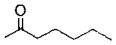
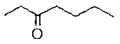
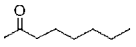
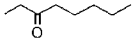
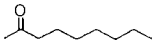
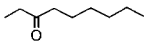
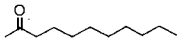
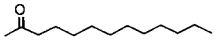
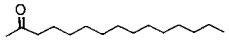
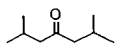
Isopropyl myristate	311	110-27-0 	Yes	No Europe: 23 USA: 0.02	NA	NA	No safety concern
Isopropyl tiglate	312	6284-46-4 	Yes	No Europe: 0.01 USA: 0.1	NA	NA	
3-Octyl acetate	313	4864-61-3 	Yes	No Europe: 0.7 USA: 30	NA	NA	
1-Ethylhexyl tiglate	448	94133-92-3 	Yes	No Europe: 0.01 USA: 29	NA	NA	
Structural class II							
2-Heptanone	283	110-43-0 	Yes	No Europe: 110 USA: 48	NA	NA	No safety concern
3-Heptanone	285	106-35-4 	No	No Europe: 4 USA: 10	NA	Yes	
2-Octanone	288	111-13-7 	Yes	No Europe: 110 USA: 67	NA	NA	
3-Octanone	290	106-68-3 	Yes	No Europe: 3 USA: 2	NA	NA	
2-Nonanone	292	821-55-6 	Yes	No Europe: 380 USA: 27	NA	NA	

Table 3 *(continued)*

Substance	No.	CAS no. and structure	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[a] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4* Adequate NOEL for substance or structurally related substance?	Conclusion based on current intake
3-Nonanone	294	925-78-0 	Yes	No Europe: 0.1 USA: 0.1	NA	NA	No safety concern
2-Undecanone	296	112-12-9 	Yes	No Europe: 380 USA: 21	NA	NA	
2-Tridecanone	298	593-08-8 	Yes	No Europe: 73 USA: 30	NA	NA	
2-Pentadecanone	299	2345-28-0 	Yes	No Europe: 21 USA: 430	NA	NA	
4-Methyl-2-pentanone	301	108-10-1 	Yes	No Europe: 7 USA: 2	NA	NA	
2,6-Dimethyl-4-heptanone	302	108-83-8 	Yes	No Europe: 0.2 USA: 0.06	NA	NA	

CAS: Chemical Abstracts Service; NA: not applicable; ND: no intake data reported.
[a] The thresholds for human intake are 1800 μg/day for Class I and 540 μg/day for Class II. All intake values are expressed in μg per day.

The Committee had evaluated five members of this group previously. Acetone (propan-2-one) was evaluated as an extraction solvent at the fourteenth meeting (Annex 1, reference *22*), when the Committee considered that, with good manufacturing practice, the residues in food would be toxicologically insignificant. The evaluation was tentative owing to a lack of relevant data. Isopropyl alcohol (2-propanol) was evaluated at the fourteenth and twenty-fifth meetings (Annex 1, references *22* and *56*); an ADI was not allocated because of a lack of data. Isopropyl acetate was evaluated at the twenty-third meeting (Annex 1, reference *50*); the Committee was unable to establish an ADI because of a lack of data on hydrolysis and other toxicological end-points. Isopropyl myristate (isopropyl tetradecanoate), evaluated at the twenty-third meeting (Annex 1, reference *50*), was not allocated an ADI owing to a lack of data. 2-Butanone (ethyl methyl ketone) was evaluated at the twenty-third and twenty-fifth meetings (Annex 1, references *50* and *56*), when the Committee concluded that the data available were not sufficient for evaluation of the substance, and no ADI was allocated.

Estimated daily per capita intake

The total annual volume of production of the 39 saturated aliphatic acyclic secondary alcohols, ketones and related esters in this group is approximately 750 tonnes in Europe and 240 tonnes in the USA. In Europe, 92% of the total annual volume is accounted for by isopropyl alcohol (no. 277) alone. In the USA, 98% of the total annual volume is accounted for by acetone (no. 139) and isopropyl alcohol. On the basis of the reported annual volumes of production, the estimated daily per capita intake of acetone resulting from its use as a flavouring agent is approximately 7.1 mg in Europe and 36 mg in the USA. Similarly, the total estimated daily per capita intake of isopropyl alcohol and its nine esters in this group (nos 304–312) resulting from their use as flavouring agents is about 99 mg in Europe and 10 mg in the USA.

Saturated aliphatic acyclic secondary alcohols, ketones and related esters are the main flavouring components of alcoholic beverages and occur naturally in a wide variety of fruits. Quantitative data on natural occurrence have been reported for 22 of the 39 substances in this group. In the USA, the intake of 20 of these 22 substances from natural sources exceeds the intake from their use as flavouring agents; for the two remaining substances, acetone (no. 139) and 3-octanol (no. 291), the intake from their use as flavouring agents exceeds that from natural sources.

Absorption, metabolism and elimination

In general, saturated aliphatic acyclic secondary alcohols and ketones are absorbed through the gastrointestinal tract. The esters in this group of flavouring agents are anticipated to be hydrolysed to their component secondary alcohols and aliphatic, saturated and unsaturated carboxylic acids, which are also readily absorbed.

Three of the esters in this group (3-octyl acetate (no. 313), 1-ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenoate; no. 448) and isopropyl tiglate (isopropyl 2-methyl-2-butenoate; no. 312)) may not be completely hydrolysed in the gastrointestinal tract, but these esters and other esters in this group of flavouring agents that reach the general circulation intact would be expected to be hydrolysed by tissue esterases to their component alcohols and carboxylic acids. The metabolism of these flavouring agents is primarily by oxidation, reduction and/or conjugation (see section 4.1).

Two of the substances in this group, 3-heptanone (no. 285) and 3-heptanol (no. 286) meet the special structural requirements for possible oxidation to form neurotoxic γ-diketone metabolites.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) to the above-mentioned saturated aliphatic acyclic secondary alcohols, ketones and related esters, the Committee assigned 28 of the 39 substances to structural class I. The remaining 11 substances, which included acyclic aliphatic 2-alkanones (nos 283, 288, 292, 296, 298, 299 and 301), 3-alkanones (nos 285, 290 and 294) and a 4-alkanone (no. 302) with four or more carbons on either side of the keto group, were assigned to structural class II.

Step 2. The available data indicated that 26 of the 28 saturated aliphatic acyclic secondary alcohols and ketones would be predicted to be metabolized to or are innocuous substances. The 11 esters in this group are anticipated to be hydrolysed to their component secondary alcohols (isopropyl alcohol or 3-octanol) and carboxylic acids (aliphatic saturated acids or the unsaturated tiglic acid). These hydrolysis products are either endogenous compounds or can be predicted to be metabolized to innocuous substances. At current levels of per capita intake, 37 of the 39 flavouring agents in this group would not be expected to saturate the metabolic pathways and were considered to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. The remaining two substances, 3-heptanone (no. 285) and 3-heptanol (no. 286), may undergo oxidation to neurotoxic γ-

diketones. The evaluation of these substances therefore proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 26 of the 28 substances in class I were below the threshold for class I (1800μg), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents. Only acetone (no. 139) and isopropyl alcohol (no. 277) had estimated intakes greater than the threshold for class I. The evaluation of these two substances therefore proceeded to step A4.

The total estimated daily per capita intake of isopropyl alcohol (no. 277) and its nine esters from their use as flavouring agents (99mg in Europe and 10mg in the USA) is greater than the threshold for human intake for class I. The intake of three additional esters of isopropyl alcohol (isopropyl benzoate, isopropyl cinnamate and isopropyl phenylacetate), which were not evaluated as part of this group, would contribute approximately an additional 15μg per day in Europe and 5μg per day in the USA to the total intake of isopropyl alcohol. This potential additional intake is relatively minor and would not alter the safety evaluation.

The estimated daily per capita intakes of all 11 substances in class II were below the threshold for class II (540μg), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents.

Step A4. Acetone (no. 139) and isopropyl alcohol (no. 277) are both endogenous components of fatty acid and carbohydrate metabolism and have been detected in the blood. Therefore, these substances were determined to be of no safety concern when used at current levels of intake as flavouring agents.

Step B3. The estimated daily per capita intakes of 3-heptanol (no. 286) and 3-heptanone (no. 285) were below the threshold for structural classes I and II, respectively. Accordingly, the evaluation of these substances proceeded to step B4.

Step B4. On the basis of neurotoxic effects seen in a limited study in which 3-heptanone (no. 285) was administered orally to male rats, the NOEL was 1000mg/kg of body weight per day. This NOEL provides a large safety margin (>1 000 000 for 3-heptanone and even higher for 3-heptanol (no. 286)) when compared with the current levels of estimated intake of 3-heptanone and 3-heptanol (which might be metabolized to 3-heptanone). Therefore, these substances were determined to be of no safety concern when used at current levels of intake as flavouring agents.

Table 3 summarizes the stepwise evaluations of the 39 saturated aliphatic acyclic secondary alcohols, ketones and related esters used as flavouring agents.

Consideration of combined intakes

In the unlikely event that all foods containing all 28 saturated aliphatic acyclic secondary alcohols, ketones and related esters of saturated and unsaturated carboxylic acids in structural class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for class I.

In the unlikely event that all foods containing all 11 saturated aliphatic acyclic secondary ketones in structural class II were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for class II.

Of the 39 substances, 37 are expected to be metabolized via well-known biochemical pathways to innocuous metabolic and/or endogenous substances; in the opinion of the Committee, the endogenous levels of these metabolites would not give rise to perturbations outside the physiological range. Accordingly, even a combined theoretical intake would be of no safety concern. For the remaining two substances that could potentially form γ-diketones, the combined intake would also be of no safety concern.

Conclusion

The Committee concluded that the substances in this group would not present safety concerns at the current levels of estimated intake.

No toxicity data were required for application of the Procedure to 37 of the 39 substances in this group. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure. For the remaining two substances, 3-heptanone (no. 285) and 3-heptanol (no. 286), the toxicity data were also consistent with the results of the safety evaluation using the Procedure.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.3 Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters

The Committee evaluated a group of 42 flavouring agents that included linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters (Table 4) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

The Committee had evaluated one member of the group, oleic acid, previously. At its twenty-ninth meeting (Annex 1, reference *70*), the Committee noted that the safety evaluation of fatty acids, including oleic acid, is based on knowledge of their metabolism and excretion and on their occurrence in edible fats and oils that have a long history of use as foods or food components. At the thirty-third meeting, ADIs "not specified" were allocated to the calcium, potassium and sodium salts of oleic acid (Annex 1, reference *83*).

Estimated daily per capita intake

The total annual volume of production of the 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, carboxylic acids and related esters is approximately 39 tonnes in Europe and 13 tonnes in the USA. Three substances in the group, *cis*-3-hexen-1-ol (no. 315), linoleic acid (no. 332) and oleic acid (no. 333) account for about 96% of the total annual volume in Europe. Four substances in the group, *cis*-3-hexen-1-ol, oleic acid, 2,6-dimethyl-5-heptenal (no. 349) and a mixture of hexyl 2-methyl-3-pentenoate and hexyl 2-methyl-4-pentenoate (no. 352) account for about 91% of the total annual volume in the USA. On the basis of the reported annual volumes of production, the estimated daily per capita intake of *cis*-3-hexen-1-ol, linoleic acid and oleic acid from their use as flavouring agents is approximately 4300 µg (72 µg/kg of body weight per day), 130 µg (2 µg/kg of body weight per day) and 970 µg (16 µg/kg of body weight per day), respectively, in Europe. Similarly, the estimated daily per capita intake of *cis*-3-hexen-1-ol, oleic acid, 2,6-dimethyl-5-heptenal and the mixture of hexyl 2-methyl-3-pentenoate and hexyl 2-methyl-4-pentenoate from their use as flavouring agents is approximately 1100 µg (18 µg/kg of body weight per day), 440 µg (7 µg/kg of body weight per day), 250 µg (4 µg/kg of body weight per day) and 530 µg (9 µg/kg of body weight per day), respectively, in the USA.

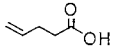
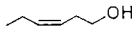
Of the 42 aliphatic unsaturated primary alcohols and unconjugated aldehydes, acids and related esters in this group, 27 are reported to occur naturally in a wide variety of foods, including fruits and vegetables, dairy products, fish and alcoholic beverages. Oleic and linoleic acids (nos 333 and 332) are some of the most common unsaturated fatty acids found in vegetable oils and animal fats, often constituting more than 50% of the total concentration of fatty acids.

Absorption, metabolism and elimination

The metabolism of flavouring agents in this group involves common pathways of intermediary metabolism which are discussed in section 4.1.

Table 4

Summary of the results of safety evaluations of 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters[a]

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
4-Pentenoic acid	314	591-80-0 	No Europe: 5 USA: 3	NR	NR	Converted to the CoA thioester, which undergoes dehydrogenation and then participates in the fatty acid and tricarboxylic acid pathways	No safety concern
cis-3-Hexen-1-ol	315	928-96-1 	Yes Europe: 4300 USA: 1100	No	Yes The NOEL of 120–150 mg/kg of body weight reported in a 98-day study in rats is >1000 times the daily per capita intake	See note 1	

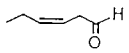
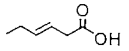
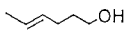
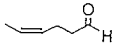
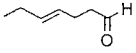
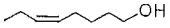
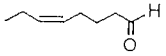
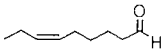
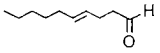
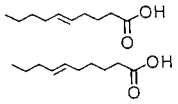
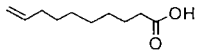
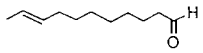
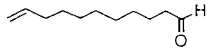
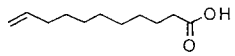
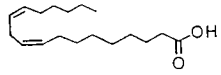
cis-3-Hexenal	316	6789-80-6 	No Europe: 5 USA: 3	NR	NR	See note 2	No safety concern
3-Hexenoic acid	317	4219-24-3 	No Europe: 11 USA: 1	NR	NR	See note 3	
4-Hexen-1-ol	318	6126-50-7 	No Europe: 3 USA: 0.04	NR	NR	See note 1	
cis-4-Hexenal	319	4634-89-3 	No Europe: 0.03 USA: 0.1	NR	NR	See note 2	
4-Heptenal	320	6728-31-0 	No Europe: 2 USA: 0.1	NR	NR	See note 2	
cis-3-Octen-1-ol	321	20125-84-2 	No Europe: 6 USA: 0.01	NR	NR	See note 1	
cis-5-Octen-1-ol	322	64275-73-6 	No Europe: 0.5 USA: 0.04	NR	NR	See note 1	
cis-5-Octenal	323	41547-22-2 	No Europe: 0.001 USA: 29	NR	NR	See note 2	
cis-6-Nonen-1-ol	324	35854-86-5 	No Europe: 3 USA: 0.1	NR	NR	See note 1	
cis-6-Nonenal	325	2277-19-2 	No Europe: 2 USA: 0.1	NR	NR	See note 2	

Table 4 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
4-Decenal	326	30390-50-2 	No Europe: 1 USA: 0.01	NR	NR	See note 2	No safety concern
Mixture of 5- and 6-decenoic acid	327	72881-27-7 	No Europe: 4 USA: 0.2	NR	NR	See note 3	
9-Decenoic acid	328	14436-32-9 	No Europe: 0.1 USA: 0.2	NR	NR	See note 3	
9-Undecenal	329	143-14-6 	No Europe: 1 USA: 0.2	NR	NR	See note 2	
10-Undecenal	330	112-45-8 	No Europe: 0.4 USA: 0.04	NR	NR	See note 2	
10-Undecenoic acid	331	112-38-9 	No Europe: 30 USA: 0.8	NR	NR	See note 3	
Linoleic acid	332	60-33-3 	No Europe: 130 USA: 6	NR	NR	See note 4	

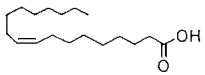
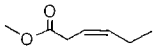
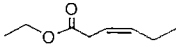
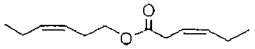
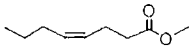
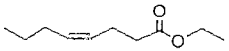
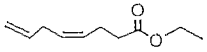
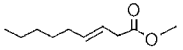
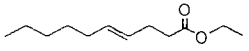
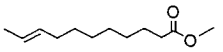
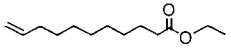
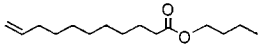
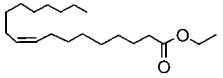
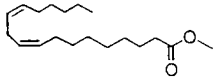
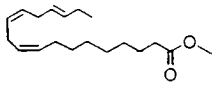
Oleic acid[c]	333	112-80-1 	No Europe: 970 USA: 440	NR	NR	See note 4	No safety concern
Methyl 3-hexenoate	334	2396-78-3 	No Europe: 0.7 USA: 0.01	NR	NR	See note 5	
Ethyl 3-hexenoate	335	2396-83-0 	No Europe: 4 USA: 1	NR	NR	See note 5	
cis-3-Hexenyl *cis*-3-hexenoate	336	6144-38-0 	No Europe: 4 USA: 0.8	NR	NR	See note 5	
Methyl *cis*-4-octenoate	337	21063-71-8 	No Europe: 0.4 USA: 0.04	NR	NR	See note 5	
Ethyl *cis*-4-octenoate	338	34495-71-1 	No Europe: 1 USA: 0.04	NR	NR	See note 5	
Ethyl *cis*-4,7-octadienoate	339	69925-33-3 	No Europe: 2 USA: 0.01	NR	NR	See note 5	
Methyl 3-nonenoate	340	13481-87-3 	No Europe: 2 USA: 76	NR	NR	See note 5	
Ethyl *trans*-4-decenoate	341	76649-16-6 	No Europe: 2 USA: 0.04	NR	NR	See note 5	

Table 4 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Methyl 9-undecenoate	342	5760-50-9 	No Europe: 39 USA: 0.4	NR	NR	See note 5	No safety concern
Ethyl 10-undecenoate	343	692-86-4 	No Europe: 2 USA: 44	NR	NR	See note 5	
Butyl 10-undecenoate	344	109-42-2 	No Europe: 0.04 USA: 5	NR	NR	See note 5	
Ethyl oleate	345	111-62-6 	No Europe: 69 USA: 3	NR	NR	See note 5	
Mixture of methyl linoleate and methyl linolenate	346	301-00-8  	No Europe: ND USA: 23	NR	NR	See note 6	

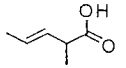
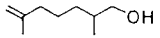
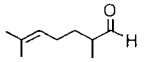
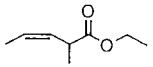
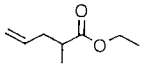
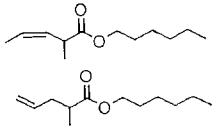
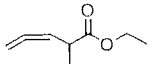
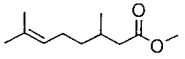
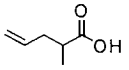
2-Methyl-3-pentenoic acid	347	37674-63-8 	No Europe: 1 USA: 2	NR	NR	See note 7	No safety concern
2,6-Dimethyl-6-hepten-1-ol	348	36806-46-9 	No Europe: ND USA: 0.1	NR	NR	See note 7	
2,6-Dimethyl-5-heptenal	349	106-72-9 	No Europe: 31 USA: 250	NR	NR	See note 7	
Ethyl 2-methyl-3-pentenoate	350	1617-23-8 	No Europe: 6 USA: 6	NR	NR	See note 5	
Ethyl 2-methyl-4-pentenoate	351	53399-81-8 	No Europe: 0.3 USA: 0.02	NR	NR	See note 5	
Mixture of hexyl 2-methyl-3- and hexyl 2-methyl-4-pentenoate	352	58625-95-9 	No Europe: 0.03 USA: 530	NR	NR	See note 6	
Ethyl 2-methyl-3,4-pentadienoate	353	60523-21-9 	— Europe: 0.01 USA: 10	NA	NA	Cannot be predicted to be metabolized to innocuous substances	Not evaluated[d]

Table 4 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Methyl 3,7-dimethyl-6-octenoate	354	2270-60-2 	No Europe: 0.2 USA: 0.1	NR	NR	See note 5	No safety concern
2-Methyl-4-pentenoic acid	355	1575-74-2 	No Europe: ND USA: 0.04	NR	NR	See note 7	

CAS: Chemical Abstracts Service; NA: not applicable; ND: no intake data reported; NR: not required for evaluation because consumption was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 1*: All of the substances in this group are in structural class I.
Step 2: All of the substances in this group are predicted to be metabolized to innocuous products, except 2-methyl-3,4-pentadienoate.

[b] The threshold for human intake of class I is 1800 µg per day. All intake values are expressed in µg per day.

[c] The ADI "not specified" for the calcium, potassium and sodium salts established at the thirty-third meeting of the Committee (Annex 1, reference *83*) was maintained.

[d] Evaluation postponed, pending review of a 90-day toxicity study not available at the present meeting.

Notes to Table 4

1. Expected to be oxidized to the corresponding aldehyde and carboxylic acid; the latter is completely metabolized in the fatty acid and tricarboxylic acid pathways.
2. Expected to be oxidized to the corresponding carboxylic acid, which is completely metabolized in the fatty acid and tricarboxylic acid pathways.
3. Metabolized in the fatty acid and tricarboxylic acid pathways.
4. Readily metabolized in the fatty acid oxidation pathway.
5. Expected to be hydrolysed to its component alcohol and carboxylic acid, which are completely metabolized in the fatty acid and tricarboxylic acid pathways.
6. Expected to be hydrolysed to their component alcohols and carboxylic acids, which are completely metabolized in the fatty acid and tricarboxylic acid pathways.
7. Undergoes β-oxidation to yield products that are completely metabolized in the tricarboxylic acid cycle.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters, the Committee assigned all 42 substances to structural class I.

Step 2. The metabolic fates of the linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, carboxylic acids and related esters in this group of flavouring agents can (with the exception of ethyl 2-methyl-3,4-pentadienoate (no. 353)) be predicted readily because of their close structural relationship to endogenous substrates and the broad substrate specificity of the relevant enzymes. The prediction that the metabolites would be innocuous was supported by available toxicity data on members of this group. The Committee considered that the available data were inadequate to predict the metabolism of ethyl 2-methyl-3,4-pentadienoate because of its terminal diene structure.

At current levels of intake, the remaining 41 substances in this group would not be expected to saturate the metabolic pathways, and all of them are predicted to be metabolized to innocuous products (see section 4.1). The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Since it could not be predicted to be metabolized to innocuous products, the evaluation of ethyl 2-methyl-3,4-pentadienoate proceeded via the right-hand side of the decision-tree.

Step A3. The current levels of intake of 40 of the 41 substances were below the threshold for class I (1800μg per day). These 40 substances were considered to be of no safety concern at this step on the basis of their structural class and low levels of estimated intake. For the remaining substance, *cis*-3-hexen-1-ol (no. 315), the estimated daily per capita intake in Europe exceeded the threshold for class I. The evaluation of this substance therefore proceeded to step A4.

Step A4. The substance *cis*-3-hexen-1-ol (no. 315) is not endogenous in humans. Accordingly, its evaluation proceeded to step A5 of the Procedure.

Step A5. A NOEL of 120–150mg/kg of body weight per day was reported for *cis*-3-hexen-1-ol (no. 315) in a 98-day study in rats. This NOEL provides a safety margin of >1000 when compared with the daily per capita intakes of this substance from its use as a flavouring agent in Europe and the USA. Therefore, this substance would not be expected to be of safety concern.

Step B3. The estimated daily per capita intake of ethyl 2-methyl-3,4-pentadienoate (no. 353) in Europe and the USA did not exceed the threshold for class I. The evaluation of this substance therefore proceeded to step B4.

Step B4. Adequate data to determine a NOEL for ethyl 2-methyl-3,4-pentadienoate (no. 353) were not available. The Committee was aware of a study in rats given ethyl 2-methyl-3,4-pentadienoate in the diet at a concentration equivalent to a daily intake of 1.0 mg/kg of body weight for 90 days; however, a full report of the study was not available. The Committee therefore postponed the evaluation of this substance, pending the review of the 90-day toxicity study.

Table 4 summarizes the stepwise evaluations of the linear and branched-chain aliphatic unsaturated alcohols and unconjugated aldehydes, acids and related esters in this group of flavouring agents.

Consideration of combined intakes

In the unlikely event that all foods containing all 41 linear and branched-chain aliphatic unsaturated alcohols and unconjugated aldehydes, acids and related esters in this group were consumed concomitantly on a daily basis, the estimated combined intake would exceed the threshold for class I. However, all 41 substances are expected to be efficiently metabolized and would not saturate the metabolic pathways. On the basis of the evaluation of the collective data, the combined intake was judged by the Committee not to raise safety concerns. The consideration of the combined intakes excludes the intake of ethyl 2-methyl-3,4-pentadienoate (no. 353) because the evaluation of this substance was deferred, pending the review of a 90-day toxicity study not available to the Committee at its present meeting.

Conclusions

The Committee concluded that 41 of the 42 linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters in this group would not present safety concerns when used at current levels of intake as flavouring agents. Data on the toxicity of *cis*-3-hexen-1-ol (no. 315) were required for the application of the Procedure. The Committee noted that these data and the available toxicity data on other substances in the group were consistent with the results of the Procedure. The evaluation of ethyl 2-methyl-3,4-pentadienoate (no. 353) was deferred, pending the evaluation of a 90-day study in rats that was not available to the Committee at its present meeting. The ADI "not specified" for the calcium, potassium and sodium salts of oleic acid established at the thirty-third meeting was maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.4 Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances

The Committee evaluated a group of 23 flavouring agents that included selected tertiary alcohols and related esters (Table 5) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Two members of the group, linalool and linalyl acetate, were previously evaluated as part of a group of other terpenoid flavouring substances that included citral, citronellol and geranyl acetate at the eleventh meeting (Annex 1, reference *14*). At that meeting, the Committee recommended that at least one member of the group be studied for the effects of long-term exposure. Linalool and linalyl acetate were re-evaluated at the twenty-third meeting (Annex 1, reference *50*). A group ADI of 0–0.5 mg/kg of body weight was established, based on the clearly defined metabolism of these substances, their rapid excretion and their low toxicity in short-term studies.

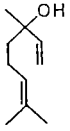
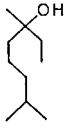
At its forty-ninth meeting (Annex 1, reference *131*), the Committee evaluated a group of 26 geranyl, neryl, citronellyl and rhodinyl esters formed from branched-chain terpenoid primary alcohols and aliphatic acyclic linear and branched-chain carboxylic acids using the Procedure for the Safety Evaluation of Flavouring Agents. The Committee concluded that these substances pose no safety concerns, based on knowledge of their metabolism and low levels of intake.

Estimated daily per capita intake

The total annual volume of production of the 23 tertiary alcohols and related esters is approximately 58 tonnes in Europe and 15 tonnes in the USA. Four substances in the group, linalool (no. 356), linalyl acetate (no. 359), α-terpineol (no. 366) and terpinyl acetate (no. 368) account for approximately 96% of the total annual volume in Europe and the USA. On the basis of the reported annual volumes of production, the total estimated daily per capita intake of linalool from use of linalool and eight of its esters as flavouring agents is approximately 4800 μg (80 μg/kg of body weight) in Europe and 1300 μg (22 μg/kg of body weight) in the USA. Similarly, the total estimated daily per capita intake of α-terpineol from use of α-terpineol and six of its esters as flavouring agents is approximately 3200 μg (53 μg/kg of body weight) in Europe and 1400 μg (23 μg/kg of body weight) in the USA.

Tertiary alcohols and related esters occur naturally in a wide variety of foods, including fruits, spices and tea. Thirteen of the substances in this group have been reported to occur naturally in foods.

Table 5
Summary of the results of safety evaluations of 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances[a]

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
Linalool[c]	356	78-70-6 	Yes Europe: 2600 USA: 1100	No	Yes The NOEL of 50 mg/kg of body weight per day reported in a 90-day study in rats is > 1000 times the daily per capita intake of linalool in Europe and the USA	Oxidation of the allylic methyl group may occur after repeated exposure. See also note 1	No safety concern
Tetrahydrolinalool	357	78-69-3 	No Europe: 55 USA: 0.1	NR	NR	See note 1	

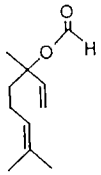
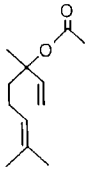
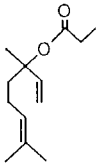
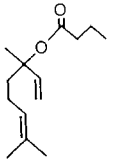
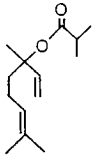
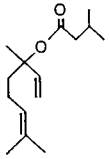
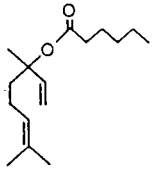
Linalyl formate	358	115-99-1 	No Europe: 8 USA: 13	NR	NR	See note 2	No safety concern
Linalyl acetate[c]	359	115-95-7 	Yes Europe: 2100 USA: 180	No	Yes The NOEL of 24 mg/kg of body weight per day reported in a 90-day study in rats is >500 times the daily per capita intake of linalyl acetate in Europe and the USA	See note 2	
Linalyl propionate	360	144-39-8 	No Europe: 16 USA: 2	NR	NR	See note 2	
Linalyl butyrate	361	78-36-4 	No Europe: 10 USA: 4	NR	NR	See note 2	

Table 5 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Linalyl isobutyrate	362	78-35-3 	No Europe: 36 USA: 1	NR	NR	See note 2	No safety concern
Linalyl isovalerate	363	1118-27-0 	No Europe: 5 USA: 6	NR	NR	See note 2	
Linalyl hexanoate	364	7779-23-9 	No Europe: 1 USA: 0.4	NR	NR	See note 2	

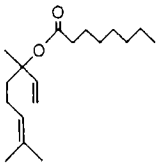
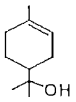
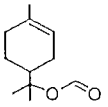
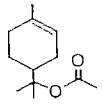
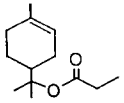
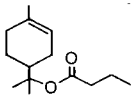
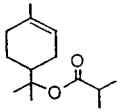
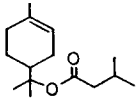
Linalyl octanoate	365	10024-64-3 	No Europe: 0.1 USA: 1	NR	NR	See note 2	No safety concern
α-Terpineol	366	98-55-5 	Yes Europe: 3000 USA: 1100	No	Yes The NOEL of 500 mg/kg of body weight per day reported in a 20-week study in rats is > 10 000 times the daily per capita intake of α-terpineol in Europe and the USA	See note 3	
Terpinyl formate	367	2153-26-6 	No Europe: 0.1 USA: 2	NR	NR	See note 4	
Terpinyl acetate	368	8007-35-0 	No Europe: 260 USA: 390	NR	NR	See note 4	

Table 5 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Terpinyl propionate	369	80-27-3 	No Europe: 0.03 USA: 1	NR	NR	See note 4	No safety concern
Terpinyl butyrate	370	80-26-2 	No Europe: 6 USA: 6	NR	NR	See note 4	
Terpinyl isobutyrate	371	7774-65-4 	No Europe: 0.7 USA: 0.02	NR	NR	See note 4	
Terpinyl isovalerate	372	1142-85-4 	No Europe: 0.1 USA: 1	NR	NR	See note 4	

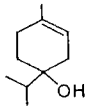
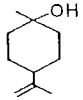
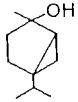
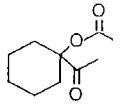
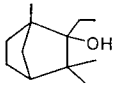
p-Menth-3-en-1-ol	373	586-82-3 	No Europe: 41 USA: 0.4	NR	NR	See note 3	
4-Carvomenthenol	439	562-74-3 	No Europe: 170 USA: 51	NR	NR	See note 3	
p-Menth-8-en-1-ol	374	138-87-4 	No Europe: 2 USA: 21	NR	NR	See note 3	No safety concern
4-Thujanol	441	546-79-2 	No Europe: 1 USA: 0.04	NR	NR	See note 1	
Methyl 1-acetoxycyclohexyl-ketone	442	52789-73-8 	— Europe: ND USA: 4	NA	No	—	Not evaluated[d]

Table 5 (*continued*)

Substance	No.	CAS no. and structure	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step B4/A5* Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II							
2-Ethyl-1,3,3-trimethyl-2-norbornanol	440	18368-91-7 	No Europe: 1 USA: 0.02	NR	NR	See note 1	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 2:* All of the substances in this group are expected to be metabolized to innocuous products, except methyl 1-acetoxycyclohexylketone.

[b] The thresholds for human intake for classes I and II are 1800 μg per day and 540 μg per day, respectively. All intake values are expressed in μg per day.

[c] The group ADI of 0–0.5 mg/kg of body weight per day established at the twenty-third meeting (Annex 1, reference *50*) for citral, geranyl acetate, citronellol, linalool and linalyl acetate expressed as citral, was maintained.

[d] This substance was not evaluated because a NOEL for this or a related substance was not available and the per capita intake exceeds 1.5 μg per day (step B5).

Notes to Table 5

1. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine.
2. Hydrolysed to linalool and the corresponding carboxylic acid. Linalool is metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group may occur after repeated exposure.
3. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group followed by hydrogenation to yield the corresponding saturated acid may occur after repeated exposure.
4. Hydrolysed to α-terpineol and the corresponding carboxylic acid. Metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group followed by hydrogenation to yield the corresponding saturated acid may occur after repeated exposure.

Absorption, metabolism and elimination

It is anticipated that the esters in this group would be readily hydrolysed to their component alcohols and carboxylic acids. The products of hydrolysis would be detoxified primarily by conjugation with glucuronic acid and excreted in the urine. Alternatively, the allyl side-chain of unsaturated alcohols may undergo ω-oxidization to yield polar metabolites, which may be conjugated and excreted. Metabolites of acyclic alcohols may be further oxidized to eventually yield carbon dioxide. Hydrolysis of esters and metabolism of the products of hydrolysis are further discussed in section 4.1.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances, the Committee assigned all but one of the 23 substances to structural class I. The remaining substance, 2-ethyl-1,3,3-trimethyl-2-norbornanol (no. 440), is a non-terpene bicyclic tertiary alcohol and was therefore assigned to structural class II.

Step 2. Adequate metabolism data were available on both linalool (no. 356) and α-terpineol (no. 366) to allow prediction of the likely pathways of metabolism of all the related compounds in the group. Although the site of oxidative metabolism would differ between compounds, the structures of linalool and α-terpineol would cover all of the functional groups present in the other members of the group of flavouring agents. On the basis of the information available on linalool and α-terpineol, the Committee concluded that, with one exception, the metabolism of compounds in this group could be predicted to give innocuous products.

Methyl 1-acetoxycyclohexylketone (no. 442) contains a sterically hindered ketone group and could not be considered *a priori* to share metabolic or toxicological similarities with other members of the group.

At their current levels of intake, 22 of the substances in this group (21 in class I and one in class II) would not be expected to saturate metabolic pathways and are predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. Methyl 1-acetoxycyclohexylketone (class I) is not predicted to be metabolized to innocuous products; its evaluation therefore proceeded via the right-hand side of the decision-tree.

Step A3. The intakes of 22 substances were evaluated at this step. Of these, 18 of the 21 substances in this group that were assigned to class

I have daily per capita intakes in Europe and the USA that are below the threshold for class I (1800μg). Therefore, these 18 substances were considered to pose no safety concern when used as flavouring agents at their current levels of estimated intake. The daily per capita intakes of the three remaining substances, linalool (no. 356), linalyl acetate (no. 359) and α-terpineol (no. 366), exceed the threshold for class I in Europe. The evaluation of these substances therefore proceeded to step A4.

The total daily per capita intakes of linalool and its esters (nos 358–365) and α-terpineol and its esters (nos 367–372) in Europe from their use as flavouring agents is above the threshold for class I. The total daily per capita intake of linalool and its esters is 4800μg in Europe and 1300μg in the USA. The total daily per capita intake of α-terpineol and its esters is 3200μg in Europe and 1400μg in the USA.

The total daily per capita intake of the class II substance, 2-ethyl-1,3,3-trimethyl-2-norbornanol (no. 440), is below the threshold for class II (540μg), indicating that this substance does not pose a safety concern when used at current levels of estimated intake as a flavouring agent.

Step A4. Linalool (no. 356), linalyl acetate (no. 359) and α-terpineol (no. 366) are not endogenous in humans and they are not predicted to be metabolized to endogenous products. The evaluation of these substances therefore proceeded to step A5.

Step A5. NOELs of 50mg/kg of body weight per day and 24mg/kg of body weight per day have been reported for linalool (no. 356) and linalyl acetate (no. 359), respectively, in 90-day studies in rats. These NOELs provide a margin of safety of >1000 for linalool and >500 for linalyl acetate and for linalool and its esters when compared with the daily per capita intakes of these substances in Europe and the USA. A NOEL of 500mg/kg of body weight per day has been reported for terpinyl acetate in a 20-week study in rats. This NOEL provides a margin of safety of approximately 10000 for α-terpineol and for α-terpineol and its esters when compared with the daily per capita intakes of these substances in Europe and the USA. A carcinogenicity bioassay in rats was conducted using a mixture of the terpenoid esters geranyl acetate and citronellyl acetate, which together with linalool and linalyl acetate, were reviewed at the twenty-third meeting of the Committee (Annex 1, reference *50*). The NOEL of 1000mg/kg of body weight per day (710mg/kg of body weight per day for geranyl acetate and 290mg/kg of body weight per day for citronellyl acetate) in this study provides a margin of safety of >10000 for linalool and its esters and for α-terpineol and its esters.

Step B3. The daily per capita intake of methyl 1-acetoxycyclohexylketone (no. 442) in the USA is below the threshold for class I (1800 μg). No intake data were reported for Europe. The evaluation of this substance therefore proceeded to step B4.

Step B4. Adequate data to determine a NOEL for methyl 1-acetoxycyclohexylketone (no. 442) or structurally related substances were not available. The evaluation of this substance therefore proceeded to step B5.

Step B5. The conditions of use of methyl 1-acetoxycyclohexylketone (no. 442) result in an intake greater than 1.5 μg per day. Accordingly, additional data on this substance were required for its evaluation.

Table 5 summarizes the evaluation of the 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances used as flavouring agents.

Consideration of combined intakes

The data were adequate to complete an evaluation of 22 of the 23 tertiary alcohols and related esters in this group. In the unlikely event that all foods containing all 22 of these substances were consumed simultaneously on a daily basis, the estimated combined intake would exceed the threshold for human intake for class I. However, these 22 substances are expected to be efficiently metabolized and would not saturate metabolic pathways. In considering combined intake, the Committee excluded intake of methyl 1-acetoxycyclohexylketone (no. 442) because additional data on toxicity were required to evaluate its safety.

Consumption of foods containing four additional esters of linalool (linalyl anthranilate, linalyl benzoate, linalyl cinnamate and linalyl phenylacetate) that were not evaluated as part of this group, would contribute an extra 10 μg or 0.2 μg to the total daily per capita intake of linalool (no. 356) in Europe and the USA, respectively. Similarly, intake of foods containing an additional ester of α-terpineol (terpinyl cinnamate) would contribute an extra 0.008 μg or 0.5 μg to the total daily per capita intake of α-terpineol (no. 366) in Europe and the USA, respectively. The potential consumption of linalool and α-terpineol from these additional esters is minor and would not be expected to lead to saturation of metabolic pathways or alter the outcome of the safety evaluation using the Procedure.

Conclusions

The Committee concluded that 22 of the 23 terpenoid tertiary alcohols and related substances in this group would not present safety

concerns when used at current levels of intake as flavouring agents. Knowledge of the pathways of metabolism of these flavouring agents and toxicity data on three substances (linalool, linalyl acetate and α-terpineol (nos 356, 359 and 366)) were required for application of the Procedure. The Committee noted that these data and the available toxicity data on other structurally related substances were consistent with the results of the safety evaluation using the Procedure. For one substance, methyl 1-acetoxycyclohexylketone (no. 442), the available metabolic data were inadequate to predict that the substance would be metabolized to innocuous products, a relevant NOEL was lacking, and intake exceeded 1.5 μg per day. According to the Procedure, the Committee concluded that additional data were required for the evaluation of methyl 1-acetoxycyclohexylketone. The group ADI established previously for linalool, linalyl acetate, citral, citronellol and geranyl acetate was maintained.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.5 Carvone and structurally related substances

The Committee evaluated carvone and eight related substances (Table 6) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). The substances in this group are terpenoid ketones, secondary alcohols and related esters containing a 2-menthyl carbon skeleton.

The Committee had previously evaluated carvone on several occasions. A conditional ADI of 0–1.25 mg/kg of body weight for the (+)- and (–)-isomers was established at the eleventh meeting (Annex 1, reference *14*). A temporary ADI of 0–1 mg/kg of body weight was established for (+)- and (–)-carvone at the twenty-third meeting (Annex 1, reference *50*), which was extended at the twenty-fifth, twenty-seventh, thirtieth and thirty-third meetings (Annex 1, references *56, 62, 73* and *83*). At its thirty-seventh meeting, the Committee determined that the (+)- and (–)-enantiomers should be evaluated separately. Owing to a lack of data on (–)-carvone *per se*, the temporary ADI for (–)-carvone was not extended. In its review of (+)-carvone, the Committee considered a long-term toxicity/carcinogenicity study in mice, short-term toxicity studies in mice and rats and *in vitro* tests for mutagenicity. On the basis of a NOEL of 93 mg/kg of body weight per day in a 3-month toxicity study in rats, the Committee established an ADI for (+)-carvone of 0–1 mg/kg of body weight per day.

Table 6
Summary of the results of safety evaluations of carvone and eight structurally related substances[a]

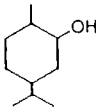
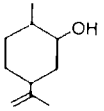
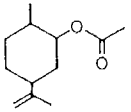
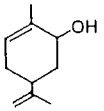
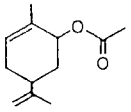
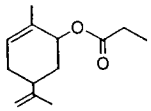
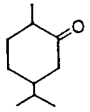
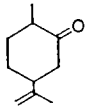
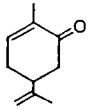
Substance	No.	CAS no. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
Structural class I						
p-Menthan-2-ol	376	499-69-4 	No Europe: 0.01 USA: 7	NR	NR	No safety concern
Dihydrocarveol	378	619-01-2 	No Europe: 3 USA: 320	NR	NR	
Dihydrocarvyl acetate	379	20777-49-5 	No Europe: 15 USA: 0.1	NR	NR	
Carveol	381	99-48-9 	No Europe: 15 USA: 140	NR	NR	
Carvyl acetate	382	97-42-7 	No Europe: 6 USA: 36	NR	NR	

Table 6 (*continued*)

Substance	No.	CAS no. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Endogenous or metabolized to endogenous substances?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
Carvyl propionate	383	97-45-0 	No Europe: ND USA: 0.04	NR	NR	No safety concern
Structural class II						
p-Menthan-2-one	375	499-70-7 	No Europe: 0.01 USA: 1	NR	NR	No safety concern
Dihydrocarvone	377	7764-50-3 	No Europe: 0.02 USA: 180	NR	NR	
Carvone[c]	380	2244-16-8 (+)- 6485-40-1 (–)- 	Yes Europe: 2800 USA: 9900	No	Yes	

CAS: Chemical Abstracts Service; ND: no intake data reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 2:* All of the substances in this group are metabolized to innocuous substances.

[b] The thresholds for human intake for classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

[c] The ADI of 0–1 mg/kg of body weight previously established for (+)-carvone at the thirty-seventh meeting of the Committee (Annex 1, reference *94*) was maintained.

Estimated daily per capita intake

The estimated total daily per capita intake of carvone (no. 380) and related substances from their use as flavouring agents is 2.8 mg in Europe and 10 mg in the USA. In Europe, carvone accounts for approximately 99% of the total annual per capita intake of this group of substances from their use as flavouring agents. In the USA, carvone accounts for approximately 96% of the total annual per capita intake of this group of substances from their use as flavouring agents.

Seven of the substances in this group have been reported to occur naturally in foods, including fruits, spices and berries. (–)-Carvone has been reported to occur in the oils of *Mentha spicata* (spearmint). (+)-Carvone has been reported in *Carum carvi* (caraway) and *Anethum graveolens* (dill). Quantitative data on the natural occurrence and consumption ratios[1] have been reported for five of these substances (nos 377, 379–382), which indicate that they are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

This group consists of three ketones (nos 375, 377, 380), three secondary alcohols (nos 376, 378, 381) and three esters (nos 379, 382, 383). The metabolism of these substances is discussed in general terms in section 4.1.

Terpenoid esters. The three esters would be expected to be hydrolysed to their corresponding alcohol and carboxylic acid by carboxylesterases, which occur predominantly in hepatocytes. Esters of carveol (no. 381) and dihydrocarveol (no. 378) would be expected to be hydrolysed to yield carveol and dihydrocarveol, respectively, and the corresponding saturated aliphatic carboxylic acids.

Terpenoid alcohols and ketones. The terpenoid alcohols resulting from hydrolysis of esters and their corresponding ketones are metabolized in the same pathways as other alicyclic terpenoid ketones and secondary alcohols. Five detoxification pathways have been identified:

— reduction of the ketone, followed by conjugation of the resulting alcohol with glucuronic acid;

[1] Defined as the ratio of the per capita intake resulting from the natural occurrence of a flavouring agent in food to the per capita intake of the flavouring agent from its intentional addition to food. Thus, a large consumption ratio signifies that the human intake from natural occurrence of the compound in food is much greater than the intake that results from its intentional addition to food. A consumption ratio of zero indicates that the compound does not occur naturally in food as far as is presently known.

— oxidation of the side-chains, yielding polar metabolites, which may be conjugated and excreted;
— conjugation of ketones with glutathione, followed by excretion;
— hydrogenation of the endocyclic double bond of carveol;
— excretion of the unchanged parent compound.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to carvone and the above-mentioned related substances, the Committee assigned six of the nine substances (*p*-menthan-2-ol, dihydrocarveol, dihydrocarvyl acetate, carveol, carvyl acetate and carvyl propionate (nos 376, 378, 379, 381–383)) to structural class I. The remaining three substances (*p*-menthan-2-one, dihydrocarvone and carvone (nos 375, 377 and 380)) were assigned to structural class II as they contain an α,β-unsaturated ketone.

Step 2. Sufficient data were available on carvone to define the major pathways of metabolism, and this compound contained all of the key structural elements and potential sites of metabolism of all other members in the group. Better data on metabolism were available on isophorone (3,4,5-trimethyl-cyclohex-2-en-1-one), which is not a member of this group of flavouring agents, but which shows close structural similarities to carvone and shares the same routes of metabolism (reduction of the carbonyl group, conjugation of the resulting alcohol with glucuronic acid, and side-chain oxidation). It is predicted that carvone and other α,β-unsaturated ketones in the group might undergo conjugation with glutathione, but this would be expected to be a minor route of metabolism because of the low reactivity *in vitro*, a conclusion supported by the toxicological data.

For the three terpenoid esters (nos 379, 382 and 383) in class I, the most likely route of metabolism is hydrolysis to carveol or dihydrocarveol. For the three terpenoid alcohols (nos 376, 378 and 381) which are also in class I, the available data indicated that the most likely route of metabolism is conjugation with glucuronic acid, followed by excretion; however, other routes of metabolism such as side-chain oxidation followed by conjugation and excretion may also be possible. In each case, metabolism yields innocuous metabolites. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

For the three terpenoid ketones (nos 375, 377 and 380) in class II, the available data indicated that the most likely route of metabolism is reduction of the ketone to the corresponding alcohol, followed by conjugation with glutathione and excretion. Other routes of metabolism are also possible, such as side-chain oxidation followed by

conjugation, and direct conjugation of the ketone with glutathione. In all cases, metabolism yields innocuous metabolites. Accordingly, the evaluation of these substances also proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of the six terpenoid esters and alcohols in class I are below the threshold of concern for this class (1800 µg per day), and they would not be expected to be of safety concern. The intakes of two of the three terpenoid ketones in class II, *p*-menthan-2-one (no. 375) and dihydrocarvone (no. 377) are below the threshold of concern for this class (540 µg per day), and these would not be expected to be of safety concern. For the remaining substance, carvone (no. 380), the intakes in both Europe and the USA are above the threshold of concern. The evaluation of this substance therefore proceeded to step A4.

Step A4. Carvone (no. 380) is not endogenous in humans. The evaluation of this substance therefore proceeded to step A5.

Step A5. A NOEL of 93 mg/kg of body weight per day was identified for (+)-carvone in a 3-month study in rats at the thirty-seventh meeting of the Committee (Annex 1, reference *94*). If it is assumed that all the carvone consumed was (+)-carvone, a margin of safety of >500 exists between this NOEL and the daily per capita intake for the (+)-isomer of carvone. Therefore this substance would not be expected to be of safety concern. The (–)-isomer of carvone would be expected to share a common metabolic pathway with (+)-carvone. A NOEL of 125 mg/kg of body weight per day was identified for carvone (isomer unspecified) at the eleventh meeting (Annex 1, reference *14*). The only material that was commercially available at that time was (–)-carvone. This NOEL was considered to apply to (–)-carvone and, if it is assumed that all the carvone intake was (–)-carvone, a margin of safety of >750 exists between this NOEL and the daily per capita intake for (–)-carvone. Accordingly, this substance would not be expected to be of safety concern.

Table 6 summarizes the evaluations of the nine substances in this group.

Consideration of combined intakes

In the unlikely event that all foods containing all six terpenoid alcohols and esters in structural class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would be below the threshold for class I.

In the unlikely event that all foods containing all three terpenoid ketones in structural class II were consumed concomitantly on a daily basis, the estimated daily per capita intake of these substances in Europe and the USA would exceed the threshold for class II.

However, all nine substances in this group are expected to be efficiently metabolized to innocuous products and would not give rise to perturbations outside the physiological range.

Conclusions

The results of the evaluations of carvone (no. 380) and related substances (nos 375–379 and 381–383) indicated that these substances would not present safety concerns at the estimated current levels of intake. In using the Procedure, the Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluation. The ADI established previously for (+)-carvone was maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

4.6 Ionones and structurally related substances

The Committee evaluated a group of 21 flavouring agents that included α- and β-ionone and structurally related substances (Table 7) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1).

Each of these substances has a cyclohexene ring with an allyl or alkyl side-chain containing a ketone or secondary alcohol functional group. With one exception, namely, 1,4-dimethyl-4-acetyl-1-cyclohexene, each contains a 2,6,6-trimethylcyclohexyl carbon skeleton, and an alkyl side-chain of 4–7 carbon atoms located at the C1 position. With the exception of γ-ionone, each of these substances has at least one endocyclic double bond. In the ionones, the carbonyl or hydroxyl group is located in the γ-position in relation to the ring, while in the damascones the carbonyl group is located in the α-position.

The Committee had previously evaluated three members of the group. α-Ionone and β-ionone were both evaluated at the twenty-eighth meeting (Annex 1, reference *66*), when a group ADI of 0–0.1 mg/kg of body weight was established for these flavouring agents, individually or in combination. Allyl-α-ionone was evaluated at the twenty-fourth meeting (Annex 1, reference *53*), when the Committee concluded that the data were inadequate for setting an ADI.

Table 7

Summary of the results of the safety evaluations of 21 ionones and structurally related substances[a]

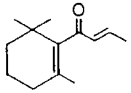
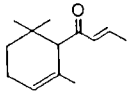
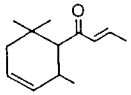
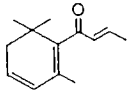
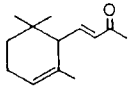
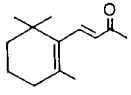
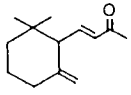
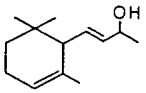
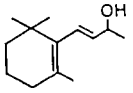
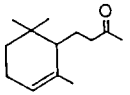
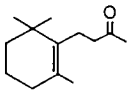
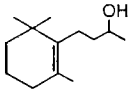
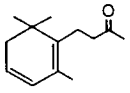
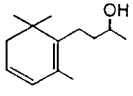
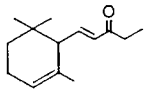
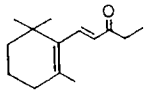
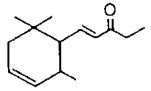
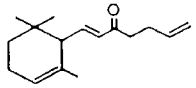
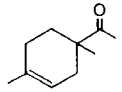
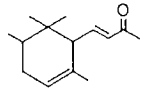
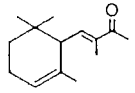
Substance	No.	CAS no. and structure	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate NOEL for substance or related substance?	Conclusion based on current intake
β-Damascone	384	23726-92-3 	No	No Europe: 43 USA: 10	Yes	No safety concern
α-Damascone	385	43052-87-5 	No	No Europe: 8 USA: 0.4	Yes	
δ-Damascone	386	57378-68-4 	No	No Europe: 0.06 USA: 0.6	Yes	
Damascenone	387	23696-85-7 	No	No Europe: 86 USA: 5	Yes	
α-Ionone[c]	388	127-41-3 	Yes	No Europe: 310 USA: 150	NA	
β-Ionone[c]	389	14901-07-6 	Yes	No Europe: 150 USA: 100	NA	
γ-Ionone	390	79-76-5 	No	No Europe: 0.01 USA: 15	Yes	

Table 7 (*continued*)

Substance	No.	CAS no. and structure	*Step 2* Metabolized to innocuous products?	*Step A3/B3*[b] Does intake exceed the threshold for human intake?	*Step B4* Adequate NOEL for substance or related substance?	Conclusion based on current intake
α-Ionol	391	25312-34-9 	Yes	No Europe: 0.7 USA: 0.06	NA	No safety concern
β-Ionol	392	22029-76-1 	Yes	No Europe: 0.9 USA: 0.1	NA	
Dihydro-α-ionone	393	31499-72-6 	Yes	No Europe: 0.7 USA: 0.02	NA	
Dihydro-β-ionone	394	17283-81-7 	Yes	No Europe: 1 USA: 0.04	NA	
Dihydro-β-ionol	395	3293-47-8 	Yes	No Europe: 0.3 USA: 0.02	NA	
Dehydrodihydroionone	396	20483-36-7 	No	No Europe: 0.1 USA: 0.08	Yes	
Dehydrodihydroionol	397	57069-86-0 	No	No Europe: 8 USA: 0.01	Yes	

Methyl-α-ionone	398	127-42-4 	Yes	No Europe: 100 USA: 7	NA	No safety concern
Methyl-β-ionone	399	127-43-5 	Yes	No Europe: 6 USA: 0.2	NA	
Methyl-δ-ionone	400	7748-98-7 	Yes	No Europe: 0.4 USA: 1	NA	
Allyl-α-ionone	401	79-78-7 	Yes	No Europe: 35 USA: 25	NA	
1,4-Dimethyl-4-acetyl-1-cyclohexene	402	43219-68-7 	—	— Europe: — USA: —	—	Not evaluated[d]
α-Irone	403	79-69-6 	Yes	No Europe: 9 USA: 3	NA	No safety concern
α-iso-Methylionone	404	127-51-5 	Yes	No Europe: 6 USA: 1	NA	

CAS: Chemical Abstracts Service; NA: not applicable.

[a] *Step 1:* All of the substances in this group are in structural class I.

[b] The threshold for human intake for class I is 1800 μg per day. All intake values are expressed in μg per day.

[c] The group ADI of 0–0.1 mg/kg of body weight established at the twenty-eighth meeting of the Committee (Annex 1, reference *66*) for α-ionone and β-ionone was maintained.

[d] 1,4-Dimethyl-4-acetyl-1-cyclohexane was not considered to have sufficient structural similarities to the ionones to be included in this group. Accordingly, the safety evaluation of this compound was not considered at the present meeting.

Estimated daily per capita intake

The estimated total daily per capita intake of all 21 ionones and related substances from their use as flavouring agents is 0.76 mg in Europe and 0.33 mg in the USA. In Europe, four substances, damascenone (no. 387), α-ionone (no. 388), β-ionone (no. 389) and methyl-α-ionone (no. 398), account for approximately 85% of the total annual per capita intake of this group of substances from their use as flavouring agents. In the USA, three substances, α-ionone, β-ionone and allyl-α-ionone (no. 401), account for approximately 85% of the total annual per capita intake of this group of substances from their use as flavouring agents.

Eleven of the substances in this group have been reported to occur naturally in foods, including raspberries, carrots, roasted almonds and herbs. Quantitative data on natural occurrence and consumption ratios have been reported for seven substances (nos 388–394), which indicate that they are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

The available data on metabolism of substances in this group are derived largely from studies on β-ionone and indicate that at least two detoxification pathways are possible (see section 4.1):

— hydroxylation of the cyclohexene ring at the C3 position followed by oxidation of the hydroxyl group to the 3-oxo derivative; and

— reduction of the ketone on the allyl side-chain to the corresponding secondary alcohol.

A combination of these reactions results in the formation of polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid. β-Ionone can also be excreted unchanged in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

1,4-Dimethyl-4-acetyl-1-cyclohexene (no. 402) was not considered to have sufficient structural similarities to the ionones to be included in this group and therefore the safety of this compound was not evaluated.

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the remaining 20 ionones and related substances, the Committee assigned all of the substances to structural class I.

Step 2. Data were available for β-ionone (no. 389), which showed that it was metabolized by reduction of the carbonyl group and hydroxy-

lation of the alicyclic ring and by conjugation of metabolites containing alcohol groups with glucuronic acid. Only limited data were available on the fate of other compounds in the group of α- and β-ionone and related flavouring agents. Other members of this group had structural characteristics similar to β-ionone, but there were differences in the number and positions of the alicyclic double bonds and the position of the carbonyl group within the side-chain and its relationship to endocyclic and exocyclic double bonds. On the basis of the similarities of the functional groups present in the flavouring agents in this group it was considered that α- and β-ionone (nos 388 and 389), their alcohol analogues, analogues with a single endocyclic double bond, and analogues with saturated side-chains or longer side-chains would be eliminated from the body by common metabolic processes, which would lead to innocuous products.

Members of the group with two endocyclic double bonds, or with the carboxyl group adjacent to the cyclohexene ring, or with an allyl double bond attached to the cyclohexene ring, might show reduced rates of elimination which might affect their toxicological potency. The Procedure takes this possibility into account to some extent since compounds that are sterically hindered are assigned to structural class II, rather than class I. However, the Committee was not able to conclude *a priori* that the products of metabolism of such compounds would be innocuous.

Substances that could be predicted to be metabolized to innocuous products fall into the following two groups:

Group 1: *α-Ionone, α-ionol, dihydro-α-ionone, methyl-α-ionone, methyl-δ-ionone, α-irone, 2-iso-methylionone and allyl-α-ionone* (nos 388, 391, 393, 398, 400, 403, 404 and 401, respectively). These substances were considered likely to share a common metabolic pathway with α-ionone, although the rate of metabolism for some of these substances may be slower than for α-ionone.

Group 2: *β-Ionol, dihydro-β-ionone, dihydro-β-ionol, methyl-β-ionone and methyl-δ-ionone* (nos 392, 394, 395, 399 and 400, respectively). These substances were considered likely to share common metabolic pathways with β-ionone, although the rate of metabolism for some of these substances may be slower than for β-ionone.

The evaluation of the substances in groups 1 and 2 proceeded via the left-hand side of the decision-tree.

The substances that could not be predicted to be metabolized to innocuous products are β-damascone, α-damascone, δ-damascone,

damascenone, γ-ionone, dehydrodihydroionone and dehydrodihydroionol (nos 384–387, 390, 396 and 397, respectively). The evaluation of these substances proceeded via the right-hand side of the decision-tree.

Step A3. The daily per capita intakes in Europe and the USA of all 13 substances in groups 1 and 2 are below the threshold for human intake for class I (1800 μg per day). Therefore, these substances would not be expected to be of safety concern.

Step B3. For β-damascone, α-damascone, δ-damascone, damascenone, γ-ionone, dehydrodihydroionone and dehydrodihydroionol (nos 384–387, 390, 396 and 397, respectively), the daily per capita intakes in Europe and the USA are below the threshold for class I. Accordingly, the evaluation of these substances proceeded to step B4.

Step B4. Information on each of the compounds considered at this step is given below.

The NOEL for β-damascone (no. 384) is 2 mg/kg of body weight per day, based on a 90-day study in rats. The margin of safety between this NOEL and the daily per capita intake under the conditions of intended use is >2000.

α-Damascone, δ-damascone and damascenone (nos 385–387) were considered likely to share common metabolic pathways with β-damascone (no. 384), although the rate of metabolism may be slower. The margins of safety between the NOEL for β-damascone and the daily per capita intakes of α-damascone, δ-damascone and damascenone are >10000, 200000 and >1000, respectively.

γ-Ionone (no. 390) was considered likely to share a common metabolic pathway with α-ionone (no. 388) and β-ionone (no. 389), for which the NOEL was 10 mg/kg of body weight per day, based on a 90-day study in rats. The margin of safety between this NOEL and the daily per capita intake of γ-ionone is 40000. It was also considered likely to share a common metabolic pathway with carvone (no. 380), for which the NOEL was 93 mg/kg of body weight per day, based on a 3-month study in rats. In this case the margin of safety between this NOEL and the daily per capita intake of γ-ionone is 370000.

Dehydrodihydroionone (no. 396) and dehydrodihydroionol (no. 397) were considered to share common metabolic pathways with α-ionone and β-ionone, for which the NOEL was 10 mg/kg of body weight per day. The margins of safety between this NOEL and the daily per capita intakes of dehydrodihydroionone and dehydrodihydroionol are 6×10^6 and 80000, respectively.

Therefore, the seven substances considered at this step would not be expected to be of safety concern.

Table 7 summarizes the evaluations of the 21 α- and β-ionones and related substances used as flavouring agents.

Consideration of combined intakes

All of the 20 ionones and structurally related substances considered in this evaluation would be expected to share common metabolic pathways. In the unlikely event that all foods containing all 20 substances were consumed simultaneously on a daily basis, the estimated daily per capita consumption in Europe and the USA would not exceed the threshold for human intake for substances in class I.

Conclusions

The Committee concluded that use of any of the 20 ionones and related substances as flavouring agents would not present safety concerns at the estimated current levels of intake.

In using the Procedure, the Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluation.

The ADIs previously established for α-ionone and β-ionone were maintained.

A monograph summarizing the safety data available on this group of flavouring agents was prepared.

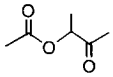
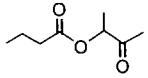
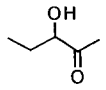
4.7 Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones

The Committee evaluated a group of 22 flavouring agents that included aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones (Table 8) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). One member of the group, diacetyl (2,3-butanedione), was previously evaluated at the eleventh meeting of the Committee (Annex 1, reference *14*). However, no ADI was allocated at that time because of a lack of data.

Estimated daily per capita intake

In the USA, aliphatic acyclic and alicyclic α-diketones and α-hydroxyketones are generally used as flavouring agents up to average maximum levels of 200 mg/kg. The total annual volume of production of the 22 substances in this group is approximately 44 tonnes in Europe and 56 tonnes in the USA. In both Europe and the USA,

Table 8
Summary of the results of safety evaluations of 22 aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones[a]

Substance	No.	CAS no. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
Acetoin	405	513-86-0 	Yes Europe: 2800 USA: 1800	Yes	NR	No safety concern
2-Acetoxy-3-butanone	406	4906-24-5 	No Europe: 0.03 USA: 23	NR	NR	
Butan-3-one-2-yl-butanoate	407	84642-61-5 	No Europe: 0.02 USA: 0.95	NR	NR	
Diacetyl	408	431-03-8 	Yes Europe: 3300 USA: 8000	Yes	NR	
3-Hydroxy-2-pentanone	409	3142-66-3 	No Europe: ND USA: 0.10	NR	NR	

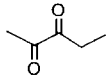
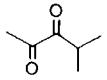
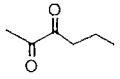
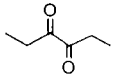
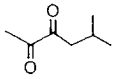
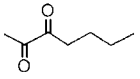
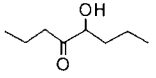
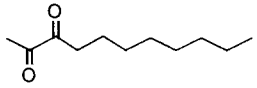
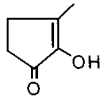
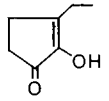
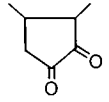
2,3-Pentanedione	410	600-14-6 	No Europe: 220 USA: 80	NR	NR	No safety concern
4-Methyl-2,3-pentadione	411	7493-58-5 	No Europe: 0.48 USA: 2	NR	NR	
2,3-Hexanedione	412	3848-24-6 	No Europe: 13 USA: 10	NR	NR	
3,4-Hexanedione	413	4437-51-8 	No Europe: 33 USA: 0.76	NR	NR	
5-Methyl-2,3-hexanedione	414	13706-86-0 	No Europe: 2 USA: 6	NR	NR	
2,3-Heptanedione	415	96-04-8 	No Europe: 2 USA: 5	NR	NR	
5-Hydroxy-4-octanone	416	496-77-5 	No Europe: 0.02 USA: 0.76	NR	NR	

Table 8 (*continued*)

Substance	No.	CAS no. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
2,3-Undecadione	417	7493-59-6 	No Europe: 0.02 USA: 0.01	NR	NR	No safety concern
Methylcyclopentenolone	418	80-71-7 	Yes Europe: 890 USA: 710	No	Yes The NOEL of 500 mg/kg of body weight per day reported in a 6-month toxicity study in rats is >30 000 times the daily per capita intake of methylcyclopentenolone	
Ethylcyclopentenolone	419	21835-01-8 	No Europe: 50 USA: 23	NR	NR	
3,4-Dimethyl-1,2-cyclopentanedione	420	13494-06-9 	No Europe: 47 USA: 2	NR	NR	

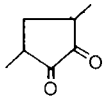
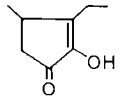
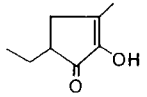
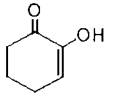
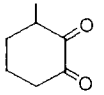
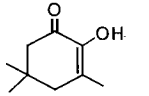
3,5-Dimethyl-1,2-cyclopentanedione	421	13494-07-0 	No Europe: 55 USA: 29	NR	NR	No safety concern
3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	422	42348-12-9 	No Europe: ND USA: 0.17	NR	NR	
5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	423	53263-58-4 	No Europe: ND USA: 0.38	NR	NR	
2-Hydroxy-2-cyclohexen-1-one	424	10316-66-2 	No Europe: 0.08 USA: 0.76	NR	NR	
1-Methyl-2,3-cyclohexadione	425	3008-43-3 	No Europe: 2 USA: 8	NR	NR	

Table 8 (*continued*)

Substance	No.	CAS no. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	426	4883-60-7 	No Europe: 2 USA: 2	NR	NR	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 (nos 406, 407, 409–417 and 419–426) or step A4 (nos 405 and 408) of the Procedure.

[a] *Step 1*: All of the substances in the group are in structural class II.
Step 2: All of the substances in this group are metabolized to innocuous products.

[b] The threshold for human intake for class II is 540 μg per day. All intake values are expressed in μg per day.

more than 95% of the total annual volume is accounted for by three substances: acetoin (3-hydroxy-2-butanone; no. 405; 19 tonnes/year in Europe and 9.2 tonnes/year in the USA), diacetyl (no. 408; 18 tonnes/year in Europe and 42 tonnes/year in the USA) and methylcyclopentenolone (no. 418; 4.7 tonnes/year in Europe and 3.7 tonnes/year in the USA). Two of these substances (acetoin and diacetyl) account for more than 90% of the total annual volume in the USA.

Nineteen of the 22 aliphatic acyclic and alicyclic α-diketones and α-hydroxyketones have been identified as natural components of a variety of foods, including fruits, vegetables, cocoa and coffee. Quantitative data on natural occurrence have been reported for six of these substances. On the basis of the information available, the levels of intake of these substances as natural components of food would appear to be greater than those from their use as flavouring agents, with one exception (diacetyl).

Absorption, metabolism and elimination

In rats and mice, orally administered aliphatic α-diketones are rapidly absorbed from the gastrointestinal tract. It is anticipated that at low levels of intake, humans will metabolize aliphatic acyclic α-diketones principally by α-hydroxylation and subsequent oxidation of the terminal methyl group to yield the corresponding ketocarboxylic acid. The acid may undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolized in the fatty acid pathway and citric acid cycle. At high levels of intake, aliphatic acyclic α-diketones may be metabolized by reduction to the diol and subsequent conjugation with glucuronic acid. Acyclic α-diketones and α-hydroxyketones without a terminal methyl group and alicyclic α-diketones and α-hydroxyketones are mainly metabolized by reduction to the corresponding diol followed by conjugation with glucuronic acid and excretion (see section 4.1).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones, the Committee assigned all 22 substances to structural class II.

Step 2. In vitro and *in vivo* data demonstrated two major routes of metabolism for diacetyl (no. 408) and acetoin (no. 405) involving complete oxidation to carbon dioxide and reduction to a diol, but data were not available on the longer-chain linear homologues or cyclic analogues. Alcohol dehydrogenases, aldehyde reductase and

carbonyl reductase are widely distributed enzymes, with broad substrate specificities, and *in vitro* data show that 1,2-cyclohexanedione is a better substrate than diacetyl for aldehyde reductase and carbonyl reductase. In consequence, it was considered that the data on the extensive reduction of acetoin can be extrapolated to other members of the group. The α-diketone group is polar, and it would be expected that all members of the group evaluated would be metabolized by a combination of oxidation (when the carbonyl group is adjacent to a methyl group), reduction of the carbonyl group and excretion of the parent compound and metabolites in urine. The products of these metabolic pathways do not raise toxicological concerns. This conclusion is supported by the toxicological data available on some members of the group.

Step A3. For 19 of the substances, the daily per capita intakes in Europe and the USA are below the threshold for human intake for substances in class II (540μg per day), indicating that they pose no safety concern when used at current levels of estimated intake as flavouring agents. Only acetoin (no. 405), diacetyl (no. 408) and methylcyclopentenolone (no. 418) have intakes greater than 540μg per day in Europe and the USA.

Step A4. Acetoin (no. 405) and diacetyl (no. 408) are endogenous in humans, but methylcyclopentenolone (no. 418) is not. The evaluation of this substance therefore proceeded to step A5.

Step A5. A NOEL of 500mg/kg of body weight per day was reported for methylcyclopentenolone (no. 418) in a 6-month toxicity study in rats. A safety margin of >30000 exists between this NOEL and the estimated daily per capita intake of methylcyclopentenolone. In addition, the results of genotoxicity tests (Ames test and test for unscheduled DNA synthesis) of methylcyclopentenolone were negative. This information indicates that methylcyclopentenolone would not be expected to be of safety concern.

The stepwise evaluations of the 22 aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones used as flavouring agents are summarized in Table 8.

Consideration of combined intakes

In the unlikely event that all foods containing all of the 22 aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones evaluated as flavouring agents were consumed simultaneously on a daily basis, the estimated total daily per capita intake of these substances in Europe and the USA would exceed the threshold for human intake for substances in class II. However, all of the flavouring agents in this

group are expected to be efficiently metabolized and excreted and would not saturate detoxification pathways. On the basis of the evaluation of the collective data, the Committee concluded that combined intake of these substances would not be expected to be of safety concern.

Conclusion

The Committee concluded that the 22 aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones evaluated do not pose a safety concern when used at current levels of intake as flavouring agents. No toxicity data were required for application of the Procedure to 21 of the 22 substances evaluated. However, the Committee noted that where toxicity data were available, they were consistent with the results of the Procedure. For the remaining substance, methylcyclopentenolone (no. 418), the toxicity data indicated that it would not be expected to be of safety concern.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.8 Substances structurally related to menthol

The Committee evaluated 13 substances structurally related to menthol (Table 9) using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1). Menthol was evaluated separately (see section 3.2.3). However, it is included here because information on this substance is integral to the evaluation of its structurally related substances.

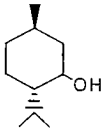
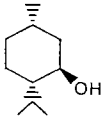
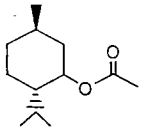
Menthol was previously evaluated at the eleventh, eighteenth and twentieth meetings of the Committee (Annex 1, references *14*, *35* and *41*). At its present meeting, the Committee allocated an ADI of 0–4 mg/kg of body weight to menthol (section 3.2.3).

Estimated daily per capita intake

The total annual volume of production of menthol and the 13 structurally related substances from their use as flavouring agents is approximately 140 tonnes in Europe and 79 tonnes in the USA. Menthol (no. 427) and menthone (no. 429) account for 97% of the total annual volume in Europe and 85% of the total annual volume in the USA.

Menthol and some structurally related substances occur naturally in a wide variety of foods, including spearmint oil, cornmint oil, peppermint oil, raspberries, rum, nutmeg and cocoa. Menthol and

Table 9
Summary of the results of safety evaluations of menthol and 13 structurally related substances[a]

Substance	No.	CAS structure and no.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
Structural class I						
Menthol[c]	427	89-78-1 	Yes Europe: 18000 USA: 10000	No	Yes The NOEL of 380 mg/kg of body weight per day reported in a 2-year toxicity/carcinogenicity study in rats is >1000 times the daily per capita intake of menthol in both Europe and the USA	No safety concern
(+)-neo-Menthol	428	2216-52-6 	No Europe: 3 USA: 27	NR	NR	
Menthyl acetate	431	16409-45-3 	No Europe: 420 USA: 560	NR	NR	

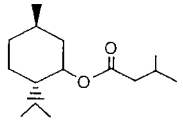
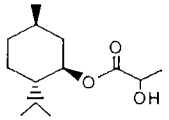
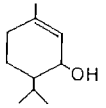
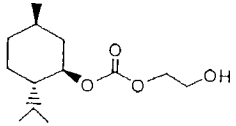
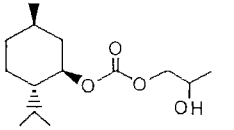
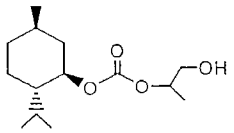
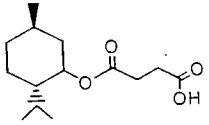
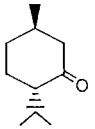
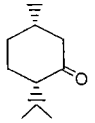
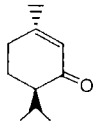
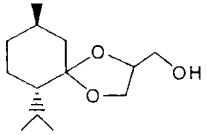
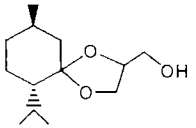
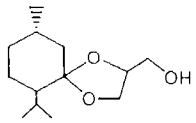
Menthyl isovalerate	432	16409-46-4 	No Europe: 9 USA: 27	NR	NR	No safety concern
(–)-Menthyl lactate	433	59259-38-0 	No Europe: 26 USA: 0.1	NR	NR	
p-Menth-1-en-3-ol	434	491-04-3 	No Europe: 0.02 USA: 0.02	NR	NR	
(–)-Menthol ethylene glycol carbonate	443	156324-78-6 	No Europe: ND USA: 760	NR	NR	
Mixture of (–)-menthol 1- and 2-propylene glycol carbonate	444	156329-82-2  	No Europe: ND USA: 380	NR	NR	

Table 9 (*continued*)

Substance	No.	CAS structure and no.	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate NOEL for substance or related substance?	Conclusion based on current intake
mono-Menthyl succinate	447	77341-67-4 	No Europe: ND USA: 22	NR	NR	No safety concern
Structural class II						
Menthone	429	89-80-5 	Yes Europe: 1000 USA: 2500	No	Yes The NOEL of 400 mg/kg of body weight per day reported in a 28-day toxicity study in rats is about 10 000 times the daily per capita intake of menthone in both Europe and the USA	No safety concern
(±)-Isomenthone	430	491-07-6 	No Europe: 200 USA: 0.1	NR	NR	

Piperitone	435	6091-50-5 	No Europe: 51 USA: 10	NR	NR	No safety concern
(–)-Menthone 1,2-glycerol ketal	445	563187-91-7 	No Europe: ND USA: 190	NR	NR	
(±)-Menthone 1,2-glycerol ketal	446	63187-91-7  	No Europe: ND USA: 190	NR	NR	

CAS: Chemical Abstract Service; ND: no intake data reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

[a] *Step 2*: All of the substances in this group are metabolized to innocuous products.

[b] The thresholds for human intake for structural classes I and II are 1800 µg per day and 540 µg per day, respectively. All intake values are expressed in µg per day.

[c] An ADI of 0–4 mg/kg of body weight was established for this substance at the present meeting.

menthone are the principal constituents of peppermint oil, accounting for 10–70% and 7–40%, respectively. Eight of the substances in this group have been reported to occur naturally in foods (nos 427–432, 434 and 435); six of the remaining substances are esters of menthol (nos 433, 443, 444 and 447) or ketals of menthone (nos 445 and 446). Quantitative data on natural occurrence and consumption ratios have been reported for five of these substances, which are consumed predominantly in traditional foods (i.e. consumption ratio >1).

Absorption, metabolism and elimination

The esters in this group (nos 431–433 and 447) would be expected to be readily hydrolysed to menthol and their respective carboxylic acids; the latter are endogenous in humans. The carbonate esters of (–)-menthol (nos 443 and 444) can be expected to be hydrolysed to menthol and carbonate and either ethylene glycol or propylene glycol. The ketals (nos 445 and 446) are hydrolysed *in vitro* to yield (–)- or (±)-menthone and simple glycols. The ketones (nos 429, 430 and 435) in this group would be reduced to their corresponding secondary alcohols which, like menthol, are conjugated with glucuronic acid and then excreted in the urine (see section 4.1).

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Menthol (no. 427) belongs to structural class I. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned substances structurally related to menthol, the Committee assigned eight of the 13 substances ((+)-neo-menthol, menthyl acetate, menthyl isovalerate, (–)-menthyl lactate, *p*-menth-1-en-3-ol, (–)-menthol ethylene glycol carbonate, the mixture of (–)-menthol 1- and 2-propylene glycol carbonate and mono-menthyl succinate (nos 428, 431–434, 443, 444 and 447, respectively)) to structural class I. The remaining five substances (menthone, (±)-isomenthone, piperitone, (–)-menthone 1,2-glycerol ketal and (±)-menthone 1,2-glycerol ketal (nos 429, 430, 435, 445 and 446, respectively)) were assigned to structural class II.

Step 2. At current levels of intake, the 14 substances would not be expected to saturate the metabolic pathways, and they are all predicted to be metabolized to innocuous products (see section 2.3.1). Consideration of this group of flavouring agents was dependent on data on the metabolic fate of menthol (no. 427) and menthone (no. 429), which are metabolized by a combination of oxidation and conjugation. The extensive conjugation of menthol with glucuronic acid and its rapid elimination in urine and bile, combined with its simple chemical structure, provided assurance that the products of

metabolism are innocuous. The metabolites of piperitone (an α,β-unsaturated ketone) were considered to be innocuous by comparison with menthone and its metabolites (saturated) and carvone (α,β-unsaturated).

Step A3. The daily per capita intakes of all of the substances in this group in structural class I, with the exception of menthol (no. 427), are below the threshold for human intake for class I (1800 μg) in both Europe and the USA, indicating that they pose no safety concerns when used at the current levels of estimated intake as flavouring agents. The intake of menthol in Europe and the USA is above the threshold for human intake for substances in class I.

The total intake of menthol in Europe and the USA from its use and use of its esters (nos 431–433, 443, 444 and 447) as flavouring agents is above the threshold for human intake for class I. The total daily per capita intake of menthol and its esters is 19000 μg in Europe and 12000 μg in the USA.

The daily per capita intakes of all the substances in structural class II, with the exception of menthone (no. 429), are below the threshold for class II (540 μg per day) in both Europe and the USA. The daily per capita intake of menthone in Europe and the USA is above the threshold for human intake for class II.

The total intake of menthone in Europe and the USA from its use and use of its ketals (nos 445 and 446) as flavouring agents is above the threshold for human exposure for class II. The total daily per capita intake of menthone and its ketals is 1000 μg in Europe and 2900 μg in the USA.

Step A4. Menthol (no. 427) and menthone (no. 429) are not endogenous in humans. The evaluation of these substances therefore proceeded to step A5.

Step A5. An ADI of 0–4 mg/kg of body weight was allocated to menthol (no. 427) at the present meeting (see section 3.2.3). For menthone (no. 429), a NOEL of 400 mg/kg of body weight per day has been reported in a 28-day toxicity study in rats. A safety margin of approximately 10000 exists between this NOEL and the daily per capita intake of menthone (42 μg/kg of body weight per day in Europe and 17 μg/kg of body weight per day in the USA) or the total daily per capita intake of menthone and its derivatives (46 and 17 μg/kg of body weight per day, respectively). This information indicates that neither menthol nor menthone would be expected to be of safety concern.

Table 9 summarizes the evaluation of menthol and the 13 structurally related substances using the Procedure.

Consideration of combined intakes

In the unlikely event that all foods containing menthol (no. 427) and the eight structurally related substances in class I were consumed concomitantly on a daily basis, the estimated total daily per capita intake in Europe and the USA would exceed the threshold for human intake for class I.

In the unlikely event that all foods containing menthone (no. 429) and the four structurally related substances in class II were consumed concomitantly on a daily basis, the estimated total daily per capita intake in Europe and the USA would exceed the threshold for human intake for class II.

All 14 substances are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. On the basis of the evaluation of the collective data, the combined intake was judged by the Committee not to raise safety concerns.

Conclusions

The Committee concluded that the use of menthol and the 13 structurally related substances evaluated as flavouring agents would not pose a safety concern at the current estimated levels of intake. The Committee noted that where toxicity data were available, they were consistent with the results of the safety evaluation using the Procedure.

A monograph summarizing the safety data on the 13 substances structurally related to menthol was prepared.

5. Intake assessments of specific food additives

In response to a request of the Twenty-Ninth Session of the Codex Committee on Food Additives and Contaminants (*8*), the Expert Committee assessed the intake of five food additives. In this section of the report, the Expert Committee recommends the review of levels of food additives in certain categories of the draft General Standard on Food Additives that is being developed by the Codex Committee. These categories are reproduced in Annex 4 for ease of reference.

5.1 Benzoates

The Committee assessed the intake of benzoates, a class of food additives generally used as preservatives. Maximum limits for benzoates have been proposed in a wide range of foods (solids and liquids) in the draft General Standard for Food Additives. A group ADI of 0–5 mg/kg of body weight, expressed as benzoic acid

equivalents, was allocated to benzyl acetate, benzyl alcohol, benzaldehyde and benzoic acid and its salts at the forty-sixth meeting of the Committee (Annex 1, reference *122*).

Information on benzoates was provided by nine Member States (Australia, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The assessments submitted were based on the budget method, "poundage" data, household economic surveys, model diets and individual dietary records (section 2.3). Most Member States provided assessments based on more than one method. Japan submitted assessments based on actual measured concentrations of benzoates in foods. The other assessments were based on benzoate levels assumed to be at the maximum limits specified in the draft General Standard for Food Additives, the maximum limits specified in national food standards or the maximum limits specified by the European Union.

Screening of benzoates using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee using the budget method had identified the benzoates as requiring further assessment. The inclusion of national estimates of the proportion of the food supply that may contain benzoates in the calculations made using the budget method resulted in the same conclusion.

Intake assessments

All the national assessments of intake evaluated by the Committee, except that from Japan, were based on the use of food additives at the maximum limits specified in the draft General Standard for Food Additives, the maximum limits specified by the European Union or the maximum limits specified in national food standards. The intake assessment from Japan was based on an analysis that combined measured levels of additives with data on food consumption to estimate the food additive intake.

Intake estimates based on "poundage" data and household economic surveys. Estimates of daily per capita intake of benzoates based on "poundage" data ranged from 0.7 to 1.4 mg/kg of body weight. These intakes were less than the ADI. The intake estimates based on household economic surveys were also less than the ADI when the maximum limits for benzoic acid specified by the European Union or in national food standards were used. The estimated daily per capita

intakes ranged from 0.4 to 0.6 mg/kg of body weight, but it should be noted that the levels that were assumed to be present in foods were lower than the maximum limits in the draft General Standard for Food Additives.

Intake estimates based on model diets. Estimates based on model diets were provided by five Member States. A number of assumptions were included in these assessments. In Japan, the intake estimates were based on levels of residues determined in a market basket survey; these estimates were substantially lower than the maximum limits in the draft General Standard for Food Additives. For example, the average benzoate concentration in non-alcoholic beverages was 190 mg/kg as compared with the maximum limit in the draft General Standard for Food Additives of 1000 mg/kg in soft drinks and 2000 mg/kg in fruit juice. The estimated daily per capita intake of benzoates in Japan was 0.18 mg/kg of body weight. In all other national assessments of intake, the maximum limits specified in national standards or the draft General Standard for Food Additives were assumed. The resulting daily per capita intakes ranged from 2.3 mg/kg of body weight (for consumers with mean intakes, based on the maximum limits in the USA) to 35 mg/kg of body weight (for consumers in the 90th percentile, based on the maximum limits in the draft General Standard for Food Additives). Intake estimates based on model diets were generally greater than the ADI, particularly when it was assumed that the benzoate levels were those proposed in the draft General Standard for Food Additives.

Intake estimates based on individual dietary records. Estimates of the intake of benzoates based on individual dietary records were submitted by four Member States. Only one assessment, the combined assessment provided by Australia and New Zealand, was based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives and individual dietary records. In that assessment, the estimated intakes exceeded the ADI for consumers with mean intakes, as well as for those in the 95th percentile. The mean intake was 20 mg/kg of body weight per day (400% of the ADI) and the intake of consumers in the 95th percentile was 36 mg/kg of body weight per day (720% of the ADI).

The intakes estimated for benzoates based on individual dietary records and maximum limits specified in national food standards were lower than the ADI for consumers with mean intakes as well as for those with high intakes (in the 95th or 97.5th percentiles) in all four Member States for which assessments were submitted (Australia and New Zealand (combined), France and the United Kingdom).

Conclusions

National intake estimates based on maximum limits in the draft General Standard for Food Additives. National estimates of intake of benzoates for consumers with mean intakes and those in the 95th percentile based on individual dietary records were below the ADI in all Member States when they were calculated using the maximum limits specified in national food standards, but above the ADI when the maximum limits and range of uses specified in the draft General Standard for Food Additives were used. This reflects the fact that the maximum limits specified in the General Standard for Food Additives represent the highest maximum limits submitted by any Member State or international nongovernmental organization.

The available data were insufficient to estimate the number of consumers who may exceed the ADI or to determine the magnitude and duration of intake above the ADI.

Because diets differ among countries, the foods contributing most to benzoate intake would be expected to vary. When foods were categorized according to the draft General Standard for Food Additives, the food category that contributed most to benzoate intake was carbonated water-based flavoured drinks (i.e. soft drinks) (category 14.1.4.1) in Australia and New Zealand (combined), France, the United Kingdom and the USA. Soy sauce was the major contributor to benzoate intake in China and the second most important contributor in Japan.

Typically, estimates of individual dietary intakes rely on short-term (1- to 7-day) surveys of food consumption, which was the case with the benzoates. Generally, estimates of long-term consumption of single foods are lower than estimates from short-term surveys. However, in the case of benzoates, the soft drinks that contribute most to intake are likely to be consumed on a regular basis. Because many consumers are likely to be regular consumers of a certain brand of soft drink, estimates of intake may reflect the levels of benzoates used in specific brands. A consumer who consumes a single bottle (0.5l) of soft drink per day would ingest 8.3mg of benzoates/kg body weight per day (170% of the ADI) if it contains the maximum limit specified in the draft General Standard for Food Additives. Thus, the estimated intake of benzoates would not be likely to be substantially lower if intake data based on a longer-term survey were available. This conclusion assumes that the levels remaining in the foods at the time of consumption are at the maximum limits. If the levels required to meet technological needs were below the maximum limits specified in the draft General Standard for Food Additives, intakes would be correspondingly lower. The data submitted by Japan from a market

basket survey suggest that this might be the case. However, further data would be required to determine the levels that are required to achieve the technological effect. In addition, benzoates are not likely to be used in all foods for which their use is permitted. Information on these aspects could be used to revise both the intake assessment and the maximum limits specified in the draft General Standard for Food Additives.

The Committee concluded that because of the potentially significant intake of benzoates based on the maximum limits specified in the draft General Standard for Food Additives, factors contributing to intakes in excess of the ADI should be further evaluated. Further information on levels in food at the time of consumption is needed.

National intake estimates based on maximum limits specified in national food standards or by the European Union. The best estimates of national mean intakes for consumers of benzoates were below the ADI (ranging from 0.18 mg/kg of body weight per day in Japan to 2.3 mg/kg of body weight per day in the USA). These estimates were based on analyses that used either model diets or individual dietary records together with maximum limits specified in national food standards or by the European Union. Intake estimates for consumers with high intakes of benzoates based on maximum limits specified in national standards were above the ADI in some cases (14 mg/kg of body weight per day in China and 7.3 mg/kg of body weight per day in the USA).

Recommendations to the Codex Committee on Food Additives and Contaminants

The Committee noted that the proposed draft General Standard for Food Additives contains several categories in which the proposed levels of use of benzoates submitted by a single country are substantially higher than those in the same or similar foods in other countries. These include the following categories (see Annex 4): ripened cheese (1.6.2), processed cheese (1.6.4), cheese analogues (1.6.5), vegetables in vinegar, oil or brine (4.2.2.3), cooked molluscs, crustaceans and echinoderms (9.2.4.2), semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms (9.3), and liquid egg products (10.2.1). The Codex Committee may wish to review the appropriate levels for these foods.

Levels of benzoates in carbonated drinks (category 14.1.4.1) that ranged from 350 to 1000 mg/kg were proposed by several countries and by one nongovernmental organization. Since soft drinks were an important contributor to the estimated intakes of benzoates for all the Member States that submitted data for this assessment (with the

exception of China), the Codex Committee may wish to further consider an appropriate level for this category.

The Committee noted that intake estimates based on maximum limits specified in national standards were below the ADI for benzoates. However, when the intake estimates are based on the maximum limits and the range of uses specified in the draft General Standard for Food Additives, the ADI is exceeded. The differences arise because the range of foods specified in the draft General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.2 Butylated hydroxyanisole (BHA)

The Committee assessed the intake of butylated hydroxyanisole (BHA), for which maximum limits have been proposed in a wide range of solid foods and water-based flavoured non-alcoholic drinks in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. An ADI of 0–0.5 mg/kg of body weight was allocated to BHA at the thirty-third meeting of the Expert Committee (Annex 1, reference *83*).

BHA is a food additive that is generally used as an antioxidant in products containing fats or oils and can be used in conjunction with butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate, providing a synergistic combination of antioxidants.

Information was provided by ten Member States (Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on "poundage" data, household economic surveys or retail sales surveys, model diets, and/or individual dietary records (see section 2.3).

Screening of BHA using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee using the budget method had identified BHA as a food additive requiring detailed assessment. The inclusion of the proportions of the national solid food or beverage supply that may contain BHA in the calculations using the budget method did not change the conclusion that the theoretical maximum levels of use of

BHA in food and beverages were lower than both the national maximum permitted levels in the five Member States that submitted such calculations and the maximum limits specified in the draft General Standard for Food Additives.

Intake assessments

All the national assessments of intake of BHA evaluated by the Committee, except that from Japan, were based on the use of food additives at the maximum limits specified in the draft General Standard for Food Additives, maximum limits specified by the European Union, or maximum limits specified in national food standards.

Intake estimates based on "poundage" data and household economic surveys or retail sales surveys. Intake estimates based on "poundage" data and on household economic surveys or retail sales surveys would generally be expected to be lower than those based on actual consumption of the additive, such as estimates based on model diets or individual dietary records. This was found to be the case for BHA.

Estimates of daily per capita intake based on "poundage" data submitted by five Member States ranged from 0.005 mg/kg of body weight in China to 0.48 mg/kg of body weight in Spain. Data from household economic surveys or retail sales surveys submitted by three Member States gave estimates of daily per capita intake which ranged from 0.02 mg/kg of body weight in France to 0.25 mg/kg of body weight in Spain.

Intake estimates based on model diets or individual dietary records. The national mean daily per capita intakes of BHA based on the best estimates of additive consumption (from model diets or individual dietary records) from countries that submitted data ranged from 0.003 mg/kg of body weight in Japan (market basket survey) to 0.39 mg/kg of body weight in Australia and New Zealand (based on individual dietary records using maximum levels specified in national food standards). The low estimate of intake for Japan was due to the use of analysed BHA levels in the foods "as consumed". All other Member States used maximum permitted levels of food additives derived from national standards.

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intake estimates based on maximum limits and the range of foods specified in the draft General Standard for Food Additives will grossly overestimate the actual intakes in any one country. This is because the maximum limits specified in the draft General Standard for Food Additives are generally compiled by adopting the highest level of use for any one

food category submitted by Member States or nongovernmental organizations. The range of uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards. National estimates of mean daily per capita intake of BHA based on maximum limits and the range of foods specified in the draft General Standard for Food Additives ranged from 0.91 to 0.94 mg/kg of body weight.

Conclusions

National intake estimates based on maximum limits specified in the draft General Standard for Food Additives. National estimates of mean intake of BHA based on maximum limits and the range of foods specified in the draft General Standard for Food Additives were available from only three Member States. The estimates of mean intake exceeded the ADI in these countries (180% of the ADI for Australia and New Zealand (combined), 190% of the ADI for the USA).

National intake estimates based on maximum limits specified in national food standards or by the European Union. All national estimates of mean intake for consumers of BHA were below the ADI (ranging from 1% of the ADI for Japan to 80% of the ADI for Australia and New Zealand (combined) and the USA). These estimates were based either on model diets or on individual dietary records submitted by seven Member States (Australia and New Zealand (combined), China, France, Japan, the United Kingdom and the USA). Intake estimates for consumers with high intakes of BHA based on maximum levels of food additives specified in national standards exceeded the ADI in some cases (ranging from 30% of the ADI for France to 260% of the ADI for Australia and New Zealand (combined)). However, the available data were insufficient to allow an estimate of the number of consumers with a high intake of BHA or the magnitude and duration of intake above the ADI.

All intake estimates, with the exception of that from Japan, assumed that BHA is the only antioxidant in foods where its use is permitted and that all such foods contain the additive at the maximum permitted levels, thus tending to overestimate the actual intake. Actual intakes of BHA will depend on the relative proportions of BHA, BHT, TBHQ and other antioxidants used in foods, on the actual levels of use according to good manufacturing practice and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Committee identified foods or food groups in the draft General Standard for Food Additives that could potentially contribute to a

high intake of BHA. These include the following categories: fats and oils and fat emulsions (type water-in-oil) (2); dried vegetables (4.2.2.2); cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (cocoa mixes (powders and syrups), cocoa-based spreads, including fillings and imitation chocolate and chocolate substitute products) (5.1.3); non-heat-treated processed comminuted meat, poultry and game products (8.3.1); frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (9.2.1); ready-to-eat soup and broths, including canned, bottled and frozen (12.5.1); and food supplements (13.6). The Codex Committee may wish to review the appropriate levels for these foods.

The Committee noted that intake estimates for BHA based on national maximum permitted levels of use were below the ADI, but exceeded the ADI when they were based on the maximum limits and range of foods specified in the draft General Standard for Food Additives. The differences arise because the range of foods specified in the General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.3 Butylated hydroxytoluene (BHT)

The Committee assessed the intake of butylated hydroxytoluene (BHT), for which maximum limits have been proposed in a wide range of solid foods in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. An ADI of 0–0.3 mg/kg of body weight was allocated to BHT at the forty-fourth meeting of the Expert Committee (Annex 1, reference *116*).

BHT is generally used as an antioxidant in products containing fats or oils. BHT can be used in conjunction with BHA, TBHQ and propyl gallate, providing a synergistic combination of antioxidants.

Information was provided by 10 Member States (Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on "poundage" data, household economic surveys or retail sales surveys, model diets, or individual dietary records (see section 2.3).

Screening of BHT using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments were required. The screening of food additives by the Codex Committee

using the budget method had identified BHT as a food additive requiring further assessment. The inclusion of the proportions of the national food supply that may contain BHT in the calculations made using the budget method did not change the conclusion that the theoretical maximum levels of use of BHT in solid foods were lower than both the national maximum permitted levels and the maximum limits specified in the draft General Standard for Food Additives.

Intake assessments

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intake estimates derived using the maximum limits specified in the draft General Standard for Food Additives will grossly overestimate the actual intakes in any country because the General Standard is generally compiled by adopting the highest level of use submitted by a Member State for any use of the food additive in a given food category. The range of uses specified in the draft General Standard for Food Additives is also much wider than in national standards. National estimates of daily per capita intake of BHT based on the maximum limits specified in the draft General Standard for Food Additives ranged from 0.70 to 0.99 mg/kg of body weight for consumers with mean intakes and from 2.0 to 6.0 mg/kg of body weight per day for consumers with high intakes.

Intake estimates based on maximum limits specified in national food standards. Intakes estimated using different methods based on national maximum permitted levels of use showed results that were relatively consistent. Estimates of daily per capita intake ranged from 0.003 to 0.11 mg/kg of body weight based on "poundage" data, from 0.052 to 0.1 mg/kg of body weight based on household economic surveys or retail sales surveys, from 0.02 to 0.09 mg/kg of body weight (except for the USA) based on model diets (for consumers with average intakes), and from 0.02 to 0.1 mg/kg of body weight based on individual dietary records.

Two exceptions were noted by the Committee. The first was that the estimates of daily per capita intake based on a model diet and the maximum permitted level of use of BHT in the USA were 0.39 and 0.78 mg/kg of body weight for mean and high levels of consumption, respectively. This result can be explained by the high maximum permitted level of use of BHT in the USA. The second exception was a study from Japan, which was based on analytical results for BHT from a market basket survey. The estimate of daily per capita intake (0.000 85 mg/kg of body weight) was lower than all the other intake estimates submitted.

Conclusions

The Committee concluded that BHT intake probably does not exceed the ADI, based on the estimated national intakes of BHT in the 10 Member States for which data were available. However, it may be possible to exceed the ADI if the proposed maximum limits specified in the draft General Standard for Food Additives are assumed.

The Committee recognized that BHT is likely to be used in conjunction with other antioxidants, such as TBHQ and BHA, which are synergistic with BHT. Consequently, the amount of BHT used in practice would be lower and it would be used in fewer foods than assumed in the estimates. All intake estimates, with the exception of that from Japan, assumed that BHT is the only antioxidant in foods where its use is permitted and that all such foods contain the additive at the maximum permitted levels. Actual intakes of BHT would depend on the relative proportions of antioxidants used in foods and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Expert Committee identified food groups in the draft General Standard for Food Additives that could potentially contribute to a high intake of BHT. These include the following categories: fats and oils and fat emulsions (type water-in-oil) (2); chewing gum (5.3); and processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2). The Codex Committee may wish to review the appropriate levels of BHT for these foods.

The Committee noted that intake estimates based on national maximum permitted levels of use for BHT were below the ADI, but exceeded the ADI when they were based on the maximum limits and range of foods specified in the draft General Standard for Food Additives. The differences arise because the range of foods specified in the General Standard for Food Additives is wider and the proposed levels of use in specific food categories are generally higher than those specified in national standards.

5.4 Sulfites

The Committee assessed the intake of sulfur dioxide and related compounds, including the following: calcium, potassium and sodium hydrogen sulfite; calcium, potassium and sodium metabisulfite; calcium, potassium and sodium sulfite; and sodium thiosulfate. These substances are used as preservatives. Maximum limits for these

substances have been proposed in a wide variety of solid foods and beverages in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. A group ADI of 0–0.7 mg/kg of body weight was allocated to sulfur dioxide and this group of related compounds at the present meeting (see section 3.5).

The sulfites are in some ways unique additives, in that the level of use typically does not reflect the level remaining in a food at the time of ingestion. This is due to losses during the processing and storage of treated foods. For example, dried vegetables, which may contain up to 5000 mg of sulfites/kg according to the draft General Standard for Food Additives, are usually rehydrated and cooked prior to ingestion, resulting in much lower levels of residual sulfite being present in the food at the time of its consumption.

Information on sulfite intake was provided by 10 Member States (Australia, China, Finland, France, India, Japan, New Zealand, Spain, the United Kingdom and the USA). A joint assessment was made by Australia and New Zealand. The intake assessments were based on "poundage" data, household economic surveys or retail sales surveys, model diets, or individual dietary records (see section 2.3).

Screening of sulfites using the budget method

Screening of sulfur dioxide and sulfites by the budget method pointed to the need for detailed assessments of their intake in solid foods and beverages.

Intake assessments

All of the national intake assessments evaluated by the Committee, except those from Japan and the USA, were based on the assumption that sulfites were used at the maximum limits specified in the draft General Standard for Food Additives, maximum levels of use specified by the European Union, or maximum limits specified in national food standards. The intake assessments from Japan and the USA were based on methods that incorporated measured levels of sulfite residues in food.

Intake estimates based on "poundage" data. The estimates of daily per capita intake of sulfites based on "poundage" data ranged from 0.07 to 1.6 mg/kg of body weight. Estimates based on "poundage" data are likely to overstate the actual per capita consumption of sulfites because of unaccounted losses during processing and storage, or household wastage of foods containing sulfites.

Intake estimates based on household economic surveys or food balance sheets. Information from household economic surveys and food

balance sheets gave estimates of daily per capita intake that ranged from 0.3 to 3 mg/kg of body weight. Since these estimates are not based on the residual levels of sulfites in foods "as consumed", they are likely to be overestimates.

Intake estimates based on model diets. Data from model diets yielded estimates of daily per capita intake that ranged from 0.03 to 30 mg/kg of body weight. The highest intake estimates, which were based on the maximum limits specified in the draft General Standard for Food Additives, were exceedingly high, but should not be considered realistic estimates of likely intake because of the losses that can be expected to occur during processing and storage of treated foods. Estimates based on national maximum permitted levels of use or measured levels of use, such as those from Japan and the USA, resulted in a much more reasonable measure of actual sulfite intake in a population.

Intakes based on individual dietary records. The data from individual dietary records produced estimates of daily per capita intake ranging from 0.3 to 15 mg/kg of body weight per day. The method, which uses national maximum permitted levels of use and the range of permitted uses, also provided the best estimates from Australia and New Zealand (combined), France and the United Kingdom.

Conclusions and recommendations to the Codex Committee on Food Additives and Contaminants

The Committee noted that all intake estimates based on national maximum levels of use for sulfites were below the ADI. However, estimates exceeded the ADI when they were based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives. The differences in intake assessments arise because the range of foods specified in the draft General Standard is wider than that in which the use of sulfites is authorized on a national basis and the proposed levels of use in specific food categories are generally higher than national maximum permitted levels.

The evaluation of the proposed maximum limits for sulfites in the draft General Standard for Food Additives, in conjunction with the data on food intake supplied by national governments, leads to the conclusion that certain foods can be identified as major contributors to overall sulfite intake. Ingestion of a 100-g portion of any food containing sulfite at a concentration of 400 mg/kg or above would result in an intake of sulfite equal to or above the ADI. The consumption of certain solid foods and some beverages can commonly lead to intakes above the ADI when the residual level of sulfites in the food approaches the maximum limit for these foods specified in the draft

General Standard for Food Additives. Such foods and beverages are included in the following categories: dried fruit (4.1.2.2); jams, jellies and marmalades (4.1.2.5); fruit preparations, including pulp and fruit toppings (4.1.2.8); dried vegetables (4.2.2.2); vegetable, nut and seed purees and spreads (4.2.2.5); white and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings (11.1); concentrates (liquid or solid) for fruit juice (14.1.2.3); wines (14.2.3); and fruit wine (14.2.4).

Some of the high maximum limits specified in the draft General Standard for Food Additives have been incorporated as a consequence of the food groupings in which one type of food in the group requiring a high level of sulfites results in the level of sulfites in the group as a whole being unrealistically high. For example, sucrose, which is included under white and semi-white sugar, requires a maximum limit of only 70mg/kg, but the group maximum limit is taken from the maximum limit for molasses of 500mg/kg. The group maximum limit for jams, jellies and marmalades of 3000mg/kg arises from a request for use in imitation fruit, but where all of the remaining levels requested are at or below 500mg/kg. The maximum limit for concentrates (liquid or solid) for fruit juice of 2000mg/kg comes from a request for use in grape juice concentrate for wine-making, whereas all of the other levels requested for fruit juice concentrates for direct use by consumers are at or below 350mg/kg. Estimates of sulfite intake by consumers of food in this group may not have taken into account either the loss in processing or dilution of the concentrates before consumption. The Codex Committee could consider separating the specific foods that require higher levels of use of sulfites in these categories in order to better differentiate specific products, resulting in lower maximum limits; this would have the potential consequence of lowering the estimated intake of sulfites.

The maximum limit specified in the draft General Standard for Food Additives for fruit preparations, including pulp and fruit toppings, of 3000mg/kg is the result of a request by only one Member State. Products of this type may not be produced using sulfites in other Member States and sulfites may not be needed. The Codex Committee could consider further examination of the need for sulfites in this type of product in order to determine an appropriate level based on good manufacturing practice, if appropriate. The above recommendations are intended to aid the Codex Committee in establishing maximum limits in the General Standard for Food Additives such that the potential for risk to consumers with a high intake of sulfites might be limited.

If intake is estimated using residual sulfite levels measured in foods at the time of consumption, as was done for the estimates from Japan and the USA, the resulting estimates would be insignificantly lower than those presented by the remaining Member States, which used various maximum permitted levels of use. The mean intakes of sulfites estimated from model diets in France, Japan and the USA based on national levels of use, as well as mean intakes from analysis of individual dietary records in the United Kingdom, suggest that the current mean intakes of sulfites worldwide are below the ADI established by the Expert Committee. Although some methods for estimating intake, notably those based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives, showed that consumers with high intakes of sulfites may exceed the ADI, the data submitted were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.

5.5 *tert*-Butylhydroquinone (TBHQ)

The Committee assessed the intake of *tert*-butylhydroquinone (TBHQ), for which maximum limits have been proposed in a wide range of food products in the draft General Standard for Food Additives being developed by the Codex Committee on Food Additives and Contaminants. The highest level of use of TBHQ specified in the draft General Standard for Food Additives is 1000 mg/kg for frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (category 9.2.1). An ADI of 0–0.7 mg/kg of body weight was allocated to TBHQ at the forty-ninth meeting of the Expert Committee (Annex 1, reference *131*).

TBHQ is generally used as an antioxidant in animal-derived food products and in fats and oils. It is often used in conjunction with BHA, BHT and propyl gallate to provide a synergistic antioxidant effect.

Information was provided by six Member States (Australia, Brazil, China, New Zealand, the United Kingdom and the USA). A combined assessment was provided by Australia and New Zealand. The intake assessments submitted were based on "poundage" data, household economic surveys or retail sales surveys, model diets, and/or individual dietary records (see section 2.3).

Screening of TBHQ using the budget method

The Committee used the budget method based on national calculations as a screening tool to identify if detailed intake assessments for TBHQ were required. The screening of food additives by the Codex Committee using the budget method had

identified TBHQ as an additive requiring further assessment. The theoretical maximum level of use of TBHQ in food was less than the national maximum permitted level of use in all of the four Member States that submitted such calculations and was also less than the highest level of use specified in the draft General Standard for Food Additives (1000 mg/kg).

Intake assessments

All the national assessments of intake of TBHQ evaluated by the Committee were based on the maximum limits specified in the draft General Standard for Food Additives, maximum limits specified by the European Union, or maximum limits specified in national food standards.

Intake estimates based on "poundage" data and household economic surveys or retail sales surveys. Estimates of per capita intake of TBHQ based on "poundage" data and household economic surveys or retail sales surveys would be expected to be lower than estimates based on actual consumption of the additive, obtained using model diets or individual dietary records. This was found to be the case for TBHQ. Estimates of daily per capita intake based on "poundage" data were available from two Member States and ranged from 0.004 mg/kg of body weight in China to 0.14 mg/kg of body weight in the USA. The estimates of daily per capita intake based on two household economic surveys in Brazil were 0.08 and 0.13 mg/kg of body weight.

Intake estimates based on model diets or individual dietary records. National estimates of mean intakes of TBHQ from four Member States based on model diets or individual dietary records ranged from 0.37 mg/kg of body weight in the USA to 0.69 mg/kg of body weight in China.

Intake estimates based on maximum limits specified in the draft General Standard for Food Additives. Intakes calculated using the maximum limits and the range of foods specified in the draft General Standard for Food Additives will generally be gross overestimates of the actual intakes in any one country. This is because the General Standard for Food Additives is generally compiled by adopting the highest level of use of TBHQ for any food category submitted by Member States and nongovernmental organizations. The range of uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards. The national daily per capita mean intake of TBHQ based on the maximum limits and range of foods specified in the draft General Standard for Food Additives was 1.2 mg/kg of body weight in Australia and New Zealand

(combined) and 0.62 mg/kg of body weight in the USA. However, for consumers with high intakes of TBHQ the estimates were 3.5 mg/kg of body weight per day in Australia and New Zealand (combined) and 1.2 mg/kg of body weight per day in the USA.

Conclusions

National intake estimates based on maximum limits specified in the draft General Standard for Food Additives. National estimates of intake based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives were available for only five Member States. The estimates of mean intake were near to or exceeded the ADI for TBHQ (ranging from 90% of the ADI for the USA to 180% of the ADI for Australia and New Zealand (combined)).

National intake estimates based on model diets or individual dietary records. All the best estimates of national mean intake of TBHQ were at or below the ADI (ranging from 50% of the ADI for the USA to 100% of the ADI for China), based on data obtained using model diets or individual dietary records.

National intake estimates based on maximum limits specified by the European Union or in national food standards. Intake estimates for consumers with high intakes of TBHQ based on maximum limits specified in national food standards exceeded the ADI in some cases (e.g. 300% for Australia and New Zealand (combined)). However, the data submitted were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.

All intake estimates assume that TBHQ is the only antioxidant present in foods in which its use is permitted and that all such foods contain the additive at maximum levels, thus tending to overestimate actual intake. The actual intakes of TBHQ will depend on the relative proportions of BHA, BHT, TBHQ and other antioxidants used in foods, on national levels of use according to good manufacturing practice, and on the proportion of foods in any one category that contain the additive.

Recommendations to the Codex Committee on Food Additives and Contaminants

The Expert Committee identified foods or food groups that potentially contribute to the high intake of TBHQ. These included the following categories: fats and oils and fat emulsions (type water-in-oil) (2); processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2); and carbonated drinks (14.1.4.1). On the basis of the assessment, the Codex Committee may wish to review

the appropriate levels of TBHQ for these categories specified in the draft General Standard for Food Additives.

The Committee noted that all national estimates of intake based on national maximum permitted levels of use for TBHQ were below the ADI. However, estimates exceeded the ADI when they were based on the maximum limits and the range of foods specified in the draft General Standard for Food Additives. The differences in intake assessments arise because the range of foods specified in the General Standard for Food Additives is wider than the range of foods in which TBHQ is authorized for use at the national level and the proposed levels of use in specific food categories are generally higher than national maximum permitted levels of use.

6. Revision of certain specifications

A total of 40 food additives were examined for specifications only (Annex 2). In general, all specifications monographs were revised in line with current policy on heavy metal contaminants (see section 2.4.6).

The existing specifications for calcium propionate were maintained.

The specifications for canthaxanthin, carnauba wax, cochineal extract, ethyl *p*-hydroxybenzoate, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, 4-hexylresorcinol, sorbitan monolaurate, potassium sorbate, calcium sorbate, polydextroses, aluminium powder, microcrystalline cellulose and sucrose esters of fatty acids were revised, with minor changes.

The specifications for ferrous gluconate were revised and designated as "tentative", with a request for information on the need for maintaining the limit test for oxalic acid and maximum limit for mercury, as well as the procedure for mercury analysis, if applicable, and for introducing maximum limits for sulfate and chloride. This information was required by 30 November 1998.

At its forty-ninth meeting (Annex 1, reference *131*), the Committee developed revised specifications for "diacetyltartaric and fatty acid esters of glycerol (DATEM)" and "tartaric, acetic and fatty acid esters of glycerol, mixed" but found it difficult to distinguish between the two substances. At that meeting, the Committee requested data that would allow these two substances to be distinguished, and stated that unless such data were provided by 31 March 1998, it would consider combining the specifications for the two substances. Since no such data were received, the Committee, at its present meeting,

decided to combine the specifications for the two substances under the name "diacetyltartaric and fatty acid esters of glycerol".

The existing monograph containing specifications for gum arabic was revised in order to distinguish clearly between gum arabic obtained from *Acacia senegal* and gum arabic obtained from *Acacia seyal*. Gums from other *Acacia* species are excluded from the revised monograph.

The existing specifications for carotenes (algae) and carotenes (vegetable) were revised in order to avoid the use of chloroform in the tests.

The existing specifications for carthamus yellow were revised to include the structural formulae of the main components, and the method of assay was improved.

The existing specifications for carob bean gum and guar gum were revised to include microbiological criteria.

The existing specifications for xanthan gum were revised in order to introduce the modified test methods for microbiological criteria in gums.

The existing specifications for nitrogen were revised and the criteria for hydrogen, carbon dioxide and moisture were deleted.

The existing specifications for talc were revised and the identification test for magnesium was replaced by a test for infrared absorption.

The existing tentative specifications for carthamus red, dichloromethane, propionic acid and petroleum jelly were revised and the "tentative" qualification was deleted.

The existing tentative specifications for shellac were revised and the "tentative" qualification was deleted. Furthermore, the title of the specifications monograph was changed to "shellac, bleached" in order to reflect better the products currently on the market for food use.

The existing tentative specifications for hexane were revised and the "tentative" designation was deleted. In order to reflect that the product defined by the monograph is a mixture of hexanes, the title was changed to "hexanes".

The existing specifications for thaumatin were revised and designated as "tentative". The Committee requested information on a specific identification test by 30 November 1998.

The existing specifications for citric acid were revised and designated as "tentative". The Committee requested information on the need for the test for oxalate and a suitable limit for this impurity by 30 March 1999.

New specifications for ferrous sulfate were prepared and designated as "tentative". The Committee requested information on the limit and an analytical method for mercury, and the need for a limit on water content. This information was required by 30 March 1999.

No specifications were prepared for sodium sorbate as no information on any commercial production was available.

The existing specifications for acetone, propan-2-ol and isobutyl alcohol (isobutanol) were revised. As these substances are used both as solvents and as flavouring agents, specifications were prepared for incorporation in the table of flavouring agents in the specifications monograph.

7. Future work

1. The two substances "diacetyltartaric and fatty acid esters of glycerol" and "tartaric, acetic and fatty acid esters of glycerol, mixed" were toxicologically evaluated at the seventeenth meeting (Annex 1, reference *32*) and were assigned different ADIs. Since the two substances are now covered by one set of specifications (see section 6), the Committee recommended that the material defined in these specifications be evaluated toxicologically.
2. The safety aspects of dietary supplementation with high levels of nutrients should be evaluated at a future meeting, preferably at the same meeting that specifications are being developed for novel forms of such nutrients.
3. Because of the increasing use of gene technology, general guidelines for food additives derived from genetically modified organisms should be developed.
4. The Committee recognized that some specifications established previously may not be adequate in the light of recently improved analytical procedures and the use of new techniques for the production of food additives. The Committee therefore endorsed the recommendation made at the forty-sixth meeting (Annex 1, reference *122*) concerning the need for the periodic updating of the *Compendium of food additive specifications* (Annex 1, reference *96*) and the *Guide to specifications* (Annex 1, reference *100*).

8. Recommendations

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role that the

recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.

2. The Committee recognized the importance of risk analysis for ensuring consistency and a sound scientific basis for food standards. It therefore recommended that FAO and WHO work with the Codex Committee on Food Additives and Contaminants, the Codex Committee on Residues of Veterinary Drugs in Foods and the Codex Committee on Pesticide Residues, together with the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues, to develop appropriate principles and procedures for risk assessment.

3. The Committee recommended that whenever chemical substances undergo toxicological evaluation, assessments of intake should be undertaken so that the risk can be characterized. When substances are referred to the Expert Committee by the Codex Committee on Food Additives and Contaminants for intake assessment, the Expert Committee should determine whether a toxicological evaluation is required.

4. To ensure timely notification of the important issues agreed upon at each meeting, the Committee recommended that draft report items dealing with issues of a general nature be included in the "Summary and conclusions" published shortly after each meeting. When a policy is developed that affects the nature of submissions for the next meeting, the Secretariat should request such information in the "Call for data" for the meeting.

5. The Committee recommended that the opinions of the Codex Committee on Nutrition and Foods for Special Uses and the Codex Committee on Food Additives and Contaminants should be sought on the need to develop specifications for vitamins and minerals for which no internationally recognized food-grade specifications exist and on priorities for the development of such specifications.

6. The Committee recognized that a number of preparations from natural sources have not yet been reviewed, although their main active components have been evaluated as food additives. The Committee recommended that such preparations that are being marketed for use in foods be evaluated.

Acknowledgements

The Committee was saddened to hear of the death of Dr J.P. Modderman, who served on the Committee and contributed greatly to its success for many years. He continued to provide support to the Committee by contributing information until his sudden death in April 1998. He will be greatly missed, both as a valued colleague and as a friend.

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Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, vol. I. *Antimicrobial preservatives and antioxidants*. Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
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5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
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11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
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13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967 (out of print).
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non-nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968 (out of print).
15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33 (out of print).
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31 (out of print).
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[1] The full text is available electronically on the Internet at http://www.who.int/pcs.

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Annex 2

Acceptable Daily Intakes, other toxicological information, and information on specifications

Substance	No.	Specifications[a]	Acceptable Daily Intakes (ADI) in mg/kg of body weight and other toxicological recommendations
Enzyme preparations			
α-Acetolactate dehydrogenase	—	R	Not specified[b]
Maltogenic amylase	—	R	Not specified[b]
Flavouring agents			
trans-Anethole	0217	S	0–2
Furfural	0450	R	No ADI allocated because insufficient data were available
Menthol	0427	R	0–4
Food colours			
Curcumin	—	R, T	0–1 (temporary)[c]
Riboflavin from genetically modified *Bacillus subtilis*	—	N	0–0.5 (group ADI with synthetic riboflavin and riboflavin-5′-phosphate)
Glazing agents			
Mineral oils (medium- and low-viscosity)		R	
Class I[d]	—		0–1 (temporary)[c]
Class II[e] and Class III[f]	—		0–0.01 (group ADI) (temporary)[c]
Preservatives			
Calcium hydrogen sulfite	—	S, T[c]	0–0.7[g]
Calcium metabisulfite	—	O	
Calcium sulfite	—	O	
Potassium hydrogen sulfite	—	O	
Potassium metabisulfite	—	R, T[c]	
Potassium sulfite	—	R, T[c]	
Sodium hydrogen sulfite	—	R, T[c]	
Sodium metabisulfite	—	R, T[c]	
Sodium sulfite	—	R, T[c]	
Sodium thiosulfate	—	R, T[c]	
Sulfur dioxide	—	R	
Sweetening agent			
Stevioside	—	O	No ADI allocated[c,h]

Substance	No.	Specifications[a]	Acceptable Daily Intakes (ADI) in mg/kg of body weight and other toxicological recommendations
Thickening agents			
Carrageenan	—	R	Not specified (group ADI) (temporary)[b,c]
Processed *Eucheuma* seaweed	—	R	
Sodium carboxymethyl cellulose, enzymatically hydrolysed	—	R	Not specified (group ADI)[b,i]
Miscellaneous substances			
γ-Cyclodextrin	—	N	Not specified (temporary)[b,c]
Glucono-δ-lactone	—	R	Not specified (group ADI)[b]
Calcium gluconate	—	R	
Magnesium gluconate	—	R, T[c]	
Potassium gluconate	—	R	
Sodium gluconate	—	R	
Polyglycitol syrup	—	N	Not specified (group ADI)[b,j]

[a] N, New specifications prepared; O, no specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not requested; T, the existing, new or revised specifications are tentative and comments are invited.

[b] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[c] See Annex 3.

[d] Including P70(H) oil.

[e] Including N70(H) and N70(A) oils.

[f] Including P15(H), N15(H) and N10(A) oils.

[g] The Committee reiterated its recommendation made at its thirtieth meeting (WHO Technical Report Series, No. 751, 1987) that, where a suitable alternative method of preservation exists, its use should be encouraged, particularly in those applications (e.g. control of enzymatic browning in fresh salad vegetables) in which the use of sulfites may lead to high levels of acute intake and which have been most commonly associated with life-threatening adverse reactions. Appropriate labelling would help to alert individuals who cannot tolerate sulfites.

[h] No ADI was allocated because insufficient data were available and specifications were not prepared.

[i] Included in the group ADI with ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, methyl ethyl cellulose and sodium carboxymethyl cellulose.

[j] Group ADI for materials conforming to the specifications for polyglycitol syrup and maltitol syrup.

Flavouring agents

The substances listed here were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents. For further details, see section 2.2.1 of the main report.

Substance[a]	No.	Specifications[b]	Conclusion based on current intake
Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters			
Structural class I			
Acetone (propan-2-one)	0139	R	No safety concern
Isopropyl alcohol (2-propanol)	0277	N	
2-Butanone (ethyl methyl ketone)	0278	N	
2-Pentanone	0279	N	
2-Pentanol	0280	N	
3-Hexanone	0281	N	
3-Hexanol	0282	N	
2-Heptanol	0284	N	
3-Heptanol	0286	N	
4-Heptanone	0287	N	
2-Octanol	0289	N	
3-Octanol	0291	N	
2-Nonanol	0293	N	
3-Decanol	0295	N	
2-Undecanol	0297	N	
3-Methyl-2-butanol	0300	N, T	
2,6-Dimethyl-4-heptanol	0303	N	
Isopropyl formate	0304	N,T	
Isopropyl acetate	0305	R	
Isopropyl propionate	0306	N, T	
Isopropyl butyrate (isopropyl butanoate)	0307	N	
Isopropyl hexanoate	0308	N, T	
Isopropyl isobutyrate (isopropyl 2-methylpropanoate)	0309	N	
Isopropyl isovalerate (isopropyl 3-methylbutanoate)	0310	N	
Isopropyl myristate (isopropyl tetradecanoate)	0311	N	
Isopropyl tiglate (isopropyl 2-methyl-2-butenoate)	0312	N	
3-Octyl acetate	0313	N	
1-Ethylhexyl tiglate (octen-3-yl 2-methyl-2-butenoate)	0448	N	
Structural class II			
2-Heptanone	0283	N	No safety concern
3-Heptanone	0285	N	
2-Octanone	0288	N	
3-Octanone	0290	N	
2-Nonanone	0292	N	
3-Nonanone	0294	N	

Substance[a]	No.	Specifications[b]	Conclusion based on current intake
Saturated aliphatic acyclic secondary alcohols, ketones and related saturated and unsaturated esters (*continued*)			
Structural class II (*continued*)			
2-Undecanone	0296	N	No safety concern
2-Tridecanone	0298	N	
2-Pentadecanone	0299	N, T	
4-Methyl-2-pentanone	0301	N	
2,6-Dimethyl-4-heptanone	0302	N	
Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters			
4-Pentenoic acid	0314	N	No safety concern
cis-3-Hexen-1-ol	0315	N	
cis-3-Hexenal	0316	N, T	
3-Hexenoic acid	0317	N, T	
4-Hexen-1-ol	0318	N	
cis-4-Hexenal	0319	N, T	
4-Heptenal	0320	N	
cis-3-Octen-1-ol	0321	N	
cis-5-Octen-1-ol	0322	N	
cis-5-Octenal	0323	N	
cis-6-Nonen-1-ol	0324	N	
cis-6-Nonenal	0325	N	
4-Decenal	0326	N	
Mixture of 5- and 6-decenoic acid	0327	N, T	
9-Decenoic acid	0328	N	
9-Undecenal	0329	N, T	
10-Undecenal	0330	N	
10-Undecenoic acid	0331	N	
Linoleic acid	0332	N	
Oleic acid[c]	0333	N, T	
Methyl 3-hexenoate	0334	N, T	
Ethyl 3-hexenoate	0335	N	
cis-3-Hexenyl *cis*-3-hexenoate	0336	N	
Methyl *cis*-4-octenoate	0337	N, T	
Ethyl *cis*-4-octenoate	0338	N, T	
Ethyl *cis*-4,7-octadienoate	0339	N, T	
Methyl 3-nonenoate	0340	N, T	
Ethyl *trans*-4-decenoate	0341	N, T	
Methyl 9-undecenoate	0342	N, T	
Ethyl 10-undecenoate	0343	N	
Butyl 10-undecenoate	0344	N, T	
Ethyl oleate	0345	N	
Mixture of methyl linoleate and methyl linolenate	0346	N	
2-Methyl-3-pentenoic acid	0347	N, T	
2,6-Dimethyl-6-hepten-1-ol	0348	N, T	
2,6-Dimethyl-5-heptenal	0349	N	
Ethyl 2-methyl-3-pentenoate	0350	N, T	

Substance[a]	No.	Specifications[b]	Conclusion based on current intake
Linear and branched-chain aliphatic unsaturated, unconjugated alcohols, aldehydes, acids and related esters (*continued*)			
Ethyl 2-methyl-4-pentenoate	0351	N	No safety concern
Mixture of hexyl 2-methyl-3- and hexyl 2-methyl-4-pentenoate	0352	N, T	
Ethyl 2-methyl-3,4-pentadienoate	0353	N, T	Evaluation deferred[d]
Methyl 3,7-dimethyl-6-octenoate	0354	N	No safety concern
2-Methyl-4-pentenoic acid	0355	N	
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances			
Structural class I			
Linalool[e]	0356	R	No safety concern
Tetrahydrolinalool	0357	N	
Linalyl formate	0358	N	
Linalyl acetate[e]	0359	R	
Linalyl propionate	0360	N	
Linalyl butyrate	0361	N	
Linalyl isobutyrate	0362	N	
Linalyl isovalerate	0363	N	
Linalyl hexanoate	0364	N	
Linalyl octanoate	0365	N, T	
α-Terpineol	0366	N	
Terpinyl formate	0367	N	
Terpinyl acetate	0368	N	
Terpinyl propionate	0369	N	
Terpinyl butyrate	0370	N, T	
Terpinyl isobutyrate	0371	N, T	
Terpinyl isovalerate	0372	N, T	
p-Menth-3-en-1-ol	0373	N, T	
4-Carvomenthenol	0439	N	
p-Menth-8-en-1-ol (β-terpineol)	0374	N, T	
4-Thujanol	0441	N, T	
Methyl 1-acetoxycyclohexylketone	0442	N, T	Not evaluated[f]
Structural class II			
2-Ethyl-1,3,3-trimethyl-2-norbananol	0440	N, T	No safety concern
Carvone and structurally related substances			
Structural class I			
p-Menthan-2-ol	0376	N, T	No safety concern
Dihydrocarveol	0378	N	
Dihydrocarvyl acetate	0379	N	
Carveol	0381	N	
Carvyl acetate	0382	N	
Carvyl propionate	0383	N, T	

Substance[a]	No.	Specifications[b]	Conclusion based on current intake
Carvone and structurally related substances (*continued*)			
Structural class II			
p-Menthan-2-one	0375	N	No safety concern
Dihydrocarvone	0377	N, T	
(+)-Carvone[g]	0380a	R	
(–)-Carvone	0380b	R	
Ionones and structurally related substances			
β-Damascone	0384	N	No safety concern
α-Damascone	0385	N	
δ-Damascone	0386	N	
Damascenone	0387	N	
α-Ionone[h]	0388	R	
β-Ionone[h]	0389	R	
γ-Ionone	0390	N, T	
α-Ionol	0391	N	
β-Ionol	0392	N, T	
Dihydro-α-ionone	0393	N	
Dihydro-β-ionone	0394	N, T	
Dihydro-β-ionol	0395	N	
Dehydrodihydroionone	0396	N, T	
Dehydrodihydroionol	0397	N, T	
Methyl-α-ionone	0398	N, T	
Methyl-β-ionone	0399	N	
Methyl-δ-ionone	0400	N, T	
Allyl-α-ionone	0401	N	
1,4-Dimethyl-4-acetyl-1-cyclohexene	0402	N, T	Not evaluated[i]
α-Irone	0403	N	No safety concern
α-Iso-methylionone	0404	N	
Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones			
Acetoin (3-hydroxy-2-butanone)	0405	N	No safety concern
2-Acetoxy-3-butanone	0406	N, T	
Butan-3-one-2-yl butanoate	0407	N, T	
Diacetyl (2,3-butanedione)	0408	N	
3-Hydroxy-2-pentanone	0409	N, T	
2,3-Pentanedione	0410	N	
4-Methyl-2,3-pentanedione	0411	N, T	
2,3-Hexanedione	0412	N	
3,4-Hexanedione	0413	N	
5-Methyl-2,3-hexanedione	0414	N, T	
2,3-Heptanedione	0415	N	
5-Hydroxy-4-octanone	0416	N, T	
2,3-Undecadione	0417	N, T	
Methylcyclopentenolone	0418	N, T	
Ethylcyclopentenolone	0419	N	
3,4-Dimethyl-1,2-cyclopentanedione	0420	N	
3,5-Dimethyl-1,2-cyclopentanedione	0421	N, T	

Substance[a]	No.	Specifications[b]	Conclusion based on current intake
Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones (*continued*)			
3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	0422	N	No safety concern
5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	0423	N	
2-Hydroxy-2-cyclohexen-1-one	0424	N, T	
1-Methyl-2,3-cyclohexadione	0425	N	
2-Hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one	0426	N	
Menthol and structurally related substances			
Structural class I			
Menthol[j]	0427	R	No safety concern
(+)-neo-Menthol	0428	N, T	
Menthyl acetate	0431	N	
Menthyl isovalerate	0432	N	
(–)-Menthyl lactate	0433	N	
p-Menth-1-en-3-ol	0434	N, T	
(–)-Menthol ethylene glycol carbonate	0443	N	
Mixture of (–)-menthol 1- and 2-propylene glycol carbonate	0444	N	
mono-Menthyl succinate	0447	N	
Structural class II			
Menthone	0429	N	No safety concern
(±)-Isomenthone	0430	N	
Piperitone	0435	N	
(–)-Menthone 1,2-glycerone ketal	0445	N	
(±)-Menthone 1,2-glycerol ketal	0446	N	

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 6, 1998). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

[b] N, new specifications prepared; R, existing specifications revised; T, the existing, new or revised specifications are tentative and comments are invited.

[c] The ADI "not specified" for the calcium, potassium and sodium salts of oleic acid established at the thirty-third meeting of the Committee (WHO Technical Report Series, No. 776, 1989) was maintained.

[d] The evaluation of this substance was deferred, pending review of a 90-day toxicity study that was not available at the meeting.

[e] The group ADI of 0–0.5 mg/kg of body weight established at the twenty-third meeting (WHO Technical Report Series, No. 648, 1980) for citral, geranyl acetate, citronellol, linalool and linalyl acetate, expressed as citral, was maintained.

[f] Methyl 1-acetoxycyclohexylketone was not evaluated because a NOEL for this or a related substance was not available and the per capita intake exceeds 1.5 µg per day.

[g] The ADI of 0–1 mg/kg of body weight established at the thirty-seventh meeting of the Committee (WHO Technical Report Series, No. 806, 1991, and corrigenda), was maintained.

[h] The group ADI of 0–0.1 mg/kg of body weight established at the twenty-eighth meeting of the Committee (WHO Technical Report Series, No. 710, 1984) for α-ionone and β-ionone, was maintained.

[i] 1-4-Dimethyl-4-acetyl-1-cyclohexane was not considered to have sufficient structural similarities to the ionones to be included in this group. Accordingly, the safety evaluation of this compound was not considered at the present meeting.

[j] An ADI of 0–4 mg/kg of body weight was established for menthol at the present meeting (see section 3.2.3).

Substance[a] (considered for specifications only)	No.	Specifications[b]
Flavouring agents		
Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids[c]		
Isobutyl alcohol (2-methyl-1-propanol)	0251	R
Isobutyraldehyde (2-methylpropanal)	0252	N
Isobutyric acid (2-methylpropanoic acid)	0253	N
2-Methylbutyraldehyde (2-methylbutanal)	0254	N
2-Methylbutyric acid (2-methylbutanoic acid)	0255	N
2-Ethylbutyraldehyde (2-ethylbutanal)	0256	N
2-Ethylbutyric acid (2-ethylbutanoic acid)	0257	N
3-Methylbutyraldehyde (3-methylbutanal)	0258	N
Isovaleric acid (3-methylbutanoic acid)	0259	N
2-Methylpentanal	0260	N, T
2-Methylvaleric acid (2-methylpentanoic acid)	0261	N
3-Methylpentanoic acid	0262	N
3-Methyl-1-pentanol	0263	N
4-Methylpentanoic acid	0264	N
2-Methylhexanoic acid	0265	N
5-Methylhexanoic acid	0266	N
2-Ethyl-1-hexanol	0267	N
3,5,5-Trimethyl-1-hexanol	0268	N
3,5,5-Trimethylhexanal	0269	N
2-Methyloctanal	0270	N, T
4-Methyloctanoic acid	0271	N, T
3,7-Dimethyl-1-octanol	0272	N
2,6-Dimethyloctanal	0273	N, T
4-Methylnonanoic acid	0274	N
2-Methylundecanal	0275	N
Aliphatic lactones[c]		
4-Hydroxybutyric acid lactone (γ-butyrolactone)	0219	N
γ-Valerolactone	0220	N
4-Hydroxy-3-pentenoic acid lactone	0221	N
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	0222	N
γ-Hexalactone	0223	N
δ-Hexalactone	0224	N
γ-Heptalactone	0225	N
γ-Octalactone	0226	N
6-Hydroxy-3,7-dimethyloctanoic acid lactone	0237	N
δ-Tetradecalactone	0238	N
ω-Pentadecalactone	0239	N, T
ω-6-Hexadecenlactone	0240	N
ε-Decalactone	0241	N, T
ε-Dodecalactone	0242	N
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	0243	N
3-Heptyldihydro-5-methyl-2(3*H*)-furanone	0244	N, T
5-Hydroxy-2,4-decadienoic acid δ-lactone	0245	N
4,4-Dibutyl-γ-butyrolactone	0227	N
δ-Octalactone	0228	N

Substance[a] (considered for specifications only)	No.	Specifications[b]
Flavouring agents (*continued*)		
Aliphatic lactones[c] (*continued*)		
γ-Nonalactone	0229	R
Hydroxynonanoic acid δ-lactone	0230	N
γ-Decalactone	0231	N
δ-Decalactone	0232	N
γ-Undecalactone	0233	R
5-Hydroxyundecanoic acid δ-lactone	0234	N
γ-Dodecalactone	0235	N
δ-Dodecalactone	0236	N
5-Hydroxy-2-decenoic acid δ-lactone	0246	N
5-Hydroxy-7-decenoic acid δ-lactone	0247	N, T
5-Hydroxy-8-undecenoic acid δ-lactone	0248	N, T
cis-4-Hydroxy-6-dodecenoic acid lactone (1,4-dodec-6-enolactone)	0249	N, T
γ-Methyldecalactone	0250	N
Mixture of 5-Hydroxy-2-decenoic acid δ-lactone, 5-hydroxy-2-dodecenoic acid δ-lactone and 5-hydroxy-2-tetradecenoic acid δ-lactone	0276	O
4-Hydroxy-3-methyloctanoic acid γ-lactone	0437	N
5-Hydroxy-2-dodecenoic acid δ-lactone	0438	N, T
Food additives		
Acetone (extraction solvent)	—	R
Aluminium powder	—	R
Calcium propionate	—	S
Calcium sorbate	—	R
Canthaxanthin	—	R
Carnauba wax	—	R
Carob bean gum	—	R
Carotenes (algae)	—	R
Carotenes (vegetable)	—	R
Carthamus red	—	R
Carthamus yellow	—	R
Citric acid	—	R, T[d]
Cochineal extract	—	R
Diacetyltartaric and fatty acid esters of glycerol (DATEM)	—	R
Dichloromethane	—	R
Ethyl *p*-hydroxybenzoate	—	R
Ferrous gluconate	—	R, T[d]
Ferrous sulfate	—	N, T[d]
Furfuryl alcohol (flavouring agent)	0451	N
Guar gum	—	R
Gum arabic	—	R
Hexanes	—	R
4-Hexylresorcinol	—	R
Isobutyl alcohol (isobutanol) (extraction solvent)	—	R
Methyl *p*-hydroxybenzoate	—	R

Substance[a] (considered for specifications only)	No.	Specifications[b]
Food additives (*continued*)		
Microcrystalline cellulose	—	R
Nitrogen	—	R
Petroleum jelly	—	R
Polydextroses	—	R
Potassium sorbate	—	R
Propan-2-ol	—	R
Propionic acid	—	R
Propyl *p*-hydroxybenzoate	—	R
Shellac, bleached	—	R
Sodium sorbate	—	O
Sorbitan monolaurate	—	R
Sucrose esters of fatty acids	—	R
Talc	—	R
Tartaric, acetic and fatty acid esters of glycerol, mixed	—	W[e]
Thaumatin	—	R, T[d]
Xanthan gum	—	R

[a] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 6, 1998). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

[b] N, new specifications prepared; O, no specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not required; T, the existing, new or revised specifications are tentative and comments are invited; W, existing specifications withdrawn.

[c] These substances were evaluated at the forty-ninth meeting of the Committee (WHO Technical Report Series, No. 884, 1998).

[d] See Annex 3.

[e] At its forty-ninth meeting (WHO Technical Report Series, No. 884, 1998), the Committee developed revised specifications for "diacetyltartaric and fatty acid esters of glycerol" and "tartaric, acetic and fatty acid esters of glycerol, mixed" but found it difficult to distinguish between the two substances. At that meeting, the Committee requested data that would allow these two substances to be distinguished, and stated that unless such data were provided by 31 March 1998, it would consider combining the specifications for the two substances. Since no data were received, the Committee, at its present meeting, decided to combine the specifications for the two substances under the name "diacetyltartaric and fatty acid esters of glycerol".

Food additives considered for evaluation of national intake assessments

Substance	Conclusions	Recommendations to the Codex Committee on Food Additives and Contaminants
Benzoates	Intake estimates based on levels specified in the draft General Standard for Food Additives[a] and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–5 mg/kg of body weight in the five Member States[b] that submitted such data. In national data submitted by nine Member States,[b] estimates of mean intake of benzoates did not exceed the ADI. The potential exists for consumers with high intakes to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — ripened cheese (1.6.2) — processed cheese (1.6.4) — cheese analogues (1.6.5) — vegetables in vinegar, oil or brine (4.2.2.3) — cooked molluscs, crustaceans and echinoderms (9.2.4.2) — semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms (9.3) — liquid egg products (10.2.1) — carbonated drinks (14.1.4.1)
BHA	Intake estimates based on levels in the draft General Standard for Food Additives[a] and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.5 mg/kg of body weight for mean intake in the three Member States[b] that submitted such data. In national data submitted by seven Member States,[b] estimates of mean intake by consumers of BHA did not exceed the ADI. The potential exists for consumers of high levels of BHA to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — dried vegetables (4.2.2.2) — cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (cocoa mixes (powders and syrups), cocoa-based spreads, including fillings, and imitation chocolate and chocolate substitute products) (5.1.3) — non-heat-treated processed comminuted meat, poultry and game products (8.3.1) — frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms (9.2.1) — ready-to-eat soup and broths, including canned, bottled and frozen (12.5.1) — food supplements (13.6)

Food additives considered for evaluation of national intake assessments (*continued*)

Substance	Conclusions	Recommendations to the Codex Committee on Food Additives and Contaminants
BHT	Intake estimates based on levels in the draft General Standard for Food Additives[a] and the range of foods in which use is allowed integrated with data on national food consumption exceeded the ADI of 0–0.3 mg/kg of body weight for mean intake in the five Member States[b] that submitted such data. In national data submitted by eight Member States,[b] estimates of mean and high intake for consumers of BHT exceeded the ADI in only one country.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — chewing gum (5.3) — processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2)
Sulfites	Intake estimates based on levels in the draft General Standard for Food Additives[a] and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.7 mg/kg of body weight for mean intake in the three Member States[b] that submitted such data. In national data submitted by six Member States,[b] estimates of mean intake for consumers of sulfites did not exceed the ADI. The potential exists for consumers of high levels of sulfites to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — dried fruit (4.1.2.2) — jams, jellies and marmalades (4.1.2.5) — fruit preparations, including pulp and fruit toppings (4.1.2.8) — dried vegetables (4.2.2.2) — vegetable, nut and seed purees and spreads (4.2.2.5) — white and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings (11.1) — concentrates (liquid or solid) for fruit juice (14.1.2.3) — wines (14.2.3) — fruit wine (14.2.4)

TBHQ	Intake estimates based on levels in the draft General Standard for Food Additives[a] and the range of foods in which use is allowed integrated with national data on food consumption exceeded the ADI of 0–0.7 mg/kg of body weight for mean intake for the three Member States[b] that submitted such data. In national data submitted by four Member States,[b] estimates of mean intake by consumers of TBHQ did not exceed the ADI. The potential exists for consumers of high levels of TBHQ to exceed the ADI, but the available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake above the ADI.	Review additive levels specified in the draft General Standard for Food Additives in the following categories: — fats and oils and fat emulsions (type water-in-oil) (2) — processed fish and fish products, including molluscs, crustaceans and echinoderms (9.2) — carbonated drinks (14.1.4.1)

BHA: Butylated hydroxyanisole; BHT: butylated hydroxytoluene; TBHQ: *tert*-butyl-hydroquinone.

[a] Intake estimates calculated using the additive levels specified in the draft General Standard for Food Additives integrated with national data on food consumption will greatly overestimate actual intakes in any one country because the levels specified in the General Standard are generally compiled by adopting the highest level of use for any one food category submitted by Member States or nongovernmental organizations. The range of food uses specified in the draft General Standard for Food Additives is also usually much wider than in national standards.

[b] Eleven Member States made submissions: Australia, Brazil, China, Finland, France, India, Japan, New Zealand, Spain, the United Kingdom and the USA. Australia and New Zealand made a joint submission.

Annex 3
Further toxicological studies and other information required or desired

Flavouring agent

Furfural

Before reviewing this substance again, the Committee would wish to have the following:

1. The results of studies of DNA binding or adduct formation *in vivo* to clarify whether furfural interacts with DNA in the liver of mice.
2. The results of a 90-day study of toxicity in rats to identify a NOEL for hepatotoxicity.

Food colour

Curcumin

The results of a reproductive toxicity study on a substance complying with the specifications for curcumin and information on the need and technological justification for alternative solvents for use in the current manufacturing processes of curcumin are required for evaluation in 2001.

Glazing agents

Mineral oils (medium- and low-viscosity)

Information requested at the forty-fourth meeting of the Committee is required for evaluation in 2002. This includes information about the compositional factors in mineral oils that influence their absorption and toxicity and a study of at least 1 year's duration of one of these materials in F344 rats, including an assessment of immune function at appropriate time periods (with a reversal period of 1 year) and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached. In addition, research on the pharmacokinetics of white mineral oils and their potential effects on immune function known to be in progress should be submitted for review at that time.

Preservatives

Calcium hydrogen sulfite

Information is required on a modified name for the compound that would be more reflective of its physical properties, the extent and functionality of its use as a food additive, and the need for limits for

selenium and arsenic and associated analytical methods. The information is required by 30 November 1998.

Potassium metabisulfite, potassium sulfite, sodium hydrogen sulfite and sodium metabisulfite

Information on iron and selenium in commercial products and on the test method for selenium is required by 30 November 1998. The existing specifications include a limit for selenium, for which the test method is no longer feasible because of a lack of availability of required reagents. The Committee also questioned the need for a limit for selenium because new processes by which sulfur is produced as a by-product of the oil industry have replaced extraction methods from sulfur mines. The Committee noted that the limit for selenium may no longer be necessary and requested information on this point.

Sodium sulfite

Information on the sources of raw materials, on the commercial use of sodium sulfite heptahydrate in food, on iron and selenium levels in commercial products, and on test methods for selenium is required by 30 November 1998.

Sodium thiosulfate

Information on iron and selenium levels in commercial products and on test methods for lead and selenium is required by 30 November 1998.

Sweetening agents

Stevioside

Before reviewing this substance again, the Committee considered that it would be necessary to develop specifications to ensure that the material tested was representative of the commercial product. Further information on the nature of the substance that was tested, data on the metabolism of stevioside in humans and the results of suitable *in vivo* genotoxicity studies with steviol would also be necessary.

Thaumatin

Information on a specific identification test is required by 30 November 1998.

Thickening agents

Carrageenan and processed Eucheuma seaweed

Information to permit clarification of the significance of the promotion of colon cancer observed in studies in rats is required for evaluation in 2001.

Miscellaneous substances

Citric acid

Information on the need for a test for oxalate and a suitable limit is required by 30 March 1999. Comments are also invited on the new test for sulfate. If no information is forthcoming, the Committee will consider retaining the specifications as they appear in the specifications monograph.

γ-Cyclodextrin

The study of human tolerance known to have been conducted should be reviewed in order to confirm the absence of adverse effects on the gastrointestinal tract at normal levels of intake. These data should be submitted for consideration by the Committee by 1999.

Ferrous gluconate

Information on the need for maintaining the limit test for oxalic acid and the maximum limit for mercury, as well as the procedure for mercury analysis, if applicable, and for introducing maximum limits for sulfate and chloride is required by 30 November 1998.

Ferrous sulfate

Information on the limit and an analytical method for mercury and the need for a limit on water content is required by 30 November 1998.

Magnesium gluconate

Information on the need for maintaining the microbiological criteria included in the specifications, and for introducing maximum limits for sulfate and chloride is required by 30 November 1998.

Annex 4
Food categorization system for the General Standard for Food Additives[1]

1 Dairy products, excluding products of category 2
- 1.1 Milk and dairy-based drinks
  - 1.1.1 Milk and buttermilk
    - 1.1.1.1 Milk, including sterilized and UHT goats' milk
    - 1.1.1.2 Buttermilk (plain)
  - 1.1.2 Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, egg-nog)
- 1.2 Fermented and renneted milk products (plain), excluding drinks
  - 1.2.1 Fermented milks (plain)
    - 1.2.1.1 Fermented milks (plain), not heat-treated after fermentation
    - 1.2.1.2 Fermented milks (plain), heat-treated after fermentation
  - 1.2.2 Renneted milk
- 1.3 Condensed milk (plain) and analogues
  - 1.3.1 Condensed milk (plain)
  - 1.3.2 Beverage whiteners
- 1.4 Cream (plain) and similar products
  - 1.4.1 Pasteurized cream
  - 1.4.2 Sterilized, UHT, whipping or whipped and reduced-fat creams
  - 1.4.3 Clotted cream
  - 1.4.4 Cream analogues
- 1.5 Milk and cream powders (plain)
  - 1.5.1 Milk and cream powders
  - 1.5.2 Milk and cream powder analogues
- 1.6 Cheese
  - 1.6.1 Unripened cheese
  - 1.6.2 Ripened cheese
    - 1.6.2.1 Total ripened cheese, including rind
    - 1.6.2.2 Rind of ripened cheese
    - 1.6.2.3 Cheese powder (for reconstitution, e.g. for cheese sauces)
  - 1.6.3 Whey cheese
  - 1.6.4 Processed cheese
  - 1.6.5 Cheese analogues
- 1.7 Dairy-based desserts (e.g. ice cream, ice milk, pudding, fruit or flavoured yoghurt)
- 1.8 Whey and whey products, excluding whey cheese

2 Fats and oils and fat emulsions (type water-in-oil)
- 2.1 Fats and oils essentially free from water
  - 2.1.1 Butter oil, anhydrous milk fat, ghee
  - 2.1.2 Vegetable oils and fats

[1] Reproduced, with minor editorial corrections, from *Draft Codex General Standard for Food Additives* (Rome, Food and Agriculture Organization of the United Nations, 1999; unpublished document FX/FAC 99/6) by permission of the publisher.

2.1.3 Lard, tallow, fish oil and other animal fats
2.2 Fat emulsions mainly of type water-in-oil
2.2.1 Emulsions containing at least 80% fat
2.2.1.1 Butter and concentrated butter
2.2.1.2 Margarine and similar products (e.g. butter–margarine blends)
2.2.2 Emulsions containing less than 80% fat (e.g. minarine)
2.3 Fat emulsions other than 2.2, including mixed and/or flavoured products based on fat emulsions
2.4 Fat-based desserts (excluding dairy-based desserts)

3 Edible ices, including sherbet and sorbet

4 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes) and nuts and seeds
4.1 Fruit
4.1.1 Fresh fruit
4.1.1.1 Untreated fruit
4.1.1.2 Surface-treated fruit
4.1.1.3 Peeled or cut fruit
4.1.2 Processed fruit
4.1.2.1 Frozen fruit
4.1.2.2 Dried fruit
4.1.2.3 Fruit in vinegar, oil or brine
4.1.2.4 Canned or bottled (pasteurized) fruit
4.1.2.5 Jams, jellies and marmalades
4.1.2.6 Fruit-based spreads other than 4.1.2.5 (e.g. chutney)
4.1.2.7 Candied fruit
4.1.2.8 Fruit preparations, including pulp and fruit toppings
4.1.2.9 Fruit-based desserts, including fruit-flavoured water-based desserts
4.1.2.10 Fermented fruit products
4.1.2.11 Fruit fillings for pastries
4.1.2.12 Cooked or fried fruit
4.2 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes) and nuts and seeds
4.2.1 Fresh vegetables
4.2.1.1 Untreated vegetables
4.2.1.2 Surface-treated vegetables
4.2.1.3 Peeled or cut vegetables
4.2.2 Processed vegetables and nuts and seeds
4.2.2.1 Frozen vegetables
4.2.2.2 Dried vegetables
4.2.2.3 Vegetables in vinegar, oil or brine
4.2.2.4 Canned or bottled (pasteurized) vegetables
4.2.2.5 Vegetable, nut and seed purees and spreads (e.g. peanut butter)
4.2.2.6 Vegetable, nut and seed pulps and preparations other than 4.2.2.5
4.2.2.7 Fermented vegetable products
4.2.2.8 Cooked or fried vegetables

5 Confectionery
- 5.1 Cocoa products and chocolate products, including imitations and chocolate substitutes
  - 5.1.1 Cocoa mixes (powders and syrups)
  - 5.1.2 Cocoa-based spreads, including fillings
  - 5.1.3 Cocoa and chocolate products other than 5.1.1, 5.1.2 and 5.1.4 (e.g. milk chocolate bars, chocolate flakes, white chocolate)
  - 5.1.4 Imitation chocolate and chocolate substitute products
- 5.2 Sugar-based confectionery other than 5.1, 5.3 and 5.4, including hard and soft candy and nougats
- 5.3 Chewing gum
- 5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces

6 Cereals and cereal products, including flours and starches from roots and tubers, and pulses and legumes, excluding bakery wares
- 6.1 Whole, broken or flaked grain, including rice
- 6.2 Flours and starches
- 6.3 Breakfast cereals, including rolled oats
- 6.4 Pastas and noodles
- 6.5 Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)
- 6.6 Batters (e.g. for fish or poultry)

7 Bakery wares
- 7.1 Bread and ordinary bakery wares
  - 7.1.1 Breads and rolls
  - 7.1.2 Crackers, excluding sweet crackers
  - 7.1.3 Other ordinary bakery products (e.g. bagels, pitta, English muffins)
  - 7.1.4 Bread-type products, including bread stuffing and breadcrumbs
- 7.2 Fine bakery wares
  - 7.2.1 Cakes, cookies and pies (e.g. fruit-filled or custard types)
  - 7.2.2 Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)
  - 7.2.3 Mixes for fine bakery wares (e.g. cakes, pancakes)

8 Meat and meat products, including poultry and game
- 8.1 Fresh meat, poultry and game
  - 8.1.1 Fresh meat, poultry and game, whole pieces or cuts
  - 8.1.2 Fresh meat, poultry and game, comminuted
- 8.2 Processed meat, poultry and game products in whole pieces or cuts
  - 8.2.1 Non-heat-treated processed meat, poultry and game products in whole pieces or cuts
    - 8.2.1.1 Cured (including salted) non-heat-treated processed meat, poultry and game products in whole pieces or cuts
    - 8.2.1.2 Cured (including salted) and dried non-heat-treated processed meat, poultry and game products in whole pieces or cuts
    - 8.2.1.3 Fermented non-heat-treated processed meat, poultry and game products in whole pieces or cuts

8.2.2 Heat-treated processed meat, poultry and game products in whole pieces or cuts
8.2.3 Frozen processed meat, poultry and game products in whole pieces or cuts
8.3 Processed comminuted meat, poultry and game products
8.3.1 Non-heat-treated processed comminuted meat, poultry and game products
8.3.1.1 Cured (including salted) non-heat-treated processed comminuted meat, poultry and game products
8.3.1.2 Cured (including salted) and dried non-heat-treated processed comminuted meat, poultry and game products
8.3.1.3 Fermented non-heat-treated processed comminuted meat, poultry and game products
8.3.2 Heat-treated processed comminuted meat, poultry and game products
8.3.3 Frozen processed comminuted meat, poultry and game products
8.4 Edible casings (e.g. sausage casings)

9 Fish and fish products, including molluscs, crustaceans and echinoderms
9.1 Fresh fish and fish products, including molluscs, crustaceans and echinoderms
9.1.1 Fresh fish
9.1.2 Fresh molluscs, crustaceans and echinoderms
9.2 Processed fish and fish products, including molluscs, crustaceans and echinoderms
9.2.1 Frozen fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms
9.2.2 Frozen battered fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms
9.2.3 Frozen minced and creamed fish products, including molluscs, crustaceans and echinoderms
9.2.4 Cooked and/or fried fish and fish products, including molluscs, crustaceans and echinoderms
9.2.4.1 Cooked fish
9.2.4.2 Cooked molluscs, crustaceans and echinoderms
9.2.4.3 Fried fish and fish products, including molluscs, crustaceans and echinoderms
9.2.5 Smoked, dried, fermented and/or salted fish and fish products, including molluscs, crustaceans and echinoderms
9.3 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms
9.3.1 Fish and fish products, including molluscs, crustaceans and echinoderms, marinated and/or in jelly
9.3.2 Fish and fish products, including molluscs, crustaceans and echinoderms, pickled and/or in brine
9.3.3 Salmon substitutes, caviar and other fish roe products
9.3.4 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms other than 9.3.1–9.3.3
9.4 Fully preserved (including canned or fermented) fish and fish products, including molluscs, crustaceans and echinoderms

10 Eggs and egg products
10.1 Fresh eggs

10.2 Egg products
10.2.1 Liquid egg products
10.2.2 Frozen egg products
10.2.3 Dried and/or heat-coagulated egg products
10.3 Preserved eggs, including alkaline, salted and canned eggs
10.4 Egg-based desserts (e.g. custard)

11 Sweeteners, including honey
11.1 White and semi-white sugar (sucrose or saccharose), fructose, glucose (dextrose), xylose, sugar solutions and syrups, and (partially) inverted sugars, including molasses, treacle and sugar toppings
11.2 Other sugars and syrups (e.g. brown sugar, maple syrup)
11.3 Honey
11.4 Table-top sweeteners, including those containing high-intensity sweeteners, other than 11.1–11.3

12 Salts, spices, soups, sauces, salads, protein products, etc.
12.1 Salt
12.2 Herbs, spices, seasonings (including salt substitutes) and condiments
12.3 Vinegars
12.4 Mustards
12.5 Soups and broths
12.5.1 Ready-to-eat soups and broths, including canned, bottled and frozen
12.5.2 Mixes for soups and broths
12.6 Sauces and similar products
12.6.1 Emulsified sauces (e.g. mayonnaise, salad dressing)
12.6.2 Non-emulsified sauces (e.g. ketchup, cheese sauce, cream sauce, brown gravy)
12.6.3 Mixes for sauces and gravies
12.7 Salads (e.g. macaroni salad, potato salad) and sandwich spreads (excluding cocoa- and nut-based spreads)
12.8 Yeast
12.9 Protein products

13 Foodstuffs intended for particular nutritional uses
13.1 Infant formulae and follow-on formulae
13.2 Foods for young children (weaning foods)
13.3 Dietetic foods intended for special medical purposes
13.4 Dietetic formulae for slimming purposes and weight reduction
13.5 Dietetic foods other than 13.1–13.4
13.6 Food supplements

14 Beverages, excluding dairy products
14.1 Non-alcoholic ("soft") beverages
14.1.1 Waters
14.1.1.1 Natural mineral waters and source waters
14.1.1.2 Table waters and soda waters
14.1.2 Fruit and vegetable juices
14.1.2.1 Canned or bottled (pasteurized) fruit juice
14.1.2.2 Canned or bottled (pasteurized) vegetable juice

14.1.2.3 Concentrates (liquid or solid) for fruit juice
14.1.2.4 Concentrates (liquid or solid) for vegetable juice
14.1.3 Fruit and vegetable nectars
14.1.3.1 Canned or bottled (pasteurized) fruit nectar
14.1.3.2 Canned or bottled (pasteurized) vegetable nectar
14.1.3.3 Concentrates (liquid or solid) for fruit nectar
14.1.3.4 Concentrates (liquid or solid) for vegetable nectar
14.1.4 Water-based flavoured drinks, including "sport" or "electrolyte" drinks
14.1.4.1 Carbonated drinks
14.1.4.2 Non-carbonated drinks, including punches and ades
14.1.4.3 Concentrates (liquid or solid) for drinks
14.1.5 Coffee, coffee substitutes, tea, herbal infusions and other hot cereal beverages, excluding cocoa
14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
14.2.1 Beer and malt beverages
14.2.2 Cider and perry
14.2.3 Wines
14.2.3.1 Still wine
14.2.3.2 Sparkling and semi-sparkling wines
14.2.3.3 Fortified wine and liquor wine
14.2.3.4 Aromatized wine
14.2.4 Fruit wine
14.2.5 Mead
14.2.6 Spirituous beverages
14.2.6.1 Spirituous beverages containing at least 15% alcohol
14.2.4.2 Spirituous beverages containing less than 15% alcohol

15 Ready-to-eat savouries
15.1 Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes)
15.2 Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit)

16 Composite foods (e.g. casseroles, meat pies, mincemeat) — foods that could not be placed in categories 1–15