This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (α-amylase from *Bacillus lichenformis* containing a genetically engineered α-amylase gene from *B. licheniformis*, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*, mixed xylanase, β-glucanase enzyme preparation produced by a strain of *Humicola insolens*, neotame, polyvinyl alcohol, quillaia extracts and xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

WHO Technical Report Series — 922

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EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

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

World Health Organization
Geneva

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Sixty-first meeting of the Joint FAO/WHO Expert Committe on Food Addtives

Rome, 10–19 June 2003

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

Safety evaluation of certain food additives and contaminants. WHO Food Additive Series, No. 52, in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, Addendum 11. FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.

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 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 Rome from 10 to 19 June 2003. The meeting was opened by Mr H. de Haen, Assistant Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr de Haen made reference to the recently completed evaluation of the work of the Joint FAO/WHO Food Standards Programme (Codex Alimentarius Commission) and of this Committee and other joint FAO/WHO activities in providing scientific advice to Member countries. He noted that at the forthcoming twenty-sixth session of the Codex Alimentarius Commission, FAO and WHO would report on steps underway to improve the work of the scientific expert committees and ad hoc consultations that provide scientific advice to Codex committees and to FAO/WHO Member countries. FAO and WHO were committed to increasing efforts and resources to improve the provision of this advice; within FAO, a significant increase of staff and non-staff resources was being negociated for the forthcoming years.

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 sixty previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the fifty-ninth meeting (Annex 1, reference *160*).

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, flavouring agents and contaminants (sections 3, 4 and 7, and Annex 2);
— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4, and Annex 2);
— to undertake a toxicological evaluation of a nutritional source of iron (section 5); and
— to undertake a toxicological evaluation of a disinfectant for drinking-water (section 6).

2.1 Modification of the agenda

Flavouring agents Nos 909, 919 and 925 were removed from the agenda because the data necessary to establish full specifications were not available.

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 (EHC 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 a number of its meetings (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116*, *122*, *131*, *137*, *143*, *149*, *152* and *154*), including the present one. Environmental Health Criteria, No. 70, contains the most important observations, comments and recommendations made, up to the time of its publication, by the Committee and associated bodies in their reports on the safety assessment of food additives and contaminants.

2.2.1 *Chemical and technical assessments of food additives*

At previous meetings, the Committee had access to documents called *Technical Data Sheets*, which were prepared for new or existing food additives and which were not published because the detailed information on manufacturing processes described therein could be commercially sensitive. These documents, however, also contain valuable information, which was not made public, on chemical and technological aspects of the compounds under discussion. At the fifty-ninth meeting (Annex 1, reference *160*), the Committee recommended that these documents should include comprehensive information on technological use levels for foods, which should also form the basis for intake assessment. Furthermore, the importance of specifications as an integral part of the risk assessment of food additives was stressed.

Taking these recommendations into consideration, the Secretariat has adapted the format and structure of the *Technical Data Sheet* and renamed it the *Chemical and Technical Assessment* (CTA), with the intention of making this document publicly available. The CTA reflects and emphasizes the role that chemical characterization plays in the risk assessment of food additives. The document is prepared by an expert assigned before the meeting and is intended to provide to the Committee the basic information regarding the identity, purity and use of the food additive, as related to its risk assessment.

The drafting expert responsible for preparing a CTA is asked to identify those sections of a confidential nature and the Secretariat will ensure that they are removed before publication. The CTAs will be available via the FAO JECFA website; it is not anticipated that they will be published in printed form.

At its present meeting, the Committee reviewed the first set of CTA for certain food additives and provided feedback to the Secretariat on the *FAO guidelines on the structure and content of the document called* "*Chemical and Technical Assessment (CTA)*[1]".

2.2.2 *Safety evaluation of flavouring agents*

Working definition of "flavouring agent"

At its fifty-ninth meeting, the Committee recognized the need for a working definition of the term "flavouring agent" and recommended that such a definition be agreed at a future meeting. At its present meeting, the Committee noted that a range of regulatory definitions of "flavouring" and similar terms exist in different countries and concluded that any definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission.

The Committee re-iterated the criteria that need to be met for an individual flavouring agent to be evaluated by the existing Procedure for the Safety Evaluation of Flavouring Agents:

- The substance should be chemically defined, such that at least 95% of the commercially used material consists either of the named chemical, or of the named chemical and identified secondary constituents.
- The substance is added to food for flavouring purposes, including the generation of active flavouring substances during storage or processing of the food.
- There is a valid estimate of current exposure to the named substance and, if appropriate, its breakdown or reaction products.

Some substances that have a use as flavouring agents may have been evaluated previously by the Committee in relation to other food additive functions. The use of such a substance, or its breakdown or reaction products, as a flavouring agent is included in the relevant, previously-established ADI.

[1] FAO guidelines on the structure and content of the document called "Chemical and Technical Assessment (CTA)": http://www.fao.org/es/ESN/jecfa/guidelines1_en.stm

Consideration of flavouring agents with high intakes, evaluated by the "B-side" of the Procedure for the Safety Evaluation of Flavouring Agents

At the present meeting, two flavouring agents, dihydrocoumarin (No. 1171) and 6-methylcoumarin (No. 1172) that were evaluated by the Procedure for the Safety Evaluation of Flavouring Agents could not be predicted to be metabolized to innocuous end products (step B2) and their intake exceeded the human intake threshold for their structural class (step B3). In application of the Procedure, more extensive data on the toxicity of these substances are required in order to complete their evaluation. In considering such substances, the Committee noted that the data required would include studies of metabolism and toxicity of the substance, and that refined estimates of intake might additionally be needed. Data on structurally related substances could also be used to support the evaluation. These studies would need to be of sufficient quality and duration to enable the flavouring agent to be evaluated at its specified intake.

The Committee noted that flavouring agents for which more extensive data were required should be clearly identified in the report of the meeting and that a complete description of the evaluation of such flavouring agents should be provided in the report item and the monograph. The Committee recommended that the guidelines for the preparation of monographs for flavouring agents be revised to ensure that a consistent approach is applied to the evaluation of such substances.

Safety evaluation of natural flavouring complexes

At its present meeting, the Committee considered a working paper outlining a revision to the safety evaluation of flavouring agents to accommodate the safety evaluation of natural flavourings that are complex mixtures (natural flavouring complexes). These flavourings are obtained from a single source material by physical processes such as distillation, or extraction with water or organic solvents. Many natural flavouring complexes consist of mixtures of individual flavouring agents, several of which have been evaluated previously by the Committee. The revised Procedure builds on the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*), organizing the components of a natural flavouring complex into congeneric groups, which become the focus of the safety evaluation. The steps in the existing Procedure have been modified to accommodate the evaluation of congeneric groups and provide for an overall evaluation of the natural flavouring complex.

In considering the revised Procedure, the Committee noted that several hundred natural flavouring complexes are currently in commercial use. These include essential oils, which are relatively well characterized in terms of their chemical composition, as well as extracts and oleoresins, some of which are currently less well characterized. Since compositional data are required to complete a safety evaluation by the revised Procedure, the Committee noted that further modification of the Procedure could be required for natural flavouring complexes that cannot be well characterized in terms of their composition.

The Committee concluded that the revised Procedure provides a potentially efficient way of evaluating natural flavouring complexes that are well characterized, such as essential oils. To determine the applicability of the revisions, the Committee recommended that a small number of natural flavouring complexes be evaluated by the revised Procedure at a future meeting.

The Committee noted that numerous products from different geographical regions are used as flavouring complexes, and the importance of ensuring that an inventory of commercial products be compiled was stressed. The Committee considered that it was necessary to take account of the range of composition of natural flavouring complexes across all regions.

The Committee was aware that different organizations have different approaches to the establishment of specifications for natural flavouring complexes. The Committee also noted that criteria would need to be developed to elaborate specifications for natural flavouring complexes.

Intake data on flavouring agents

The Committee discussed the data requirements for substances to be evaluated by the Procedure for the Safety Evaluation of Flavouring Agents. For those substances with current usage in food, poundages used for intake assessments should be reported using no more than two significant figures. Flavouring agents without reported poundage data will not be evaluated by the Committee.

2.3 Joint FAO/WHO Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food

The Committee was informed about the progress of this Project and recognized its importance. The Committee noted that several issues being considered by this Project were of particular relevance to some of their present evaluations:

— dose–response modelling of endpoints, both carcinogenic and non-carcinogenic, which cannot be assigned a threshold;
— probabilistic modelling for estimation of intake;
— biomarkers of effect and their relationships to disease outcome;
— relevance of reversible, non-progressive, treatment-related effects;
— longer tolerable intake periods, e.g. provisional tolerable monthly intake (PTMI), for contaminants with longer biological half-lives;
— revision of the approach to the safety evaluation of flavouring agents, in order to accommodate natural flavours;
— approaches for the development of specifications for complex mixtures, particularly those of natural origin.

2.4 Provision of scientific advice by FAO and WHO

The Committee was informed about a consultative process initiated by FAO and WHO, which would consider the provision of scientific advice by both organizations to the Codex Alimentarius Commission and to Member countries.

Such advice may be elaborated by committees, such as JECFA, ad hoc consultations or consultants. This consultative process is designed to improve the scientific advice provided with regard to quality, independence, integrity, transparency, timeliness, efficiency and sustainability. The outcome of the process would be a set of recommendations, addressed to the Directors-General of FAO and WHO, for the development of a consistent, harmonized and flexible overarching framework (an "umbrella"), which is realistic, feasible and acceptable to all stakeholders.

The Committee noted that this exercise would take into consideration and build upon the experience of and the improvements already being implemented by the Secretariat of this Committee.

The Committee was informed that Maria Lourdes Costarrica (FAO) and Wim van Eck (WHO) were responsible for the coordination of this consultative process.

2.5 Food additive specifications

2.5.1 Compendium of Food Additive Specifications *and* Guide to Specifications

At its forty-sixth and fifty-fifth meetings, the Committee had recommended the revision of the *Compendium of Food Additive Specifications* (Annex 1, reference *96*) and the *Guide to Specifications* (Annex 1, reference *100*). At the present meeting, the Secretariat presented a project that had been proposed recently to FAO, with the following objectives:

- The current edition of the *Guide to Specifications* will be updated and published together with a consolidated edition of the *Compendium of Food Additive Specifications* as one document in two volumes.
- The update shall reflect state-of-the-art analytical methodologies and practice by regulators and industry. These methods should also respect the fact that they are applied by laboratories in developing and developed countries with varying levels of equipment and expertise.
- The update shall consider the general guidelines laid out by this Committee and the Joint FAO/WHO Conference on Food Additives (summarized in EHC 70) and the work of other relevant standard-setting bodies
- The update shall be available in print and electronically.

Depending on the availability of funds, the project will start during 2003 and will terminate in 2005.

2.5.2 *Residual solvents*

Several of the specifications for food additives under review at the present meeting include limits for residual solvents. In some cases, the methods of analysis to be used are included in the specifications and in others reference is made to the General Method included in the *Guide to Specifications*, FNP 5 (Annex 1, reference *100*). The Committee noted that the General Method described in FNP 5 refers to obsolete gas chromatographs with packed columns, and that it may be difficult to obtain such chromatographs, since injectors for packed columns are no longer available.

It was also noted that a variety of gas chromatographic methods for the determination of residual solvents were included in the specifications. The Committee concluded that specifications containing limits for residual solvents should refer to the same General Method in FNP 5 wherever possible. The Committee recommended that FNP 5 be revised to include modern methodology.

At its present meeting, the Committee formulated a general method of analysis for residual solvents, using head-space gas chromatography with flame ionization detection (FID). This method is to be published in Section E of FNP 52, Add 11.

2.5.3 *Specifications of purity for flavouring agents*

The Committee agreed to replace the now outdated Council of Europe numbers with the recently introduced European Commission "FLAVIS database" numbers.

2.6 Intake assessment of food additives

2.6.1 *Use of proposed maximum limits in the intake assessment of food additives*

The Committee assesses dietary exposure to food additives using a tiered approach, according to the JECFA Guidelines[1]. One of these tiers consists of combining estimated food intakes from various geographical regions with the draft proposed maximum levels (draft MLs) of additives for the Codex General Standard on Food Additives (GSFA).

The Committee observed that, in most cases, the MLs in Codex standards are higher than the typical use levels reported by governments and industry. For example, at the present meeting the Committee evaluated annatto extracts and noted that they are used at a concentration of 35 mg/kg in Mimolette cheese. However, an ML of 600 mg/kg is proposed in the GSFA for all cheese. This example also illustrates that some food additives are listed in the GSFA for use in very broad food categories when in reality they are used in a very limited number of applications.

When draft MLs are the only available information on additive use levels in food, the estimation of the intake using high proposed GSFA levels has resulted in unrealistic estimates. In some cases, these intake estimates were many times the corresponding ADI. Consequently, the Committee suggests that the Codex Committee on Food Additives and Contaminants (CCFAC) might wish to review MLs with the aim of lowering them or restricting their use to food subcategories, as appropriate. Alternatively, CCFAC may consider providing the Committee with typical use levels to allow for more realistic exposure assessments.

2.6.2 *Consideration of the Guidelines*

The *Guidelines* for the preparation of working papers on the intake of food additives were given further consideration and revisions were suggested by the Committee. In the revised version, the *Guidelines* indicate how to verify whether the assumptions on which the budget method is based are adequate. In addition, the limitations of market-share data, and the need for data on levels of use by industry in order to make intake assessments, are stressed.

[1] JECFA Guidelines, http://www.who.int/pcs/jecfa/jecfa_gls.htm

3. Specific food additives (other than flavouring agents)

The Committee evaluated six food additives for the first time and re-evaluated a number of others. Information on the safety 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 Safety evaluations

3.1.1 *α-Amylase from* Bacillus licheniformis *containing a genetically engineered α-amylase gene from* B. licheniformis

The enzyme preparation under evaluation contains the enzyme LE399 α-amylase from the genetically modified *Bacillus licheniformis*. LE399 α-amylase has not been evaluated previously by the Committee. The enzyme is thermostable and active at a relatively low pH and low calcium concentration. These characteristics make the enzyme particularly suitable for use in starch hydrolysis conducted at high temperatures, for example, for the liquefaction of starch used in the production of nutritive sweeteners.

LE399 α-amylase is produced by pure culture fermentation of a strain of *B. licheniformis* that is non-pathogenic and non-toxigenic and which has been genetically modified to carry a genetically engineered gene coding for α-amylase. The enzyme is subsequently partially purified and concentrated, resulting in a liquid enzyme concentrate (LEC). In the final preparation, this LEC is stabilized and standardized/formulated with methionine, sodium chloride, and glucose/sucrose.

The α-amylase protein was developed by changing four amino acids in the polypeptide chain of another genetically engineered thermostable α-amylase, "Termamyl LC". These modifications were accomplished by introducing appropriate mutations into the DNA sequence encoding the Termamyl LC α-amylase. The engineered gene, designated as the LE399 α-amylase gene, was introduced into the host strain SJ5550.

The host strain was developed from a parent strain DN2717, a derivative of a natural *B. licheniformis* isolate. The DN2717 strain was genetically engineered to inactivate the following native genes: the *apr* gene encoding the "Alkalase" protease; the *amyL* gene encoding the Termamyl α-amylase; the *xyl* gene encoding xylose isomerase; and the *gnt* gene encoding gluconate permease. The inactivated *amyL*, *xyl*, and *gnt* genes were replaced with three copies of the

LE399 α-amylase gene. In a separate step, the gene encoding C-component protease was deleted. The resulting strain was designated as MOL2083 and used as a production strain The aim of these genetic modifications was to produce the LE399 α-amylase, to prevent the synthesis of proteases that might hydrolyse the LE399 α-amylase, and to avoid the production of the Termamyl α-amylase.

The genetic material introduced into the production strain has been well characterized and does not contain any sequences that would encode for proteins resulting in the production of toxic or undesirable substances. The LE399 α-amylase gene is stably integrated into the *B. licheniformis* chromosome. The production strain does not contain genes encoding proteins that inactivate antibiotics.

The LE399 α-amylase was assessed for potential allergenicity by amino acid sequence comparison with known allergens listed in publicly-available protein databases. No immunologically-significant sequence homology was detected.

Toxicological studies were conducted on the LEC. The materials added to the LEC for stabilization and formulation/standardization have either been evaluated previously by the Committee or are common food constituents and do not raise safety concerns. In a 13-week study in rats, no significant treatment-related effects were seen when the LEC was administered by oral gavage at doses of up to and including 10 ml LEC/kg of body weight per day, the highest dose tested. Therefore this highest dose (equivalent to 1.02 g total organic solids (TOS)/kg of body weight per day) was considered to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

The α-amylase preparation is intended for use in starch liquefaction in the production of sweetener syrups, alcoholic beverages and beer. The absence of the α-amylase protein in the final (purified) sweetener syrup has been confirmed experimentally. In the spirits industry, no LE399 α-amylase or other organic solids are expected to be carried over to the final product because ethanol is removed by distillation from the fermentation mash containing the enzyme preparation. In the brewing of beer, the enzyme preparation is added during the mashing process and is denatured and inactivated during the subsequent wort-boiling stage. The beer filtration process is likely to remove the denatured enzymes along with other insoluble materials. In conclusion, no residual LE399 α-amylase is expected to be present in food processed using this enzyme preparation.

Nevertheless, very conservative estimates of daily intakes were performed on the assumption that all the TOS would persist in the final products, giving an estimated daily intake of 12 mg TOS/day (equivalent to 0.2 mg TOS/kg of body weight per day) for sugar and syrups, 3 mg TOS/day (equivalent to 0.05 mg TOS/kg of body weight per day for a 60 kg person) for beer and 10.8 mg TOS/day (equivalent to 0.18 mg TOS/kg of body weight per day) for spirits. Compared to the NOEL of 1020 mg TOS/kg of body weight per day derived from the 13-week study of oral toxicity, the margin of safety is >2000.

The Committee allocated an ADI "not specified" to α-amylase from this recombinant strain of *B. licheniformis*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.1.2 *Annatto extracts*

Annatto extracts have been used for over two centuries as a food colour, especially in cheese, and various types are now used in a wide range of food products. Annatto extracts are obtained from the outer layer of the seeds of the tropical tree *Bixa orellana.* The principal pigment in annatto extract is *cis*-bixin, which is contained in the resinous coating of the seed itself. Processing primarily entails the removal of the pigment by abrasion of the seeds in an appropriate suspending agent. Traditionally, water or vegetable oil is used for this purpose, although solvent extraction is also employed to produce annatto extracts with a higher pigment content. Microcrystalline bixin products of 80–97% purity have been developed in response to the need for more concentrated annatto extracts.

Annatto extracts were evaluated by the Committee at its thirteenth, eighteenth and twenty-sixth meetings (Annex 1, references *19*, *35*, *59–61*).

At its eighteenth meeting, the Committee considered the results of long-term and short-term tests in experimental animals fed an annatto extract containing 0.2–2.6% pigment expressed as bixin. A long-term study in the rat provided the basis for evaluation; the NOEL in this study was 0.5% in the diet, the highest dose tested, equivalent to 250 mg/kg of body weight. A temporary ADI was established of 0–1.25 mg annatto extract/kg of body weight.

The Committee re-evaluated annatto extract at its twenty-sixth meeting, when the results of the metabolic studies that had been requested became available. Studies of mutagenicity, additional long-term

(1-year) studies in the rat, and observations of the effects of annatto extract in humans were also considered. No evidence was found for the accumulation of annatto pigments in the tissues of rats fed at low concentrations (20–220 mg/kg of body weight per day of annatto extracts containing up to 2.3% bixin/norbixin mixture) for a year. Studies in both rats and humans showed that although annatto pigments are absorbed from the intestine into the blood, clearance from the plasma is rapid.

The NOEL in the original long-term rat study was determined as 0.5% in the diet, equivalent to 250 mg/kg of body weight, and the ADI was set at 0–0.065 mg/kg of body weight of annatto extract expressed as bixin. In this re-evaluation, the Committee considered the highest concentration of bixin in the material tested (i.e. 2.6%) and established an ADI on the basis of the content of bixin.

B. orellana is grown in many countries and various procedures are used to produce annatto extracts from the seeds for commercial use. The following extracts were considered for evaluation:

Annatto extract (solvent-extracted bixin): Annatto B[1]

The seeds are extracted with solvent to dissolve the pigment. The extract is filtered to remove insoluble material. Subsequent processing involves removal of fats and waxes, solvent removal, crystallization and drying.

Annatto extract (solvent-extracted norbixin): Annatto C

The seeds are extracted with solvent to dissolve the pigment. The extract is filtered to remove insoluble material. Subsequent processing involves removal of fats and waxes, removal of the solvent, crystallization and drying. Aqueous alkali is added to the resultant powder, which is heated to hydrolyse the pigment and then cooled. The aqueous solution is filtered, and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled to give a granular powder.

Annatto extract (oil-processed bixin suspension): Annatto D

The seeds are abraded in hot vegetable oil to remove the pigment.

[1] To ensure clarity, the Committee adopted the designations B, C, D, E, F, G, as employed in the submitted information, to refer to the different annatto extracts under evaluation

Annatto extract (aqueous-processed bixin): Annatto E
The seeds are abraded in cold aqueous alkali (potassium or sodium hydroxide) to remove the pigment. The resulting suspension is acidified to precipitate the bixin. The precipitate is filtered, washed, dried and milled to give a granular powder.

Annatto extract (alkali-processed norbixin): Annatto F
The seeds are abraded in cold aqueous alkali (potassium or sodium hydroxide) to remove the pigment. Additional alkali is added to the resultant suspension, which is heated to dissolve the pigment and then cooled. Fats and waxes are removed. The aqueous solution is filtered, and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled to give a granular powder.

Annatto extract (alkali-processed norbixin, not acid-precipitated): Annatto G
The seeds are abraded in cold aqueous alkali (potassium or sodium hydroxide) to remove the pigment. Additional alkali is added to the resultant suspension, which is heated to dissolve the pigment, and then cooled. Fats and waxes are removed. The aqueous solution is filtered, and dried. Potassium carbonate may be added.

Bixin and norbixin, the main pigments contributing to the colour of annatto extracts, are present at different concentrations in different commercial preparations. These preparations are traded between primary processors of annatto seeds and the final vendors of colour products to the food companies. They are, however, too concentrated to add directly to foods and require dilution with carriers such as vegetable oil (with emulsifiers), propylene glycol or alkali. The non-pigment fractions of the concentrated extracts are not well characterized.

At its present meeting, the Committee evaluated new studies involving several concentrated preparations containing bixin and norbixin. The new studies consist primarily of 28-day and 90-day studies, disposition studies, studies of effects on microsomal oxidation enzymes and studies of genotoxicity with these formulations, one study of developmental toxicity and data on the potential allergenicity of annatto extract.

The pigment content, expressed as bixin and norbixin, of the extracts considered for evaluation is as follows:

Annatto B: solvent-extracted annatto extract containing 92% pigment, of which 97% was bixin and 1.7% norbixin;
Annatto C: solvent-extracted annatto extract containing 91.6% norbixin;

Annatto D: oil-processed annatto extract containing 10.8% pigment, of which 94% was bixin and 1.7% norbixin;

Annatto E: aqueous-processed annatto extract containing 26% pigment, of which 90% was bixin and 4.2% norbixin;

Annatto F: alkali-processed annatto extract containing 41.5% norbixin;

Annatto G: alkali-processed annatto extract, sodium and potassium salts containing 17.1% norbixin.

Toxicological studies. New toxicological data were made available for four of these extracts: Annatto B, C, E and F.

The new data confirmed earlier findings that there appears to be at least partial absorption of bixin and norbixin, and that the colours in water-soluble preparations of annatto are more readily absorbed than the oil-soluble preparations.

Bixin was not detected in plasma after oral administration of norbixin to rats, suggesting that norbixin is not converted to bixin in the body. *Cis*-norbixin appears to be readily converted to *trans*-norbixin. The more polar acid norbixin is absorbed to a greater extent than the less polar bixin. The presence of norbixin in plasma after administration of Annatto B and E suggests that bixin may be converted to norbixin in the body, but these preparations also contain norbixin which could have accounted for the norbixin levels in plasma. No bixin was detected in the urine following administration of any of the annatto extracts, but extremely small amounts of norbixin (<3% of the dose) were found in urine after administration of Annatto F, and traces (<0.01%) after administration of Annatto E. Bixin and norbixin are mostly cleared from the plasma within 24 hours. The total percentage of the dose of bixin and norbixin absorbed cannot be determined from the available data. Although studies have shown that about half of the administered dose appears in the faeces, it is not possible to determine whether this is because the pigments are not absorbed and pass through the gastrointestinal tract, or whether part of the dose is absorbed and is excreted via the bile.

In humans given a commercial preparation containing 16 mg bixin and 0.5 mg norbixin, the concentrations of norbixin in blood were higher than those of bixin and persisted for longer, since bixin could not be detected in the plasma 8 hours after a single oral dose whereas norbixin reached a peak after 4 hours and was still detected after 48 hours. It is unclear whether conversion of bixin to norbixin occurs in the body.

Examination of cytochrome P450 (CYP450) enzymes in liver samples at the end of 10-week studies in rats fed with one of several annatto preparations (Annatto B, E or F) revealed that Annatto B and E are inducers of CYP1A2. There was no evidence that any of the annatto extracts was a phenobarbital-type inducer, or was an inducer of CYP2E1. There were only slight increases in CYP3A1 and CYP3A2. All three annatto extracts induced CYP4A, particularly in male rats. The pattern of induction of CYP1A2 and CYP4A by different preparations in different sexes indicate that these effects are independent. Annatto F caused the greatest induction of CYP4A and there was an increase in the number of mitochondria observed by electron microscope with Annatto C, observations which are consistent with the action of peroxisome proliferators. Liver weight increases were not related to the increase in cytochrome P450 enzymes. The absence of any further hypertrophy between days 28 and 90 of treatment, and the absence of pathological changes in the liver, may be compatible with metabolic adaptation.

Studies in mongrel dogs fed a chloroform-extracted preparation of annatto in glucose and similar studies in rats and mice fed an ethanol-extracted preparation of annatto, were considered not to be relevant to the extracts being evaluated.

In vitro studies of genotoxicity revealed equivocal and inconsistent positive results only at concentrations that exceeded solubility or at concentrations that were cytotoxic. Since the results of tests on analytical grade bixin and norbixin were negative, some weak positive results obtained with the concentrated annatto extracts in bacterial tests in the absence of an endogenous metabolic activation system were considered to be caused by other components in the annatto preparations. Results of tests for mutagenicity in mammalian cells and for chromosomal aberration were inconsistent. Weak positive results at toxic concentrations were noted for some preparations in tests for mutagenicity in mammalian cells in the absence of an endogenous metabolic activation system, whereas weak positive results were noticed only in the presence of an endogenous metabolic activation system in tests for chromosomal aberration. Studies in mice receiving Annatto B, E and F preparations did not demonstrate any potential to cause genetic damage in a test for micronucleus formation in bone marrow in vivo.

In its previous evaluations, the Committee concluded that annatto extracts are not carcinogenic. This conclusion was based on the results of tests with annatto preparations containing low concentrations of bixin. No new studies of carcinogenicity have become available, but in

a study of the initiation/promotion of liver carcinogenesis, Annatto C did not increase the incidence of preneoplastic lesions. Together with the results of the tests for genotoxicity and the absence of proliferative lesions in the short-term tests for toxicity, this is supportive of earlier conclusions.

A study of developmental toxicity in rats fed an annatto extract with a bixin content (28%) comparable to that of Annatto E at doses of up to 500 mg/kg of body weight per day (equal to 140 mg of bixin/kg of body weight per day) confirmed the absence of developmental toxicity at this dose.

Studies of subchronic toxicity demonstrated that the annatto extracts tested, Annatto B, C, E and F, have low toxicity, as only non-specific toxicity was reported at the higher doses tested. As is the case with genotoxicity, it is not clear whether the non-specific toxicity was attributable to bixin or norbixin or to other components present in the extracts. A common feature was the increase in absolute and relative liver weights at high and intermediate doses, which was accompanied in some cases by centrilobular hepatocellular hypertrophy.

The NOEL for Annatto B was identified as 16 000 mg/kg diet (equal to 1311 mg and 1446 mg of extract/kg of body weight per day for males and females respectively, corresponding to 1170 mg or 1290 mg of bixin/kg of body weight per day, and 21 mg or 23 mg of norbixin/kg of body weight per day) on the basis of urinary effects (elevated concentrations of protein in urine and crystals in urine sediment).

The NOEL for Annatto C was identified as 1000 mg/kg diet (69 mg and 76 mg/kg of body weight per day for males and females respectively, corresponding to 63 mg or 70 mg of norbixin/kg of body weight per day) on the basis of increases in liver weight accompanied by hepatocellular hypertrophy and necrosis.

The NOEL for Annatto E was identified as 10 000 mg/kg in the diet (734 mg and 801 mg/kg of body weight per day for males and females respectively, corresponding to 172 mg or 180 mg of bixin/kg of body weight per day, and 8 mg or 8.8 mg of norbixin/kg of body weight per day) on the basis of increases in thyroid and kidney weights and decreased spleen weights.

The NOEL for Annatto F was identified as 1000 mg/kg in the diet (79 mg and 86 mg/kg body weight per day for males and females respectively, corresponding to 33 mg and 36 mg of norbixin/kg of body weight per day) on the basis of increased kidney weights, haematological changes and alterations in serum proteins.

The differences in NOEL for Annatto B (bixin) and Annatto C (norbixin) indicate that extracts that mainly contain norbixin are more potent than those containing mainly bixin. The potencies of the different extracts cannot be explained on the basis of their bixin and/or norbixin contents. Other components in the extracts might contribute to or be responsible for the effects noted, and/or the differences in potency might have arisen from differences in bioavailability of the extracts.

A number of studies on possible allergic potential in humans were available, but the results of oral challenges were inconclusive because of inadequate study design or lack of statistical significance.

A revision of the previous intake estimate was performed on the basis of typical use levels of extracts expressed as bixin and norbixin provided by industry. These levels were combined with various national intake data. The resulting average intake is observed to be between 0.03 mg and 0.40 mg/day. Estimated intake for high consumers reaches 1.50 mg/day, on the basis of data from the United Kingdom.

Evaluation. The Committee could not establish a generic ADI for the various annatto extracts on the basis of the data submitted and therefore established a temporary ADI for each of the individual preparations tested. With the application of a 200-fold safety factor to the NOEL for each of the annatto preparations, the following temporary ADIs were allocated:

Annatto B: 0–7.0 mg/kg of body weight (based on NOELs of 1311 mg and 1446 mg/kg of body weight per day in male and female rats respectively).

Annatto C: 0–0.4 mg/kg bw (based on NOELs of 69 mg and 76 mg/kg of body weight per day in male and female rats respectively).

Annatto E: 0–4.0 mg/kg of body weight (based on NOELs of 734 mg and 801 mg/kg of body weight per day in male and female rats respectively).

Annatto F: 0–0.4 mg/kg of body weight (based on NOELs of 79 mg and 86 mg/kg of body weight per day in male and female rats respectively).

No data on the potential toxicity of Annatto D or Annatto G were available, and no ADI could be established. An additional safety factor of 2 was applied to the NOELs, because of deficiencies in the database.

Comparison of the estimated intakes with the temporary ADI values were performed assuming that each annatto extract was a unique

source of bixin/norbixin. These simulations show in each case that the estimated exposure for adults is <20% of the corresponding temporary ADI.

The Committee requested additional information to clarify the role that the non-pigment components of the extract play in the expression of the qualitative and quantitative differences in toxicity of the various extracts. In addition, the Committee requested data on the reproductive toxicity of an extract, such as Annatto F, that contains norbixin.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared.

Specifications. The principle purpose of specifications is to ensure that the material of commerce is comparable to the material that has been biologically tested. This Committee adopted tentative specifications for the four annatto extracts tested, with the following minimum assay values:

Annatto extract (solvent-extracted bixin) — Annatto B: not less than 85% pigment (as bixin, of which not more than 2.5% is norbixin)

Annatto extract (solvent-extracted norbixin) — Annatto C: not less than 85% pigment (as norbixin)

Annatto extract (aqueous-processed bixin) — Annatto E: not less than 25% pigment (as bixin, of which not more than 7% is norbixin)

Annatto extract (alkali-processed norbixin) — Annatto F: not less than 35% pigment (as norbixin)

The Committee also adopted tentative specifications with minimum assay values as proposed for the commercial products Annatto D and G, which had not been tested biologically.

3.1.3 *Curcumin*

The food colour curcumin (turmeric yellow) is obtained by solvent extraction of turmeric, i.e. the ground rhizomes of *Curcuma longa* L. (*C. domestica* Valeton), with purification of the resultant extract by crystallization. The commercial product consists essentially of curcumins: the colouring principle (1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) and its desmethoxy and bisdesmethoxy derivatives in varying proportions. The total content of colouring matter (curcuminoids) in curcumin is not less than 90%. Minor amounts of oils and resins that occur naturally in turmeric may also be present.

The term "curcumin" in this report refers to the material for which specifications exist. The principal colouring component, 1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione, is often referred to as curcumin in the literature; to avoid confusion, the Committee decided that this report would not use the term curcumin when referring to this substance. A common synonym for this substance is diferuloylmethane, and this name will be used below when it is necessary to refer to the principal colouring component of curcumin.

Turmeric oleoresin, the product of solvent extraction of turmeric containing <90% of total colouring matter (curcuminoids), and curcumin were evaluated by the Committee at its thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth, thirty-ninth, forty-fourth, fifty-first and fifty-seventh meetings (Annex 1, references *19, 35, 47, 53, 59, 73, 88, 101, 116, 137* and *154*). At its eighteenth meeting, the Committee established a temporary ADI of 0–0.1 mg/kg of body weight for curcumin, based on the then existing ADI for turmeric oleoresin (0–2.5 mg/kg of body weight) and an assumed average concentration of 3% curcuminoids in turmeric. The temporary ADI for curcumin was extended at the twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth and thirty-ninth meetings, whereas the ADI for turmeric oleoresin was withdrawn at the thirty-fifth meeting. At the thirty-ninth meeting, the Committee requested the results of studies of carcinogenicity in mice and rats fed turmeric oleoresin and the results of a study of reproductive and developmental toxicity associated with curcumin. At its forty-fourth meeting, the Committee evaluated the results of studies of carcinogenicity in rats and mice given turmeric oleoresin containing 79–85% curcuminoids, and new biochemical and genotoxicity data. The Committee concluded that data on developmental toxicity were no longer required but reiterated its request for a study of reproductive toxicity. On the basis of the NOEL of 220 mg/kg of body weight per day for liver enlargement observed in the study of carcinogenicity 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 submission of the results of a study of reproductive toxicity with curcumin to be reviewed in 1998.

At its fifty-first meeting, the Committee evaluated the results of studies of fertility in rats and mice treated with turmeric oleoresin (68.0–76.5% curcuminoids). The low survival rate of pups in the mouse study and the low rates of pregnancy in rats led the Committee to conclude that these studies did not provide assurance that the potential reproductive effects of curcumin had been adequately investigated. The Committee again extended the temporary ADI, pending

submission of the results of a study of reproductive toxicity with a substance complying with the specifications for curcumin, for review in 2001. At its fifty-seventh meeting, the Committee was informed that a multigeneration study in the rat was in progress, and thus extended the temporary ADI of 0–1 mg/kg of body weight until 2003.

The results of the multigeneration study were available to the Committee for evaluation at the current meeting. In addition, the Committee reviewed the results of two new clinical trials investigating either an extract of *Curcuma* or diferuloylmethane as potential anticancer agents.

Biological data. In the multigeneration study of reproductive toxicity, Wistar rats were fed diets containing curcumin (comprising 80% diferuloylmethane and 99% total curcuminoids) at doses equal to 0, 130–140, 250–290 and 850–960 mg/kg of body weight per day in males, and 0, 160, 310–320 and 1000–1100 mg/kg of body weight per day in females. The total period of treatment was 21 weeks for the parental generation and 24 weeks for the F_1 generation. Transient minor decreases in maternal body-weight gain were observed during gestation days 10–15 in the parental, but not the F_1 generation, at the intermediate and high doses. There were no significant differences in maternal body weights at the end of the gestation period and no adverse effects were observed in the F_1 offspring. This change was therefore considered to be incidental. Significant decreases in the average weights of the F_2 generation pups were observed at days 1 and 7 at the intermediate dose, and days 7, 14 and 21 at the high dose. These decrements represented <10% of the average weight of the concurrent controls and were reported to be within the range of the historical control data. There were no other effects on general health, body weight, pup survival or fertility indices in either generation. The effect on pup weight seen at the intermediate dose (equal to 250–320 mg/kg of body weight per day) was likely to be incidental. The changes seen at the high dose (equal to 960–1100 mg/kg of body weight per day for the F_1 parental generation) could be an indication of a persistent decrement in body-weight gain. The Committee therefore concluded that the NOEL for decreased pup body weight was 250 mg of diferuloylmethane/kg of body weight per day.

Two clinical trials were conducted in patients with cancer, or at high risk of cancer. In pharmacokinetic studies in these patients, diferuloylmethane could be detected in plasma after oral diferuloylmethane (99.3%) doses of >2000 mg/day (>33 mg/kg of body weight per day, for a 60 kg adult), but not after lower doses. Diferuloyl-

methane, and in one patient diferuloylmethane sulfate, was detected in the faeces but not in the urine.

These clinical trials provided limited information of relevance to the assessment of toxicity of curcumin. Fifteen patients receiving an extract of *Curcuma* (18 mg of diferuloylmethane and 2 mg of the desmethoxy derivative suspended in 200 mg of essential oils derived from *Curcuma* spp.) at daily doses of 26–180 mg of diferuloylmethane for up to 4 months, were monitored for adverse effects by physical examination and tests for haematological parameters. In the course of the study, one patient (receiving 108 mg/day diferuloylmethane) experienced nausea and two patients (receiving 72 and 180 mg/day diferuloylmethane) experienced diarrhoea. There were no reported adverse effects in a study of twenty-five patients taking diferuloylmethane (99.3%) at doses of up to 8000 mg/day for 3 months. The Committee considered that these ancillary studies could not be used to derive an ADI for curcumin.

The new multigeneration study in rats that were fed with curcumin for periods of up to 24 weeks met the Committee's requirements. Additionally, this material met the specification developed at this meeting. Decreased weight gain in the F_2 generation was observed at doses equal to 960–1100 mg/kg of body weight per day of curcumin; the NOEL was 250–320 mg/kg of body weight per day. The Committee noted that the previous temporary ADI was derived from a study on turmeric oleoresin (79–85% curcuminoids) that did not comply with the current specification. Taking into account all of the data evaluated previously, the Committee withdrew the temporary designation and allocated an ADI of 0–3 mg/kg of body weight for curcumin, on the basis of the NOEL of 250–320 mg/kg of body weight per day in the multigeneration study in rats, and the application of a safety factor of 100.

Assessment of intake. The Committee received an estimate of intake only from Food Standards Australia New Zealand (FSANZ). This estimate, combining maximum curcumin use levels from the draft General Standard for Food Additives (GSFA) with food consumption data, is an unrealistic overestimate of the exposure. The report stated that FSANZ was not able to provide national estimates of intake for Australia or New Zealand owing to the regulatory status of curcumin, which is allowed at levels consistent with good manufacturing practice in all foods. Therefore the Committee concluded that adequate data were not available to accurately assess the exposure.

An addendum to the toxicological monograph and a chemical and technical assessment (CTA) were prepared.

Specifications. The existing specifications were revised. Ethyl acetate and carbon dioxide were added as alternative solvents. Ethyl acetate has been evaluated previously by the Committee as a carrier solvent. A residual limit for ethyl acetate of 50 mg/kg was included in the specification monograph. Carbon dioxide, as a supercritical fluid, is recognized as an extraction solvent. Because carbon dioxide is a gas at ambient conditions, no limit for residual carbon dioxide is needed in this case.

3.1.4 *Diacetyltartaric and fatty acid esters of glycerol*

Diacetyltartaric and fatty acid esters of glycerol (DATEM) were reviewed by the Committee at its tenth and seventeenth meetings (Annex 1, references *13* and *32*). At its seventeenth meeting, the Committee allocated an ADI of 0–50 mg/kg of body weight on the basis of the results of studies of biochemical and metabolic parameters and tests in animals receiving DATEM in the diet. At the same meeting, the Committee also reviewed fatty acid esters of glycerol with acetic, citric, lactic and tartaric acids and allocated a collective ADI "not limited" to this group, with the provision that the intake of tartaric acid should not exceed 30 mg/kg of body weight per day.

At its fifty-first meeting (Annex 1, reference *137*), the Committee established specifications for both the above-mentioned products under the name "diacetyltartaric and fatty acid esters of glycerol", as the Committee was aware that the two products could not be distinguished analytically. At that meeting, the Committee recommended that the material defined in the specifications be evaluated toxicologically.

New data were evaluated at the fifty-seventh meeting and the previous ADI of 0–50 mg/kg of body weight was made temporary, pending submission of additional information concerning adrenal medullary and cardiac lesions observed in the 2-year study in rats. Significant increased incidences of both lesions in the group treated with a high dose of DATEM and in the reference control group receiving an equivalent quantity of monoglyceride had been noted in this study. No differences in survival between the control and DATEM-treated groups were reported.

At the present meeting, the Committee considered information provided on the reassessment of the histopathological data related to the adrenal and cardiac lesions from the 2-year study in the rat. Higher incidences of these lesions were observed in all groups in the reassessment compared to the earlier analysis, with the exception of adrenal medullary tumours (designated as phaeochromocytomas) in male

rats. The incidence of adrenal medullary hyperplasia and adrenal medullary tumours in DATEM-treated groups was not higher than in untreated controls.

The results of the reassessment indicated that 2 years of treatment with DATEM did not affect the incidence of myocardial fibrosis. There was an increase in the incidence of an inflammatory lesion, myocarditis, in the groups receiving 10% reference substance (monoglycerides) or 10% DATEM compared with untreated controls. This lesion was considered to reflect an earlier step in the process leading to myocardial fibrosis. No difference in the incidence of myocarditis was observed between the reference control group and the group receiving 10% DATEM.

The Committee considered that the NOEL for DATEM was 10% in the diet (equal to 4100 mg/kg of body weight per day in males and 6100 mg/kg of body weight per day in females) corresponding to the highest dose tested in the 2-year combined study of toxicity/carcinogenicity in the rat. The Committee removed the temporary designation and allocated an ADI of 0–50 mg/kg of body weight, on the basis of the established NOEL with the application of a 100-fold safety factor, and with the provision that the total intake of tartaric acid from food additives should not exceed the ADI for tartaric acid (0–30 mg/kg of body weight).

3.1.5 *D-Tagatose*

D-Tagatose is a ketohexose, an epimer of D-fructose inverted at C-4. It is obtained from D-galactose by isomerization under alkaline conditions in the presence of calcium. Its properties permit its use as a bulk sweetener, humectant, texturizer and stabilizer.

D-Tagatose was evaluated by the Committee at its fifty-fifth and fifty-seventh meetings (Annex 1, references *149* and *154*). At its fifty-fifth meeting, the Committee concluded that D-tagatose was not genotoxic, embryotoxic or teratogenic. It also concluded that an ADI could not be allocated for D-tagatose because of concern about its potential to induce glycogen deposition and hypertrophy in the liver and to increase the concentrations of uric acid in serum. At its fifty-seventh meeting, the Committee evaluated the results of four studies in experimental animals, the results of a study in volunteers and some publications concerning the increased uric acid concentrations in serum after intake of D-tagatose, other sugars, and other food components.

The Committee decided to base its evaluation on the human data reviewed in the course of the two meetings. A NOEL of 0.75 g/kg of

body weight per day was identified from a 28-day study in which no effects were observed in humans receiving three doses of 15 g of D-tagatose per day. An ADI of 0–80 mg/kg of body weight for D-tagatose was established on the basis of this NOEL and a safety factor of 10.

At the present meeting, the Committee reviewed the results of two new studies of toxicity conducted in rats, and of two new studies of concentrations of plasma uric acid in human volunteers.

Studies of D-tagatose administered to rats in the diet, reviewed previously by the Committee, focused on the hepatic effects of D-tagatose, in particular, increased liver weight and hypertrophy. These studies indicated that these effects were caused, at least in part, by glycogen accumulation, and that Sprague-Dawley rats were more sensitive to these effects than Wistar rats. The new 28-day study investigating the effects of 20% D-tagatose in the diet has shown that, of six rat strains, the largest increase in liver weight occurred in Sprague-Dawley rats, and the smallest increase occurred in Wistar rats, confirming the previous observation of strain differences. The role of glycogen, however, was not specifically investigated.

In a 2-year study in Wistar rats, the administration of diets containing 2.5, 5 or 10% D-tagatose, 20% fructose, or 10% D-tagatose plus 10% fructose did not result in histological changes in the liver, although increased liver weights were reported in male and female rats fed on 10% D-tagatose. Increased absolute and relative adrenal weights were observed in female rats at all doses of D-tagatose, but not in those receiving fructose alone. Increased adrenal weights were also reported in male rats fed on 5% and 10% D-tagatose. The weights of the kidneys in females, the testes in males, and the caecum in each sex were also increased in animals fed on 10% D-tagatose, and in some cases, on 5% D-tagatose. In the absence of histopathological confirmation of the nature of the changes induced by D-tagatose in the adrenals, kidneys and testes, it is not possible to assess their toxicological significance to humans.

Two new human studies have shown that a single dose of 30 g D-tagatose to small numbers of healthy volunteers, or 15 g D-tagatose to hyperuricaemic individuals, had no biologically significant effect on uric acid production or excretion, and no recorded gastrointestinal effects. At its forty-eighth meeting, the Committee noted that D-fructose increases uric acid production by accelerating the degradation of purine nucleotides, probably by hepatocellular depletion of inorganic phosphate resulting from accumulation of ketohexose-1-phosphate. The degradation of D-tagatose-1-phosphate is slower than

that of D-fructose-1-phosphate, and therefore the hyperuricaemic effect of D-tagatose may be greater than that of D-fructose; hyperuricaemic individuals are therefore potentially vulnerable to the adverse effects of D-tagatose. The new study demonstrated no increase in serum concentrations of uric acid within 4 hours of consumption of 15g of D-tagatose by this vulnerable group. In studies reviewed previously by the Committee, the maximum increases in serum uric acid and D-tagatose and the maximum decrease in serum ATP were seen within one hour of ingesting D-tagatose. It is therefore anticipated that no effect would be observed in hyperuricaemic individuals following repeated consumption of 15 g of D-tagatose at subsequent meals.

The Committee concluded that the results of the 2-year study in rats established that the previously-reported liver glycogen deposition and hypertrophy observed after long-term administration of D-tagatose did not result in histopathological changes, and thus addressed concerns expressed at the fifty-fifth meeting. However, this study also identified new effects, namely increased adrenal, kidney and testes weights. The Committee considered that these changes might have been due to high osmotic load resulting from the high dietary doses administered, but this could not be confirmed in the absence of histopathological examination of these tissues. Pending provision of the histopathology data, the Committee confirmed that the human data provided the most relevant basis for assessing the acceptable intake of D-tagatose.

At the fifty-seventh meeting, the Committee identified a NOEL for healthy individuals of 45 g D-tagatose per day in three divided doses. The study on hyperuricaemic individuals discussed at the current meeting indicated that the NOEL is also applicable to this vulnerable group. The Committee considered that a safety factor of 3 would be appropriate to allow for interindividual variation. In view of the additional uncertainty regarding the nature of the effects observed in the adrenals, kidneys and testes in the 2-year study in rats, the Committee concluded that the ADI should be temporary and applied an additional safety factor of 2. The previous ADI was thus removed, and on the basis of the NOEL of 0.75 g/kg of body weight per day, and a safety factor of 6, the Committee allocated a temporary ADI of 0–125 mg D-tagatose/kg of body weight.

The temporary ADI does not apply to individuals with hereditary fructose intolerance caused by deficiency in 1-phosphofructoaldolase (aldolase B) or fructose 1,6-diphosphatase. The Committee requested information on the histological examination of the adrenals, kidneys and testes of the rats from the 2-year study, by 2006.

The intake assessment prepared by the Committee at its fifty-seventh meeting is still valid.

An addendum to the toxicological monograph and a chemical and technical assessment (CTA) were prepared. The existing specifications were maintained

3.1.6 *Laccase from* Myceliophthora thermophila *expressed in* Aspergillus oryzae

The enzyme preparation under evaluation contains the active enzyme laccase, which has not been evaluated previously by the Committee. Laccase is an enzyme that catalyses the oxidation of phenolic compounds such as ortho- and para-diphenols to their corresponding quinones, with the concomitant reduction of oxygen to water. This enzyme is marketed for use in the brewing of beer to prevent the formation of off-flavour compounds, such as *trans*-2-nonenal. Laccase scavenges oxygen which otherwise would react with fatty acids, amino acids, proteins, and alcohols to form off-flavour precursors.

The laccase evaluated by the Committee is produced by pure culture fermentation of a strain of *Aspergillus oryzae* that is non-pathogenic and non-toxigenic and which has been genetically modified to carry a gene coding for laccase derived from *Myceliophthora thermophila*. This production strain was obtained from *A. oryzae* host strain How B711 (derived from the A 1560 strain) using recombinant DNA techniques and traditional mutagenesis. After fermentation, the enzyme is partially purified and concentrated, resulting in a liquid enzyme concentrate (LEC). In the final preparation, this LEC is stabilized and standardized/formulated with sorbitol, glucose, glycine, sodium lactate, potassium sorbate, and sodium benzoate.

The *A. oryzae* production strain, designated as Mt, was developed by transfection of the *A. oryzae* host strain How B711 (derived from the A 1560 strain) with two plasmids, pRaMB17.WT and pToC90. The pRaMB17.WT plasmid contains the laccase gene from the thermophilic fungus, *M. thermophila,* that is found in decaying organic matter. The laccase gene is linked to DNA regulatory sequences, a promoter and a terminator. The pRAMB17.WT plasmid also contains the *bla* gene, which confers resistance to ampicillin, and other well-characterized DNA sequences. The pToC90 plasmid contains the *amdS* gene that encodes acetamidase, which enables *A. oryzae* to metabolize acetamide in the absence of other sources of carbon or nitrogen and which is used as a selection marker. The pToC90 plasmid also contains the *bla* gene.

The selection of transformants was achieved by growing on a medium containing acetamide as the sole nitrogen source and screening for ability to produce laccase. One colony was selected and subjected to chemical mutagenesis and screening for high yield of laccase. A transformant producing an adequately high quantity of laccase was selected for use as the laccase production strain *A. oryzae* Mt.

The genetic material introduced into the production strain has been well characterized using known molecular biology methods and does not contain any sequences that would encode proteins that are toxic or that produce toxic or undesirable substances. The laccase gene is stably integrated into the *A. oryzae* genome. Although the introduced DNA contains the *bla* gene, this gene is not expressed because it is under the control of a bacterial promoter that is not functional in the eukaryotic fungus *A. oryzae*. Furthermore, the *bla* gene is stably integrated into the host genome. Thus, the laccase preparation does not contain the *bla* gene product, i.e. the enzyme β-lactamase that hydrolyses and inactivates ampicillin. No *bla* DNA was detected in the laccase preparation.

The *M. thermophila* laccase enzyme was assessed for potential allergenicity by amino acid sequence comparison with allergens listed in publicly available protein databases. No immunologically-significant sequence homology was detected. The LEC did not show skin sensitizing potential in a repeated patch test in humans.

Toxicological studies were conducted on the LEC. The materials added to the LEC for stabilization and formulation/standardization have either been evaluated previously by the Committee or are common food constituents and do not raise safety concerns. In a 13-week study in rats, no significant treatment-related effects were seen when the LEC was administered by oral gavage at doses of up to and including 10 ml LEC/kg of body weight per day. Therefore this highest dose tested (equivalent to 1700 mg total organic solids (TOS)/kg of body weight per day) is considered to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro. Studies of skin and eye irritation in rabbits did not reveal treatment-related effects.

In the brewing of beer, the laccase preparation is added during the mashing process and is denatured and inactivated during the subsequent wort-boiling stage. The beer filtration process is likely to remove the denatured enzymes along with other insoluble materials. Thus no residual LE399 α-amylase is expected to be present in food processed using this enzyme preparation.

Nevertheless, a very conservative estimate of daily intake from beer was performed with the assumption that all the TOS would persist in the final product, giving an estimated daily intake of 9 mg TOS/day (equivalent to 0.15 mg TOS/kg of body weight per day). Moreover, since the Committee is aware that laccases are receiving increasing interest for various applications other than brewing, e.g. in chewing gum, mouthwash, breath mints and toothpaste, conservative estimates of daily intakes resulting from these uses were performed, resulting in the following values: 2 mg TOS/day (equivalent to 0.35 mg TOS/kg of body weight per day for a 60 kg person) for chewing gum; 16 mg TOS/day (equivalent to 0.26 mg TOS/kg of body weight per day for a 60 kg person) for mouthwash; 12.8 mg TOS/day (equivalent to 0.21 mg TOS/kg of body weight per day for a 60 kg person) for breath mints and 3.2 mg TOS/day (equivalent to 0.05 mg TOS/kg of body weight per day for a 60 kg person) for toothpaste. The ratio between the NOEL of 1700 mg TOS/kg of body weight per day from the 13-week study of oral toxicity and the cumulative intake deriving from all these conservative estimates is nearly 2000.

The Committee allocated an ADI "not specified" to laccase from this recombinant strain of *A. oryzae*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.1.7 *Mixed xylanase, β-glucanase enzyme preparation, produced by a strain of* Humicola insolens

The mixed β-glucanase and xylanase preparation under evaluation is produced by fed-batch, submerged, pure culture fermentation of a strain of *Humicola insolens* that is non-pathogenic and non-toxigenic. This enzyme mixture has not been evaluated previously by the Committee. The enzyme preparation contains two main activities, β-glucanase and xylanase, and several secondary activities, including cellulase, hemicellulase, pentosanase and arabinase. The preparation is used in beer brewing to hydrolyse β-glucans, pentosans and other gums in order to reduce the viscosity of the solution and thereby increase the filtration rate of both wort and beer and improve beer clarity. The production strain has been selected for improved enzyme production.

The manufacturing procedure comprises a fermentation process, a purification process, a formulation process and finally quality control of the finished product. The cell mass and other solids are separated from the broth by filtration or centrifugation. Ultrafiltration and/or

evaporation are used for concentration and further purification. The liquid enzyme concentrate (LEC) is then stabilized and formulated/standardized by the addition of sorbitol, glycerol, and potassium sorbate.

Toxicological studies were conducted on the LEC. The materials added to the LEC upon stabilization and formulation/standardization have either been evaluated previously by the Committee or are common food constituents and do not raise safety concerns. In a 13-week study in rats, no significant treatment-related effects were seen when LEC was administered by oral gavage at doses of up to and including 10.2 g LEC/kg body weight per day. Therefore this highest dose (equivalent to 0.62 g total organic solids (TOS)/kg of body weight per day) is considered to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

The enzyme preparation is added during the mashing process of beer-making and the enzymes are denatured and inactivated during the subsequent wort-boiling stage. The beer filtration process is likely to remove the denatured enzymes along with other insoluble materials. The preparation may also be used in the spirits industry; again, in this case no enzymes or other organic solids are expected to be carried over into the final product because ethanol is removed by distillation from the fermentation mash containing the enzyme preparation. In conclusion, no residual enzymes are expected to be present in food processed using this enzyme preparation. The Committee is not aware of any other uses for this enzyme mixture in which the enzymes might persist in the final product.

Nevertheless, very conservative estimates of daily intakes were performed with the assumption that all the TOS would persist in final products, giving an estimated daily intake of 5.1 mg TOS/day (equivalent to 0.085 mg TOS/kg of body weight per day) for beer and 2.7 mg TOS/day (equivalent to 0.04 mg/kg of body weight per day for a 60 kg person) for spirits. Compared to the NOEL of 0.62 g TOS/kg of body weight per day from the 13-week study of oral toxicity, the margin of safety is nearly 5000.

The Committee allocated an ADI "not specified" to mixed β–glucanase/xylanase from the production strain *H. insolens*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.1.8 *Neotame*

Neotame is a dipeptide methyl ester that is intended for use in food as a sweetener and flavour enhancer in a variety of applications. Neotame, the common name for *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester, is chemically related to aspartame. It has a sweetness potency of 7000–13 000 times that of sucrose and 30–60 times that of aspartame, depending on the food matrix in which it is used. Neotame, a white/off-white powder, is manufactured from aspartame and 3,3-dimethylbutyraldehyde in a one-step chemical synthesis, which includes a reductive alkylation, followed by purification, drying, and milling. Neotame has not been evaluated previously by the Committee.

The metabolism and pharmacokinetics of neotame have been examined in mice, rats, dogs, rabbits and humans. Approximately 20–30% of orally-administered neotame is absorbed in all species studied. The major metabolic pathway for both absorbed and non-absorbed neotame is de-esterification to *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine and methanol, a reaction which is mediated by non-specific esterases. More than 95% of orally-administered neotame is metabolized. De-esterified neotame accounted for approximately 80% of the neotame administered, while other metabolites each accounted for <5%.

In studies in rats given radiolabelled neotame by gavage, radioactivity was found to be primarily confined to the stomach, gastrointestinal tract, liver, kidney, and bladder, with lower concentrations of radioactivity detected throughout the rest of the body. There was no evidence of accumulation of radioactivity in any tissue. In a study undertaken in pregnant rats, radioactivity was not detected in the fetus. Following oral administration of radiolabelled neotame to rats and dogs, 90–95% of the radioactivity was recovered in the urine and faeces within 48 hours. The major metabolite found in the urine and faeces of the rat and dog was de-esterified neotame. Unchanged neotame was not detected in rat urine but was present at 1–6% of the administered dose in the urine of dogs. Unchanged neotame was not detected in the faeces of rats or dogs. Pharmacokinetic analysis of plasma metabolites after oral administration of radiolabelled neotame to rats indicated that peak plasma concentrations occurred after 0.5 hours, followed by a rapid decline with a $t_{1/2}$ of approximately 1 hour.

Pharmacokinetic analysis of absorbed neotame in human plasma after oral administration of radiolabelled neotame indicated rapid absorption, with a maximum plasma concentration at 0.4 hours followed by rapid clearance (t, 0.6 hours). Concentrations of de-

esterified neotame in plasma peaked at 1 hour and declined with a $t_{1/2}$ of 1.5 hours. After oral administration of radiolabelled neotame to humans, 98% of the radioactivity was recovered in the urine and faeces within 72 hours. De-esterified neotame was the major metabolite in both urine and faeces. The unchanged neotame detected in the urine represented 3.3% of the administered dose, while no unchanged neotame was found in the faeces. A metabolite detected initially only in human urine (and subsequently also in female rat urine) was identified as 3,3-dimethyl-butanoyl-L-carnitine and represented 0.5–3.4% of the administered dose of neotame. On the basis of the data evaluated, the Committee considered that neotame is rapidly but only partially absorbed in all species studied, and that both absorbed and non-absorbed neotame are metabolized via well-characterized pathways to non-toxic metabolites. The major metabolite, de-esterified neotame, is itself eliminated rapidly via the urine and faeces, with no evidence of tissue accumulation.

Short-term and long-term studies on neotame have been conducted in mice, rats and dogs using a body-weight adjusted constant dose regime. In all of these studies, the major effect observed was a treatment-related decrease in body-weight gain, which was linked in most cases to a measurable decrease in food consumption, particularly at high doses. The Committee considered that this effect was due to reduced palatability of the neotame-containing diet rather than neotame-induced toxicity. This conclusion is supported by several observations. First, in the 13-week study in mice fed neotame in the diet, the high incidence of food scattering is indicative of reduced palatability of the diet. Second, the body-weight changes observed in the rat and dog 13-week studies were partially reversible when the animals were returned to a basal diet during the 4-week period at the end of the study. Third, reduced food consumption often occurred at the start of treatment at all doses, followed by some degree of adaptation as the animals adjusted to the diet. Fourth, studies were conducted over a wide range of doses (50–1000 mg/kg of body weight per day) and body-weight changes were not closely related to dose, as would be expected if the observed changes were a manifestation of treatment-related toxicity. Fifth, in the 1-year rat study, there were no changes in body-weight gain or food conversion efficiency in rapidly growing rats consuming neotame-containing diets. Sixth, when the effect of neotame on palatability of the diet was specifically examined in a preference study comparing basal diets with and without neotame at concentrations of 50–15 000 mg/kg diet, the rats showed a clear preference for the diet without neotame when the neotame concentration was ≥150 mg/kg diet. There was no significant decrease in body

weight observed in this study, but body-weight gain was reduced in males at 5000 mg/kg diet and above, when the reduced palatability significantly decreased food consumption. In the light of the above information, the Committee agreed that the NOELs for the various short-term and long-term studies of toxicity should not be assigned on the basis of decreases in body weight or body-weight gain.

Aside from the palatability-related decreases in body-weight gain, neotame was well tolerated in all species at high doses in the diet in both the short-term and long-term studies, with no clinical signs of toxicity. In the 13-week study in mice, the NOEL was 1000 mg neotame/kg of body weight per day in the diet, on the basis of an increase in liver weight relative to body weight. In the 13-week study in rats, there was a small but significant increase in serum alkaline phosphatase activity at 1000 and 3000 mg neotame/kg of body weight per day in the diet at week 13. On the basis of these changes, the NOEL was 300 mg neotame/kg of body weight per day. However, in the 1-year study in rats following exposure in utero, no increases in alkaline phosphatase activity were observed and the NOEL, based on the highest dose tested, was 1000 mg neotame/kg of body weight per day.

In the 13-week study in dogs, there were changes in clinical chemistry parameters in dogs fed ≥600 mg neotame/kg of body weight per day, including significant increases in serum alkaline phosphatase activity, which isoenzyme analysis confirmed to be of hepatic origin. The small increase in alkaline phosphatase activity observed at 200 mg/kg of body weight per day in 3 out of 4 female dogs was not considered to be toxicologically significant and a NOEL of 200 mg neotame/kg of body weight per day was established. In the 1-year study in dogs, the only significant change was an increase in alkaline phosphatase activity (hepatic isoenzyme) that was observed only at the high dietary dose (800 mg/kg of body weight per day) in both males and females. The increase in alkaline phosphatase activity was rapidly reversible and was not accompanied by changes in other parameters indicative of cholestasis or other hepatotoxicity. Nevertheless, the Committee considered this to be indicative of a treatment-related effect and it was therefore used as the basis of the NOEL of 200 mg neotame/kg of body weight per day.

In the 2-year study of carcinogenicity in mice, there was no treatment-related increase in tumour incidence at doses of up to 4000 mg neotame/kg of body weight per day. In the 2-year study of carcinogenicity undertaken in rats following exposure in utero, there was no treatment-related increase in tumour incidence at doses of up to 1000 mg neotame/kg of body weight per day.

In a study of reproductive toxicity in rats, there were no treatment-related effects on reproductive parameters (estrus cycle, mating performance, fertility, gestation time, parturition, and gestation index) or on litter size, sex ratio, offspring viability, physical development or learning at doses of up to 1000 mg neotame/kg of body weight per day. The major effect observed was a reduction in body-weight gain in treated animals compared to controls. As in the short- and long-term studies, the Committee considered this to be related to a decrease in food consumption as a result of the reduced palatability of the diet containing neotame. Mean litter body weights in treated groups in both the F_1 and F_2 generations were also reduced at day 21, probably as a result of a decrease in food consumption, compared to controls during the pre-weaning days 14–21 when pups begin to consume solid food. The significant increase in the swim time for F_1 males in a water-filled Y-maze at 1000 mg/kg of body weight per day was within the variability seen in studies of this type and was likely to be related to reduced body weight rather than reduced learning ability. The Committee considered that the NOEL in this study, on the basis of the highest dose tested, was 1000 mg neotame/kg of body weight per day.

The developmental toxicity of neotame was examined in rats and rabbits at doses of up to 1000 mg neotame/kg of body weight per day and 500 mg neotame/kg of body weight per day, respectively. In neither species was there any evidence of embryotoxicity or teratogenicity. In rats, there was an immediate but transitory decrease in food consumption resulting in lower body-weight gain in the treated animals; however, there was no significant effect on body weight or body-weight gain during gestation. In rabbits, there was no significant effect on overall food consumption, body weight or body-weight gain.

Studies of genotoxicity have examined the ability of neotame to induce gene mutations in both bacterial and mammalian cells, as well as chromosome aberrations in vitro in Chinese hamster ovary cells and in vivo in mouse bone marrow cells. There was no evidence of genotoxicity in any of the tests.

The potential pharmacological effects of neotame on the gastrointestinal system in rats, on the autonomic nervous system in guinea pig ileum, on the parameters associated with the cardiovascular, respiratory or renal systems in dogs, and on the hexobarbital-induced sleeping time in rats were examined. These studies demonstrated no evidence of pharmacological activity associated with neotame.

Studies of toleration in humans included a single-dose study in adult males, 2-week studies in both diabetic and non-diabetic male and

female adults, and a 3-month study in male and female adults. Single doses of 0.5 mg neotame/kg of body weight per day were tolerated without treatment-related signs or symptoms. In both the 2-week studies on diabetic and non-diabetic adults, there were no signs or symptoms associated with the administration of neotame. In the study in diabetic adults, treatment with neotame had no effect on plasma glucose or insulin concentrations. In both of these 2-week studies, neotame was well tolerated at doses of up to 1.5 mg/kg of body weight per day. In the 3-month study of toleration in non-diabetic adults, there were no signs or symptoms associated with the administration of neotame at doses of up to 1.5 mg/kg of body weight per day.

The major degradation product of neotame under normal storage conditions was de-esterified neotame, which accounted for 7% of the initial neotame concentration after 8 weeks storage. Three minor degradation products were formed which represented <1% of the initial neotame concentration. All of the degradation products have low acute toxicity and gave negative results in tests for genotoxicity. Furthermore, no treatment-related adverse effects were observed in a 4-week study in rats fed with a mixture of the three minor degradation products. The Committee also noted that the safety studies conducted in animals and humans with neotame would have included low levels of these degradation products.

Evaluation. On the basis of the available studies, the Committee considered neotame to be a substance of low toxicity across a range of species, including humans. Appropriate studies indicated that neotame is not carcinogenic, mutagenic, teratogenic or associated with any reproductive/developmental toxicity. The only consistent treatment-related effect observed was an increase in serum alkaline phosphatase activity in the 13-week and 1-year studies in dogs fed neotame in the diet. While the increase in alkaline phosphatase was moderate, reversible, and was not accompanied by other evidence of liver toxicity, the observed change was reproducible, of high statistical significance and treatment-related. The Committee agreed there were insufficient data to discount this effect and therefore accepted the dog as the most sensitive species with a NOEL for neotame of 200 mg/kg of body weight per day, on the basis of the 1-year study in dogs fed neotame in the diet. Studies of toleration in humans confirmed the lack of any treatment-related signs or symptoms at doses of up to 1.5 mg neotame/kg of body weight per day in diabetic and non-diabetic subjects. Although a 1-year study is not considered to be a long-term study in dogs, an additional safety factor was not considered necessary, in light of the human data.

The Committee established an ADI of 0–2 mg/kg of body weight for neotame on the basis of a NOEL of 200 mg/kg of body weight per day in a 1-year study in dogs and a 100-fold safety factor.

Assessment of intake. Neotame is intended for use as a tabletop sweetener as well as in a large variety of solid and liquid foods. Conservative calculations based on its lowest sweetness potency (7000 times that of sugar) suggest that intakes of 2 mg neotame/kg of body weight per day would correspond to the replacement of 840 g of sugar in the diet of a 60 kg adult. Therefore even a total replacement of sugar with neotame would not lead to the ADI being exceeded.

The Committee agreed that the ADI also applied to those individuals with phenylketonuria since the formation of phenylalanine from the normal use of neotame would not be significant in relation to this condition.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.1.9 *Polyvinyl alcohol*

Polyvinyl alcohol is a synthetic resin that is prepared by polymerization of vinyl acetate, followed by partial hydrolysis of the resulting ester in the presence of an alkaline catalyst. The number of acetate groups in polyvinyl alcohol is determined by the degree of hydrolysis (86.5–89.0% hydrolysis for this food additive specification). Polyvinyl alcohol is used as a coating, binder, sealing and surface finishing agent in food products such as dairy-based desserts, confectionery and cereal products and dietary supplement tablets, in the range of 0.2–1.8% by weight.

Polyvinyl alcohol has not been evaluated previously by the Committee.

The Committee examined a large database of studies of the toxicity of polyvinyl alcohol after administration by various routes to a number of species. Much of the information was found to be dated, not relevant to oral administration, or from studies that were not conducted in accordance with GLP principles or that were conducted with material that did not comply with the specification for polyvinyl alcohol as prepared at the current meeting. Nonetheless, the Committee was able to conclude that polyvinyl alcohol was very poorly absorbed after oral administration, that the acute oral toxicity was generally very low and that, taken as a whole, the results were consistent with very low toxicity and showed no evidence for carcinogenicity.

The Committee also considered a number of recent studies, which had been performed with preparations of polyvinyl alcohol complying with the food additive specification, and which met appropriate GLP standards. A 90-day study of toxicity in rats treated orally revealed no toxicity at doses of up to 5000 mg of polyvinyl alcohol/kg of body weight per day. No significant differences in body weight, neurobehaviour, haematology, blood coagulation, clinical chemistry, urinalysis, organ weight or macroscopic pathology were observed. Microscopic examination of an extensive range of organs and tissues, including the gastrointestinal tract, from the control group and the groups given high doses showed no evidence for treatment-related pathology. This was the only short-term study provided, in any species, that was considered to be directly relevant to the safety evaluation of oral exposure to polyvinyl alcohol.

No toxicity was observed in a two-generation study of reproduction in rats in which the parental, first and second generations received a maximum dose of polyvinyl alcohol of 5000 mg/kg of body weight per day.

In both the 90-day and the two-generation studies, the most notable observations were loose or unformed stools in the groups given higher doses of polyvinyl alcohol, this being attributed to the high intestinal concentration of unabsorbed test material and increased food consumption in these groups. The Committee considered that these observations did not represent adverse effects.

There was no evidence for genotoxicity in a battery of tests undertaken with preparations of polyvinyl alcohol complying with the food additive specification.

The Committee also noted a report that more polyvinyl alcohol was absorbed after intravaginal than oral administration, and reviewed a study involving the intravaginal administration of polyvinyl alcohol to mice, 5 days per week for 104–105 weeks, that provided no evidence for local or systemic carcinogenic activity.

Evaluation. The Committee identified a NOEL of 5000 mg/kg of body weight per day for polyvinyl alcohol, on the basis of the maximum dose tested from both the 90-day and the two-generation studies in rats. The Committee noted the lack of reports of any apparent toxic or carcinogenic effects in studies concerning polyvinyl alcohol as a whole, the very poor absorption of preparations of polyvinyl alcohol complying with the specification following oral administration and the absence of any effects on the gastrointestinal tract in the 90-day study in rats. Despite the absence of long-term studies or studies in a second

species, the Committee considered the data adequate for the establishment of an ADI. The Committee therefore established an ADI for polyvinyl alcohol of 0–50 mg/kg body weight per day, on the basis of the NOEL of 5000 mg/kg body weight per day from the 90-day and two-generation studies in rats, with a safety factor of 100.

Assessment of intake. The intake estimate based on use levels provided by the sponsor and national food consumption data (from the USA) shows a mean ingestion of around 0.5 g/day, equivalent to 8.3 mg/kg of body weight per day for a 60 kg adult. Extreme intakes based on Australian and New Zealand consumption data during one day were shown to reach 2–2.5 g/day at the 97.5th percentile, corresponding to 33 and 42 mg/kg of body weight per day respectively.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.1.10 *Quillaia extracts*

Unpurified quillaia extracts were reviewed by the Committee at its twenty-sixth and twenty-ninth meetings (Annex 1, references *59*, *70*). An ADI of 0–5 mg/kg of body weight for unpurified quillaia extracts was allocated at the latter meeting. At the fifty-seventh meeting (Annex 1, reference *154*), the Committee reviewed quillaia extracts and adopted tentative specifications. Owing to outstanding queries concerning the relevance of a 90-day study in rats that had become available to the Committee after the ADI was allocated, and to a lack of information on the composition of the saponin and non-saponin fractions of the preparations used in this study and in the previous 90-day studies, the ADI was made temporary pending further clarification. No ADI was allocated to the semi-purified product.

At its present meeting, the Committee reviewed new information related to the chemical charaterization of quillaia extracts and further information related to the specifications, as requested at the fifty-fifth and the fifty-seventh meetings.

The Committee emphasized again that the evaluation of (unpurified) quillaia extract at its twenty-sixth and twenty-ninth meetings was limited to the commercial product that had been tested at that time. It was agreed that two separate specifications should be developed, one for quillaia extract (type 1) previously designated as “unpurified”, the other for quillaia extract (type 2), previously designated as “semi-purified”, to replace the existing specifications.

A chemical and technical assessment (CTA) for products of type 1 and type 2 was prepared.

Quillaia extract (type 1)

Quillaia extract (type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Q. saponaria* Molina (family Rosaceae), followed by clarification and purification. The product contains triterpenoid saponins consisting predominantly of glycosides of quillaic acid. Other substances that occur in bark tissue, such as polyphenols, tannins, carbohydrates and salts, are also present.

The Committee noted that a considerable amount of data was available that characterizes the composition of the saponin fraction in more detail. Four saponins (QS-7, QS-17, QS-18, QS-21) have been identified as the major constituents of that fraction. These saponins are also considered to be representative of the total saponin content and their quantification is henceforth the basis for the assay, with a total saponin content of 20–26%. As quillaia extract is a product of natural origin, it was recognized that the proportions of these four compounds (and other saponins) may vary.

The non-saponin fraction was described in the literature as consisting of 10–16% polyphenols, ~7% tannins, ~14% salts (mainly calcium oxalate), ~32% carbohydrates (mainly sugars) and ~5% fat. The Committee noted that these figures were only approximate and understood that the content of these plant constituents might vary between batches.

The question of whether the product that had been tested toxicologically was representative of the material currently being marketed was considered by the Committee, with reference to a recent study (*3*) on the variability of saponins in quillaia extracts. Bark extracts obtained from thirty different natural, not cultivated, trees were screened in a search for plant varieties that exhibit a specific saponin spectrum. The authors identified two separate profiles in this random group of isolates, of which one (profile A), showing two predominant saponin peaks, appeared to be a subset of the other (profile B). Profile A and profile B were each observed in 50% of the trees sampled. Comparison with the saponin profile of a commercial extract revealed that the latter displayed a mixture of both profiles. This mixed profile was attributed to the mixing of barks from both tree types during processing. The observed variation in saponin profiles between trees was attributed by the authors to genetic factors, as neither soil, altitude, or age of trees or sampled tissues correlated with the composition of saponins; in addition, it was observed that trees from the same location could display different profiles. The authors showed that the two major peaks present in profile A were identical to those of the saponins QS-18 and QS-21, described 10 years earlier.

The Committee also received confirmation from a European manufacturer that no significant changes had been introduced to the manufacturing process since 1975, the year when purity criteria for quillaia extract were laid down by the Emulsifiers and Stabilisers in Food Regulations of the United Kingdom. The material tested before 1982 complied with these specifications.

Assessment of intake. The intake estimates made on the basis of consumption data for soft drinks likely to contain quillaia extract (type 1) were prepared by the Committee at its fifty-seventh meeting and remain valid.

Evaluation. In view of the new data suggesting that the variability of the saponin content in single trees is determined genetically, a feature which is assumed not to change significantly within a period of 20 years within a particular population of trees, and considering further that mixing of batches with different saponin profiles would take place during processing, the Committee agreed that the material tested toxicologically was representative of the material specified as quillaia extract (type 1). The "temporary" assignment for the ADI of 0–5 mg/kg of body weight was removed.

Quillaia extract (type 1) is used in food applications, primarily for its foaming properties. Most of the products available are diluted with carriers, such as lactose, maltodextrin or maltitol, in order to standardize the saponin concentration and thereby achieve a consistent range of foam-building strength. Concentrations at which the undiluted extract is used in soft drinks were reported to be approximately 100 ppm (dry basis). For a more recently developed category of frozen carbonated beverages, concentrations of up to 250 ppm have been reported.

New specifications were prepared for quillaia extract (type 1).

Quillaia extract (type 2)

The initial manufacturing steps of quillaia extract (type 2) are the same as those for type-1-extract: an aqueous extraction of the inner bark or wood of *Q. saponaria* Molina (family Rosaceae) is subjected to several clarification and purification steps. Three- to four-fold concentrations of saponins are subsequently achieved by ultra-filtration or affinity chromatography, yielding more purified extracts with saponin contents of 75–80%. The non-saponin fraction of the type-2 extracts contains same minor components as that of the type-1-extracts.

The Committee noted that the saponin profile of quillaia extract (type 2) obtained using ultra-filtration was similar to the saponin profile

(QS-7, QS-17, QS-18, QS-21) displayed when the extract was tested according to the assay method described in the specifications. As quillaia extract is a product of natural origin, it was also recognized that these four compounds (and other saponins) may be present in varying proportions, however, at higher concentrations. No information on saponin profile was available for the material after affinity chromatography, since the manufacturer applied a method involving partial hydrolysis of the saponins in the sample. Concentrations of saponins obtained using this method could not be related to those obtained using the method described in the specifications.

Limited data were available on the composition of the non-saponin fraction. The manufacturer claimed that the extract produced by ultra-filtration contained <8% polyphenols and <8% tannins. It was estimated that the extract produced using affinity chromatography contained <10% polyphenols and tannins, <5% salts and <5% sugars.

Quillaia extract (type 2) is used in Japan as an emulsifier for preparations containing lipophilic colours or flavours that are added to, for example, soft drinks, fermented vegetables and dressings. The typical concentration of type-2 extract in these food products was claimed to be not more than 10 ppm.

Assessment of intake. The Committee recommended that production data for quillaia extract (type 2) and consumption data for products containing the additive should be collected, in order to assess the per capita intake of the additive in different regions. In addition, marketing data related to the percentage of consumers of the products would be necessary in order to refine the estimate.

Evaluation. The Committee concluded that the limited information on the qualitative and quantitative composition of the type-2 extracts prevented the Committee from considering the establishment of an ADI or assessing the need for additional toxicological studies.

New specifications were prepared for quillaia extract (type 2).

3.1.11 *Xylanase from* Thermomyces lanuginosus *expressed in* Fusarium venenatum

The enzyme preparation under evaluation contains the enzyme xylanase, which has not been evaluated previously by the Committee. Xylanase is produced by submerged fermentation of a strain of *Fusarium venenatum* that is non-pathogenic and non-toxigenic (under conditions consistent with good manufacturing practice), and which has been genetically modifed to carry a gene encoding a xylanase from *Thermomyces lanuginosus,* inserted by recombinant DNA tech-

niques. The enzyme is subsequently partially purified and concentrated, resulting in a liquid enzyme concentrate (LEC). In the final preparation, this LEC is stabilized and standardized/formulated with sodium chloride, dextrin, sorbitol, and wheat solids.

The enzyme produced is an endo-xylanase, which hydrolyses xylosidic linkages in the arabinoxylans into smaller oligosaccharides. The enzyme preparation is used in baking applications to increase the elasticity of the gluten network, improving handling and stability of the dough. The enzyme is denatured and inactivated during bread baking.

The production strain LyMC4.B was developed from the *F. venenatum* host strain MLY3. The MLY3 strain is a spontaneous mutant of the CC1-3 strain that, in turn, is a spontaneous mutant of the *F. venenatum* strain used in the production of mycoprotein marketed for human consumption since 1985 under the trade name "Quorn." The MLY3 strain was transfected with the expression plasmid pjRoy36 containing the xylanase gene from *T. lanuginosus* and the *bar* gene from *Streptomyces hygroscopicus*. The *bar* gene confers resistance to the herbicide phosphinothricin and serves as a selectable marker. A single transformed colony producing xylanase was selected. The selected strain was designated as JRoy36-19.B.

The JRoy36-19.B strain was subsequently transfected with DNA containing the *amdS* gene from *Aspergillus nidulans*. The *amdS* gene was flanked by specific sequences of the *F. venenatum tri5* gene and replaced the *tri5* gene in the JRoy36-19.B strain. One of the transformants was designated as LyMC4.B and was used as a xylanase production strain.

Deletion of the *tri5* gene served to inactivate the biochemical pathway by which mycotoxins (trichothecenes) are synthesized. To confirm that the *tri5* gene had been deleted, the transformed strain was evaluated for production of diacetoxyscirpenol (DAS), the major trichothecene produced by non-engineered strains of *F. venenatum*: no DAS was detected. *F. venenatum* is capable of producing other secondary metabolites, such as culmorins, enniatins and fusarins. Analyses performed on conventional *F. venenatum* under conditions known to be optimal for production of these secondary metabolites revealed them to be present only at very low concentrations. As it is very unlikely that the production strain LyMC4.B produces these secondary metabolites to the same extent under industrial fermentation conditions, the concentrations of these secondary metabolites, if present, are considered to be of no toxicological relevance.

The xylanase was assessed for potential allergenicity by amino acid sequence comparison with known allergens listed in publicly-available protein databases. No immunologically-significant sequence homology was detected.

Toxicological studies were conducted on the LEC. The materials added to the LEC for stabilization and formulation/standardization have either been evaluated previously by the Committee or are common food constituents and do not raise safety concerns. In a 13-week study in rats, no significant treatment-related effects were seen when LEC was administered by oral gavage at doses of up to and including 10 ml LEC/kg of body weight per day. Therefore this highest dose tested (equivalent to 1.1 g total organic solids (TOS)/kg of body weight per day) was considered to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

Conservative estimates of daily intakes resulting from the use of xylanase in bakery goods are 6.9 mg TOS/day (equivalent to 0.12 mg TOS/kg of body weight per day). Compared to the NOEL of 1.1 g TOS/kg of body weight per day derived from the 13-week study of oral toxicity, the margin of safety is nearly 10 000.

The Committee allocated an ADI "not specified" to xylanase from this recombinant strain of *F. venenatum*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and specifications were established.

3.2 Revision of specifications

3.2.1 *β-Carotene from* Blakeslea trispora

At the fifty-seventh meeting, the specifications for β-carotene from *Blakeslea trispora* were made tentative and the Committee requested additional information regarding the analysis of residual solvents. At the present meeting, the Committee considered the methods supplied, and formulated a general method of analysis for residual solvents, using head-space gas chromatography with flame ionization detection (FID). The existing tentative specifications were revised to refer to this general method and the "tentative" designation was removed.

3.2.2 *Magnesium silicate (synthetic)*

This additive was on the agenda of the sixty-first meeting in response to a request by the Codex Committee on Food Additives and

Contaminants at its Thirty-fourth Session (*4*) that the Expert Committee examine evidence for its functional use as a filtering aid and adsorbent.

The evidence provided showed that magnesium silicate removes free fatty acids and other polar compounds from used cooking oils. If magnesium silicate is mixed into a used frying oil, polar compounds (such as fatty acids) are adsorbed and held for removal by filtration. Regular filtration through magnesium silicate can extend the usage time of a frying oil by ≥50%, depending on the oil used and the type and amount of food fried. Restaurants typically use ≤1% by weight of this filtering aid, whereas industrial fryers use ≤2% by weight. The magnesium silicate is discarded after it has been used to remove impurities.

A chemical and technical assessment (CTA) was prepared; new information on the method of manufacture was provided and was also incorporated into the revised specifications.

3.2.3 *Monomagnesium phosphate and trisodium diphosphate*

At its fifty-seventh meeting (Annex 1, reference *154*), the Committee designated the specifications for monomagnesium phosphate and trisodium diphosphate as tentative and information was requested on limits and methods for the loss on drying and loss on ignition, and on the assay method for the hydrates.

For trisodium diphosphate, information on limits and method for loss on ignition for the monohydrate and on loss on drying for the anhydrous material was received. The specifications were revised and the Committee maintained the tentative status, pending information on levels and the method for loss on drying for the monohydrate.

For monomagnesium phosphate, no information was received. The tentative status of the specifications was maintained.

The information on the method for loss on drying for the hydrates is necessary in order to express the assay for these additives in terms of dry weight.

The Committee decided that the tentative specifications for the two additives would be withdrawn if the requested information was not received before the end of the year 2004.

A chemical and technical assessment (CTA) for both phosphates was prepared.

3.2.4 *Natamycin*

The tentative specifications for natamycin were revised in response to the queries raised by the Committee at its fifty-seventh meeting (Annex 1, reference *154*). After receipt of information regarding the measurement of pH, loss on drying, specific rotation, lead content and the method of assay, the Committee decided to modify the method by which pH is measured, by removing the step using dimethylformamide in favour of a method using a suspension in water. The Committee removed the "tentative" designation from the specifications.

3.2.5 *Sucrose esters of fatty acids*

Sucrose esters of fatty acids consist of mono-, di- and tri-esters of sucrose with fatty acids commonly occurring in food. They are prepared from sucrose and methyl or ethyl esters of food fatty acids by esterification in the presence of a catalyst, or by extraction from sucroglycerides. The substance was placed on the agenda in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-fourth Session that the specifications should be revised. Methods of analysis for the determination of residual levels of solvents (dimethylformamide, methanol, isopropanol, isobutanol, ethyl acetate and methyl ethyl ketone) used in the manufacture of the substance were revised to include more modern gas chromatographic conditions than those included in the current specifications, i.e. including the use of capillary columns. The revised methods submitted to the Committee have been thoroughly validated and the Committee found them suitable for their intended purposes. Methods of analysis for the determination of free sucrose and solvent residues in addition to those noted above (dimethyl sulfoxide and propylene glycol) that are currently included in the specifications may need revision to bring them up to date. For example, the methods specify the use of packed gas chromatography columns and instruments that are now considered to be obsolete (see general consideration 2.5.2). The existing specifications were revised.

3.2.6 *Talc*

Talc was placed on the agenda of the present meeting following a request for the revision of the specifications. After considering the information provided, the Committee revised the method of analysis for the determination of acid-soluble substances to include temperature conditions for the digestion of the sample. A new provision and method of analysis for amphiboles and serpentines for the detection of asbestos was also included in the specifications.

3.3 Revision of limits for metals in food additives

The Committee reviewed the limits for heavy metals in the specifications for a group of food additives, continuing the 5-year programme initiated at the fifty-fifth meeting (Annex 1, reference *149*). At its present meeting, the Committee reviewed limits for heavy metals in 39 food additives. The functional uses of the additives reviewed included absorbent, antioxidant, antifoaming, carrier solvent, clouding agent, colour retention, emulsifier, and sequestrant applications. The outdated tests for heavy metals (as lead) in these food additive specifications were replaced with appropriate limits for individual metals of concern (see Table 1). Where no data were submitted, the Committee followed the guidelines established at previous meetings.

Eighty-eight substances remain to be reviewed at future meetings.

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

Five groups of flavouring agents and additional compounds for two previously published groups were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154* and *160*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference 122). The structural classes are as follows:

- Class I. Flavouring agents that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity by the oral route.
- Class II. Flavouring agents 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. Flavouring agents that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity.

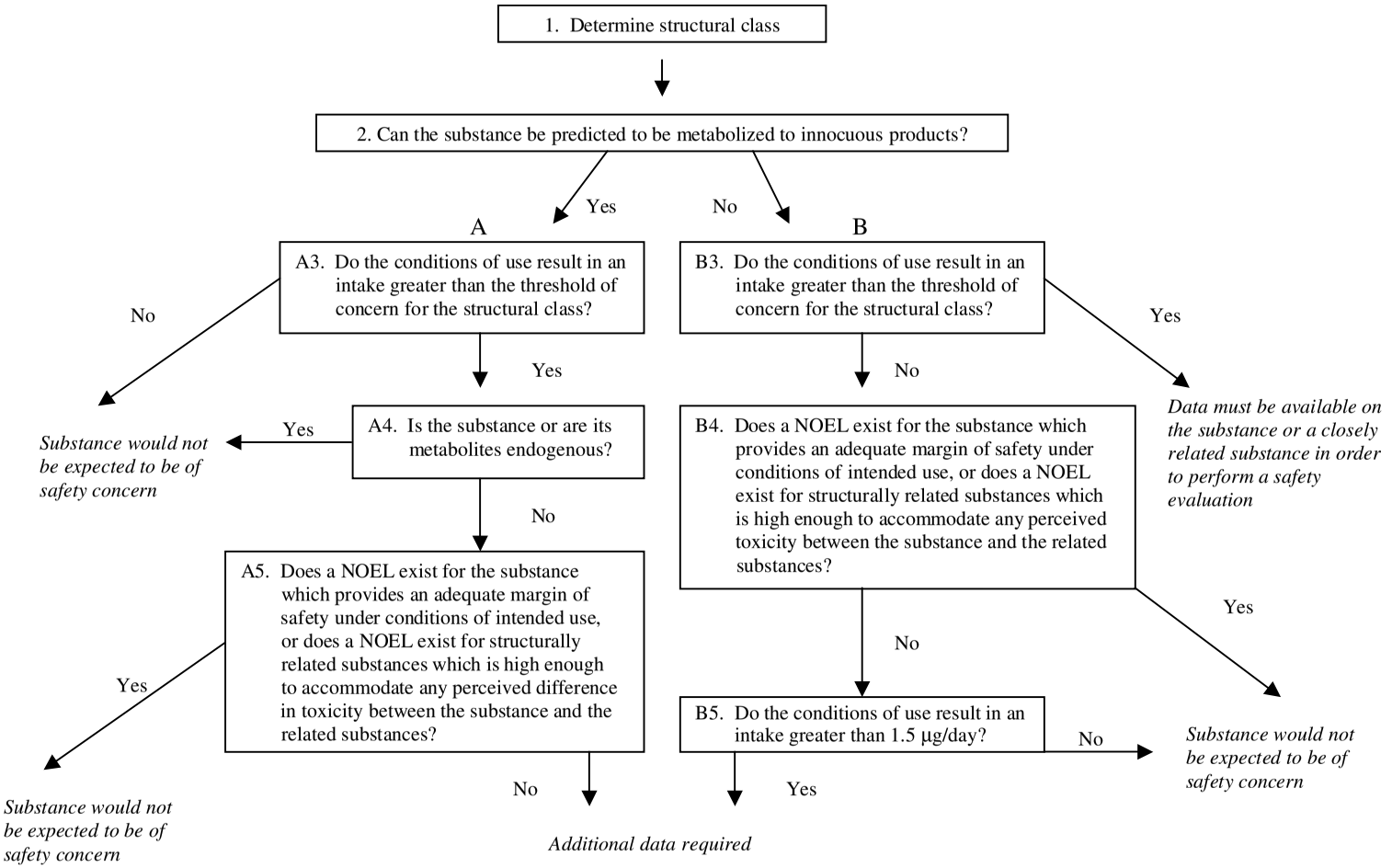
A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting:

Table 1
Limits for metals in food additives

Additive name	INS No.	Limits (mg/kg)			
		Arsenic	Lead	Cadmium	Mercury
Activated carbon	—	3	5	—	—
Aluminium potassium sulfate	522	—	5	—	—
Aluminium sulfate (anhydrous)	520	—	5	—	—
Ascorbic acid	300	—	2	—	—
Ascorbyl palmitate	304 (i)	—	2	—	—
Ascorbyl stearate	304 (ii)	—	2	—	—
Bone phosphate	542	3	2	—	—
Butylated hydroxyanisole (BHA)	320	—	2	—	—
Butylated hydroxytoluene (BHT)	321	—	2	—	—
Calcium ascorbate	302	—	2	—	—
Calcium disodium ethylenediaminetetraacetate	385	—	2	—	—
Cupric sulfate	519	—	10	—	—
Dilauryl thiodipropionate	389	—	2	—	—
Disodium ethylenediaminetetraacetate	386	—	2	—	—
Dodecyl gallate	312	—	2	—	—
Erythorbic acid	315	—	2	—	—
Ethyl protocatechuate	—	—	2	—	—
Ferrous lactate	585	—	1	—	—
Isopropyl citrate mixture	384	—	2	—	—
Lecithin	322	—	2	—	—
Octyl gallate	311	—	2	—	—
Polydimethyl siloxane	900	—	1	—	—
Polyethylene glycols	1521	—	1	—	—
Potassium lactate solution	326	—	2	—	—
Potassium polyphosphates	452 (ii)	3	4	—	—
Propyl gallate	310	—	2	—	—
Sodium aluminium phosphate, acidic	541 (i)	3	2	—	—
Sodium ascorbate	301	—	2	—	—
Sodium caseinate	—	—	2	—	—
Sodium erythorbate	316	—	2	—	—
Sodium lactate (solution)	325	—	2	—	—
Stannous chloride	512	—	2	—	—
Sucrose acetate isobutyrate	444	—	2	—	—
Tertiary butylhydroquinone	319	—	2	—	—
Thiodipropionic acid	388	—	2	—	—
Tocopherol concentrate, mixed	307 b	—	2	—	—
Tocopherol concentrate, mixed	307 a	—	2	—	—
Tocopherol, dl-α	307 c	—	2	—	—
Triethyl citrate	1505	—	2	—	—

INS: International Numbering System; —: not applicable.

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



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

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. 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 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 using the data from these surveys to estimate intakes of flavouring agents, it was assumed that only 60% of the total amount used is reported in Europe and 80% of the amount used is reported in the USA and that the total amount used in food is consumed by only 10% of the population.

$$\text{Intake (μg per person per day)} = \frac{\text{annual volume of production (kg)} \times 10^9 (\mu g/kg)}{\text{population of consumers} \times 0.6 \text{ or } 0.8 \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

Several of the flavouring agents that were evaluated at the present meeting were not included in the above surveys or were placed on the market after the surveys were conducted. Intakes of these flavouring agents were estimated on the basis of anticipated use by the manufacturer in the USA, and the standard formula was applied.

4.1.1 *Alicyclic, alicyclic-fused and aromatic-fused ring lactones*

The Committee evaluated a group of 16 flavouring agents (see Table 2) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). This group included:

— a group of four aliphatic lactones (Nos 1157–1160) that form open-chain acyclic hydroxycarboxylic acids upon hydrolysis[1];
— a group of six γ- or δ-lactones fused to an alicyclic ring (cyclohexyl or decalin ring systems) (Nos 1161–1166); and
— a group of six γ- or δ-lactones fused to a benzene ring (Nos 1167–1172).

The Committee had previously evaluated one member of the group, dihydrocoumarin (No. 1171), at its thirty-fifth meeting (Annex 1, reference *88*), but no ADI was established. The Committee noted that metabolites of dihydrocoumarin have been identified in rabbit urine. The toxicological data considered were derived from studies of acute toxicity in mice, rats, and guinea pigs, a 14-week study in rats in which the dosage was uncertain owing to loss of the test substance during storage, a 90-day study in rats treated with a single dose, and a study in which three dogs were treated with one of two doses for 2 years (without a control group). No adverse effects were reported, but the Committee considered these data to be inadequate. At the thirty-fifth meeting, the Committee stated that the results of a short-term study in a rodent species and metabolic studies to determine the extent of conversion of dihydrocoumarin to coumarin would be needed before dihydrocoumarin could be re-evaluated.

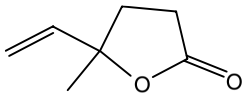
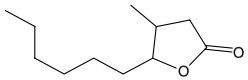
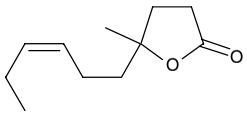
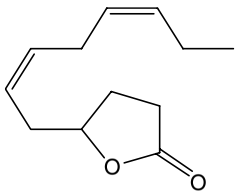
Eight of the 16 flavouring agents in this group (Nos 1157, 1162–1164, 1168–1171) have been reported to occur naturally in foods and have been detected in rose-apple, celery stalks, soya bean, black and green tea, and peppermint oil.

Estimated daily per capita intake

The total annual volume of production of the 16 flavouring agents in this group is approximately 12 200 kg in Europe and 10 000 kg in the

[1] A group of 35 aliphatic lactones of similar structure was evaluated at the forty-ninth meeting of the Committee (Annex 1, reference 132).

Table 2
Summary of results of the safety evaluations of alicyclic, alicyclic-fused and aromatic-fused ring lactones used as flavouring agents[a]

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[b]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments based on predicted metabolism	Conclusion based on current intake
Structural class I						
4-Hydroxy-4-methyl-5-hexenoic acid γ-lactone	1157	1073-11-6 	No Europe: ND USA: 3	NR	See note 1	No safety concern
(+/–) 3-Methyl-γ-decalactone	1158	67663-01-8 	No Europe: ND USA: 5	NR	See note 1	No safety concern
4-Hydroxy-4-methyl-7-*cis*-decenoic acid γ-lactone	1159	70851-61-5 	No Europe: ND USA: 13	NR	See note 1	No safety concern
Tuberose lactone	1160	153175-57-6 	No Europe: ND USA: 11	NR	See note 1	No safety concern

Structural class III

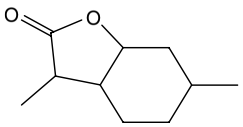
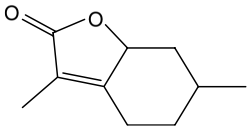
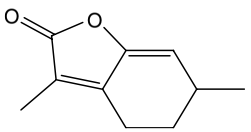
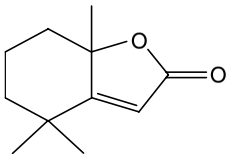
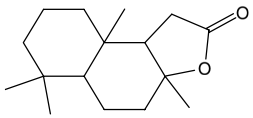
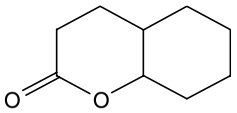
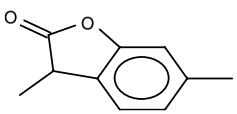
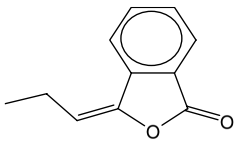
Dihydromintlactone	1161	92015-65-1 	No Europe: ND USA: 12	NR	See note 2	No safety concern
Mintlactone	1162	13341-72-5 	No Europe: 4 USA: 9	NR	See note 2	No safety concern
Dehydromenthofurolactone	1163	75640-26-5 	No Europe: 2 USA: 9	NR	See note 3	No safety concern
(+/–)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid γ-lactone	1164	15356-74-8 	No Europe: ND USA: 0.9	NR	See note 2	No safety concern
Sclareolide	1165	564-20-5 	No Europe: 1 USA: 6	NR	See note 2	No safety concern

Table 2 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[b]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments based on predicted metabolism	Conclusion based on current intake
Octahydrocoumarin	1166	4430-31-3 	No Europe: ND USA: 0.07	NR	See note 4	No safety concern
Structural class III						
2-(4-Methyl-2-hydroxyphenyl)propionic acid γ-lactone	1167	65817-24-5 	No Europe: ND USA: 2	Yes. The NOELs of 5.42 (males) and 6.55 (females) mg/kg of body weight per day for the related substance 3-propylidenephthalide (No. 1168) are >100000 times the estimated intake of 2-(4-methyl-2-hydroxyphenyl)propionic acid γ-lactone in the USA (0.03μg of body weight per day) when used as a flavouring agent	See note 5	No safety concern
3-Propylidenephthalide	1168	17369-59-4 	No Europe: 20 USA: 52	Yes. The NOELs of 5.42 (males) and 6.55 (females) mg/kg of body weight per day are >1000 times the estimated intakes of 3-	See note 6	No safety concern

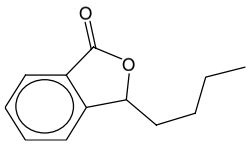
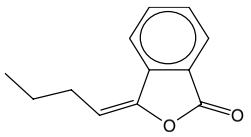
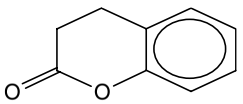
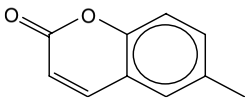
				propylidenephthalide in Europe (0.3μg/kg of body weight per day) and in the USA (0.9μg/kg of body weight per day) when used as a flavouring agent		
3-*n*-Butylphthalide	1169	6066-49-5 	No Europe: 0.6 USA: 0.4	Yes. The NOELs of 5.42 (males) and 6.55 (females) mg/kg of body weight per day for the related substance 3-propylidenephthalide (No. 1168) is >100000 times the estimated intakes of 3-*n*-butylphthalide in Europe (0.01μg/kg of body weight per day) when used as a flavouring agent	See note 6	No safety concern
3-Butylidenephthalide	1170	551-08-6 	No Europe: 10 USA: 7	Yes. The NOELs of 5.42 (males) and 6.55 (females) mg/kg of body weight per day for the related substance 3-propylidenephthalide (No. 1168) are >10000 times the estimated intakes of 3-butylidenephthalide in Europe (0.2μg/kg of body weight per day) and in the USA (0.1mg/kg of	See note 6	No safety concern

Table 2 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[b]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments based on predicted metabolism	Conclusion based on current intake
				body weight per day) when used as a flavouring agent		
Dihydrocoumarin	1171	119-84-6 	Yes Europe: 1415 USA: 1111	Yes. Several safety studies are available as discussed in the text	See note 7	No safety concern
6-Methylcoumarin	1172	92-48-8 	Yes Europe: 298 USA: 96	Yes. Several safety studies are available as discussed in the text	See note 8	No safety concern

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

[a] *Step 2*: Ten flavouring agents (Nos 1157–1166) in this group are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the decision tree. The aromatic-fused ring lactones (Nos 1167–1172) are in structural class III; limited metabolic data exists for this subgroup of flavouring agents. The evaluation of these six flavouring agents therefore proceeded via the B-side of the decision tree.

[b] The thresholds for human intake for structural classes I and III are 1800 and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intake of flavouring agents in structural class I is 32 μg per day in the USA. The combined per capita intake of flavouring agents in structural class III is 1751 μg per day in Europe and 1305 μg per day in the USA.

Notes to Table 2:

[1] Hydrolysed to a hydroxycarboxylic acid, followed by β-oxidative cleavage to yield metabolites that are completely metabolized in the citric acid cycle.

[2] Hydrolysed to a hydroxycarboxylic acid, followed by excretion; oxidation of ring substituents or the ring itself to yield polar hydroxylated metabolites that may be excreted in the urine.

[3] Hydrolysed to a ketoacid, followed by excretion as the glucuronic acid conjugate or oxidation of ring substituents to yield polar hydroxylated metabolites that may be excreted in the urine.

[4] Hydrolysed to a hydroxycarboxylic acid, followed by β-oxidative cleavage to a polar hydroxyacid that may be excreted free or conjugated with glucuronic acid.

[5] Hydrolysed to hydroxycarboxylic acid, followed by glucuronic acid or glycine conjugation.

[6] Hydrolysed to hydroxycarboxylic acid or keto carboxylic acid, followed by excretion as the glucuronic acid or glycine conjugate.

[7] Hydrolysed followed by β-oxidation to yield *o*-hydroxybenzoic acid which is excreted primarily in the urine unchanged or as the glycine conjugate.

[8] Oxidized to yield 7-hydroxy-6-methylcoumarin via ring hydroxylation or coumarin-6-carboxylic acid via methyl group oxidation. Excretion of these conjugates either in the free form or as glycine conjugates.

USA. More than 80% of the total annual volume of production in Europe and more than 84% in the USA is accounted for by dihydrocoumarin (No. 1171). The estimated daily per capita intakes in Europe and the USA for dihydrocoumarin are 1.4 and 1.1 mg, respectively. The daily per capita intakes of all the other flavouring agents in the group are in the range of 0.07–298 μg, most values being at the lower end of this range. The daily per capita intake of each agent in Europe and in the USA is reported in Table 2.

Absorption, distribution, metabolism and elimination

Lactones are formed by acid-catalysed intramolecular cyclization of 4- or 5-carbon hydroxycarboxylic acids to yield five-(γ–) or six-(δ–) membered lactone rings, respectively. The stability of the lactone ring in an aqueous environment is pH-dependent. In blood, lactones would hydrolyse rapidly to the open-chain hydroxycarboxylic acid. On the basis of the results of studies of structurally related lactones, the four aliphatic lactones (Nos 1157–1160) in the group can be expected to hydrolyse to the corresponding hydroxycarboxylic acid, and then undergo β-oxidative cleavage to yield metabolites that are completely metabolized in the citric acid cycle.

The metabolic options open to lactones fused to alicyclic rings (Nos 1161–1166) include excretion as the open-chain hydroxycarboxylic acid derivative, hydroxylation of ring alkyl substituents producing polar metabolites that may be excreted, or oxidative degradation of the carboxylic acid side-chain to yield polar alicyclic or aromatic carboxylic acids that are excreted unchanged or in conjugated form.

Metabolic pathways available to aromatic fused-ring lactones (Nos 1167–1172) include excretion as the glycine or glutamine conjugates of the open-chain hydroxycarboxylic acid derivative, or oxidation or reduction of the side-chain and subsequent excretion as the glucuronic acid conjugate.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned four of the 16 agents (Nos 1157–1160) to structural class I. The remaining 12 agents (Nos 1161–1172) were assigned to structural class III.

Step 2. Ten flavouring agents (Nos 1157–1166) in this group are expected to be metabolized to innocuous products. The evaluation of

these flavouring agents therefore proceeded via the A-side of the decision-tree.

Limited metabolic data exist for the aromatic-fused ring lactones (Nos 1167–1172). The evaluation of these six flavouring agents therefore proceeded via the B-side of the decision tree.

Step A3. The estimated daily per capita intakes of all four of the flavouring agents in structural class I (Nos 1157–1160) and six agents (Nos 1161–1166) in structural class III are below the threshold for concern (i.e. 1800 μg/day for class I and 90 μg/day for class III). The Committee concluded that these 10 substances would not be expected to be of safety concern at current estimated levels of intake as flavouring agents.

Step B3. The estimated daily per capita intakes of four of the flavouring agents in structural class III are below the threshold for concern for their class (i.e. 90 μg/day). Accordingly, the evaluation of these four agents proceeded to step B4.

The estimated daily per capita intakes of the remaining two substances in structural class III, dihydrocoumarin (No. 1171) and 6-methylcoumarin (No. 1172), exceed the threshold of concern for their class (i.e. 90 μg/day). The estimated intake of dihydrocoumarin is 1415 μg/person per day in Europe and 1111 μg/person per day in the USA. The estimated intake of 6-methylcoumarin (No. 1172) is 298 μg/person per day in Europe and 96 μg/person per day in the USA. In accordance with the Procedure, more extensive data are needed to perform a safety evaluation of flavouring agents exceeding the threshold for their structural class at step B3.

Step B4. The NOELs of 5.42 and 6.55 mg/kg of body weight per day for males and females respectively for 3-propylidenephthalide (No. 1168) are 1000 times greater than its estimated intake of 0.3 μg/kg of body weight per day in Europe and 0.9 μg/kg of body weight per day in the USA.

The NOELs for 3-propylidenephthalide are appropriate to evaluate 2-(4-methyl-2-hydroxyphenyl)propionic acid γ-lactone (No. 1167), 3-n-butylphthalide (No. 1169), and 3-butylidenephthalide (No. 1170) because these substances are structurally related and undergo similar pathways of metabolism. The NOELs of 5.42 and 6.55 mg/kg of body weight per day for males and females respectively for 3-propylidenephthalide are 100 000 times greater than the estimated intake from use as a flavouring agent of 2-(4-methyl-2-hydroxyphenyl)propionic acid γ-lactone in the USA (0.03 μg/kg of body weight per day), 100 000 times greater than the estimated intake

of 3-*n*-butylphthalide in Europe (0.01 µg/kg of body weight per day) and in the USA (0.006 µg/kg of body weight per day), and 10 000 times greater than the estimated intake of 3-butylidenephthalide in Europe (0.2 µg/kg of body weight per day) and in the USA (0.1 µg/kg of body weight per day). The Committee concluded that these substances would not pose a safety concern at currently estimated levels of intake. Table 2 summarizes the evaluations of the members of this group.

Consideration of flavouring agents with high intakes evaluated by the B-side of the decision-tree

Dihydrocoumarin (No. 1171)

More extensive data on metabolism and toxicity were considered to complete the safety evaluation of dihydrocoumarin (No. 1171).

Dihydrocoumarin is a δ-lactone fused to a benzene ring and is not a member of the class of aromatic coumarin derivatives. Dihydrocoumarin lacks α,β-unsaturation in the lactone ring, which is a key structural feature in the metabolism of coumarin. Coumarin is principally metabolized in humans and primates by electrophilic substitution (i.e. 7-hydroxylation), and in rats and several other species by epoxidation of the alkene function to form mainly 3-hydroxycoumarin. The absence of a double bond in dihydrocoumarin precludes the formation of the reactive epoxide and subsequent metabolites. The metabolic fate of dihydrocoumarin closely resembles that of simple aliphatic δ-lactones. Dihydrocoumarin and aliphatic lactones hydrolyse to the corresponding ring-opened hydroxy-acids. Hydrolysis of dihydrocoumarin yields a substituted 3-phenylpropanoic acid derivative that is expected to undergo either conjugation or side-chain oxidation to yield the corresponding benzoic acid derivative.

In a 13-week study, groups of B6C3F_1 mice received dihydrocoumarin in corn oil by gavage at doses of up to 1600 mg/kg of body weight, once daily, 5 days a week. No gross or microscopic lesions were observed in either sex at any dose, although body-weight gain was reduced and males and females at the highest dose and females at 800 mg/kg of body weight showed were changes in organ weights. The results of this study show that the NOEL for dihydrocoumarin is 400 mg dihydrocoumarin/kg of body weight per day for B6C3F_1 mice.

In a study of the potential carcinogenicity of dihydrocoumarin, B6C3F_1 mice received dihydrocoumarin in corn oil by gavage at doses

up to 800 mg/kg of body weight, once daily, 5 days per week for 2 years. No significant differences in survival rates, final mean body weights or clinical findings were reported in the treated animals as compared with the controls. The only increase in neoplasia associated with the administration of dihydrocoumarin were increases in the incidences of hepatocellular adenoma and hepatocellular adenoma and carcinoma (combined), seen at all doses in females only. This effect reflects the high incidence of spontaneous liver tumours in this hybrid mouse and thus the heightened sensitivity to enhancement of liver neoplasia. In view of the nature of the findings, the Committee concluded that observations of hepatic neoplasms in this bioassay in mice are not relevant to the safety of dihydrocoumarin in humans at low levels of intake from use as a flavouring agent.

In a 13-week study, groups of Fischer 344/N rats received dihydrocoumarin in corn oil by gavage at doses of up to 1200 mg/kg of body weight, once daily, 5 days per week. In males only, body weight was decreased at the highest dose. Changes in enzymes and other constituents of blood plasma were reported at doses ≥300 mg/kg of body weight. Increases in liver and kidney weights were observed at the two higher doses. Centrilobular hepatocellular hypertrophy, ranging in severity from minimal to mild, was reported in the livers of animals of each sex at doses of 300 mg/kg of body weight and above. No adverse treatment-related effects were observed in either male or female rats receiving 75 and 150 mg dihydrocoumarin/kg of body weight.

In a study of the potential carcinogenicity of dihydrocoumarin, Fischer 344/N rats received doses of up to 600 mg/kg of body weight in corn oil by gavage, on 5 days per week for 2 years. A significant dose-related decrease in the survival of male rats, attributed to progressive degenerative nephropathy leading to renal failure, was reported after week 92. Nephropathy was reported in control and treated rats of each sex. Although the incidence of nephropathy was greater in male rats, the findings were significant only in females at the two higher doses. Microscopic examination revealed a statistically significant, dose-related increase in renal tubule hyperplasia in male rats only. A significant increase in the incidence of renal tubule adenomas was observed in males treated with 600 µg dihydrocoumarin/kg of body weight when compared to the control group, but there was no evidence of malignant renal tubule neoplasms in male rats at any dose. Increases in the incidence of renal tubule hyperplasia or renal tubule adenomas were not observed in female rats. The Committee concluded that the renal hyperplastic and neoplastic effects observed are sex- and species-specific and not dose-related, and that these effects

reflect the sensitivity of the male rat kidney to chronic progressive nephropathy and neoplasia. The NOEL was 300 mg/kg of body weight per day.

In a study in rats fed diets containing 0.76% dihydrocoumarin (equivalent to 580 mg/kg of body weight), relative liver weights were significantly increased in the treated group compared with the controls, but no microscopic abnormalities were observed. Dihydrocoumarin did not markedly affect the activities of carnitine acetyltransferase and palmitoyl-coenzyme A oxidation, indicating that dihydrocoumarin is unlikely to be a rodent liver peroxisome proliferator.

As noted by the Committee at its thirty-fifth meeting, no effects were reported in three short-term studies in rats fed dihydrocoumarin in the diet. Although it was not possible to determine a NOEL from these studies, they did provide additional data that support the safe use of dihydrocoumarin as a flavouring agent at its current level of intake.

As also noted at the thirty-fifth meeting, no effects were reported in a long-term study in dogs fed dihydrocoumarin in the diet. The data obtained in this study were limited by the small number of animals tested.

Dihydrocoumarin was tested in various assays for genotoxicity in vitro. The results of the assays for reverse mutation and unscheduled DNA synthesis were negative. Cytotoxicity was reported in a mouse lymphoma assay in the presence of an endogenous metabolic activation system, however, similar assays for forward mutation without metabolic activation produced negative results. Negative results were obtained in six out of seven studies of chromosomal aberration in Chinese hamster ovary cells. A dose-related increase in sister chromatid exchange was found in the same cell line, but this isolated positive result was not considered evidence for genotoxicity. A test for micronucleus formation in rats in vivo gave negative results. The Committee concluded that the data indicated that dihydrocoumarin is not genotoxic.

6-Methylcoumarin (No. 1172)

More extensive data on metabolism and toxicity were considered in order to complete the safety evaluation of 6-methylcoumarin (No. 1172).

It is anticipated that humans will metabolize 6-methylcoumarin via methyl group oxidation to the corresponding benzoic acid deriva-

tive, which can also be readily excreted. 6-Methylcoumarin may also undergo ring hydroxylation to form the corresponding 7-hydroxy metabolite, followed by excretion as the glucuronic acid conjugate. At high doses, metabolism via the 3,4-epoxide is at most a minor pathway, even in individuals exhibiting decreased activity of CYP2A6.

When 6-methylcoumarin was administered daily by gavage to male and female B6C3F$_1$ mice for 13 weeks at doses of up to 800 mg/kg of body weight, no toxicologically significant changes were reported in clinical, macroscopic or microscopic examinations at any dose. Prostration, bradycardia, bradypnoea, hypoactivity, hypothermia, and loss of the grasping reflex were reported at the highest dose only.

In a 13-week study, 6-methylcoumarin was given to rats by gavage at doses of up to 1200 mg/kg body weight per day. All rats receiving the highest dose of 1200 mg/kg of body weight and one male rat receiving 600 mg/kg of body weight died during week 1 of the study. Decreases in body weight were reported in males and females receiving 600 mg 6-methylcoumarin/kg of body weight, relative to controls. The clinical effects, including hypoactivity, lachrymation, ataxia, impaired righting reflex and decreased limb tone, were reported in animals of each sex at 600 mg/kg of body weight. A decrease in serum cholinesterase activity was reported in females receiving 300 mg/kg of body weight. No other changes were reported in haematological, serum biochemical or urinary parameters at any dose. Necropsy of all animals receiving 1200 mg/kg of body weight revealed microscopic hepatic lesions that varied in the degree of congestion, degeneration, necrosis and hepatitis. Increased mean absolute and relative liver weights were reported in males and females receiving doses of 300 and 600 mg/kg of body weight; however, these changes were not accompanied by any substance-related macroscopic observations. No treatment-related effects were reported in animals receiving a dose of 150 mg/kg of body weight per day.

In a 14-week study, groups of weanling Osborne-Mendel rats were fed diets containing 6-methylcoumarin at a concentration of 0, 1000 or 10 000 ppm (equivalent to 0, 100 and 1000 mg 6-methylcoumarin/kg of body weight). No significant differences in general health and behaviour, or body weight and food consumption were reported in the treated animals as compared with the controls. Haematological examinations performed at the end of the study did not reveal any treatment-related effects. No effects on organ weights, or macroscopic or microscopic changes in the tissues were reported at any dose.

In a 2-year feeding study, Osborne-Mendel rats were fed diets containing 6-methylcoumarin at concentrations up to 15000 ppm (equivalent to 750 mg/kg of body weight). Depression in growth rates was noted in males receiving doses of 375 or 750 mg/kg of body weight, but in females only at the higher dose. Hepatic effects in males and females receiving a dose of 750 mg/kg of body weight included fatty metamorphosis, very slight bile duct proliferation, and focal telangiectasis. No treatment-related effects were observed at doses up to and including 175 mg/kg of body weight per day in males and 375 mg/kg of body weight per day in females.

The results of a 13-week study conducted in rats fed diets containing 0.82% 6-methylcoumarin (corresponding to 695 mg/kg of body weight) revealed a slight vacuolation of hepatocytes in three out of eight treated animals; however, no increases in plasma aminotransferase activity and no bile-duct hyperplasia or cholangiofibrosis were reported. 6-Methylcoumarin treatment increased mixed function oxidase activity (i.e. 7-ethoxycoumarin O-deethylase activity), but it did not markedly affect the activities of carnitine acetyltransferase and palmitoyl-coenzyme A oxidation, indicating that 6-methylcoumarin is unlikely to be a rodent liver peroxisome proliferator.

No effects were reported in a limited study in dogs that received 6-methylcoumarin at a dose of 200 mg/kg body weight per day in gelatine capsules for 2 or 4 weeks. In a long-term study, no effects were reported in dogs fed diets containing 6-methylcoumarin at a concentration resulting in 50 mg/kg of body weight per day . The data obtained in these studies are limited because only one or two dogs were tested per group.

6-Methylcoumarin was not genotoxic in a number of assays for reverse mutation with *Salmonella typhimurium* strains; marginally positive results were reported in a single assay. Negative results were reported in a mouse lymphoma assay. No increase in the frequency of mutation was observed in a test for sex-linked recessive lethal mutation in *Drosophila melanogaster*. A test for micronucleus formation in mice in vivo gave negative results in females and equivocal results in males; the positive results were not confirmed in a similar study. The Committee concluded that the data indicated that 6-methylcoumarin is not genotoxic.

In a 13-week study in rats given dihydrocoumarin, a NOEL of 150 mg/kg of body weight per day was identified. This NOEL is about 5000 times more than the estimated per capita intake of dihydrocoumarin in Europe (24 µg/kg of body weight per day) and in the USA (19 µg/kg of body weight per day). In rats, the NOEL in the 2-year study by

gavage was 300 mg/kg of body weight per day for dihydrocoumarin. These NOELs are 10 000 times more than the estimated intake of dihydrocoumarin in Europe (24 µg/kg of body weight per day) and in the USA (19 µg/kg of body weight per day). In a 13-week study in rats, a NOEL of 150 mg/kg of body weight per day was found. This NOEL is 30 000 times more than the estimated intake of 6-methylcoumarin in Europe (5 µg/kg of body weight per day) and in the USA (2 µg/kg of body weight per day). Understanding of their metabolism and the available data on toxicity led the Committee to conclude that the safety of dihydrocoumarin and 6-methylcoumarin would not be expected to present a safety concern at current levels of intake (Table 2).

Consideration of secondary components

Three members of this group of flavouring agents (Nos *1158, 1160,* and *1164*) have minimum assay values of <95%. Information on the safety of the secondary components of these three compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less). The secondary components of No. 1158 (heptan-1-ol) and No. 1160 (γ-dodecalactone and 2(3*H*)-furanone, dihydro-5-(2-octenyl)-(Z)) were evaluated at the forty-ninth meeting. None of the secondary components was considered to present a safety concern at current levels of intake. The secondary components of No. 1164 (2,9-dimethyl 3,8-decanedione and 4-hydroxy-5,6-oxo β-ionone) have not been evaluated previously. However, compounds that are structurally related to the secondary components of No. 1164 (3,4-hexandione and β-ionone) were evaluated at the fifty-first meeting, and were considered not to present a safety concern at current levels of intake.

On this basis, the secondary components of No. 1164 were considered not to present a safety concern at current levels of intake.

Consideration of combined intakes from use as flavouring agents

All 16 agents in this group are expected to be efficiently metabolized and would not saturate metabolic pathways. Evaluation of all the data indicated no safety concern associated with combined intake.

Conclusions

The Committee concluded that none of the 16 flavouring agents in this group of alicyclic, alicyclic-fused and aromatic-fused ring lactones would present safety concerns at the current estimated levels of intake. More extensive data on metabolism and toxicity were

considered in the evaluations of dihydrocoumarin (No. 1172) and 6-methylcoumarin (No. 1171) in accordance with the application of the Procedure in the case of flavouring agents with high intakes evaluated by the B-side of the decision-tree. Other data on the toxicity and metabolism of these ring lactones were consistent with the results of the safety evaluation.

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

4.1.2 *Aliphatic, alicyclic, linear, α,β-unsaturated, di- and trienals and related alcohols, acids and esters*

The Committee evaluated a group of 26 *α,β*-unsaturated flavouring agents (see Table 3) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). This group included 12 dienals (Nos 1173, 1175, 1179, 1181, 1182, 1185–1187, 1190, 1195–1197), a trienal (No. 1198), five dienols (Nos 1174, 1180, 1183, 1184 and 1189), a dienoic acid (No. 1176) and seven related esters (Nos 1177, 1178, 1188, 1191–1194), all of which contain unsaturation in the 2,3-position and some of which contain saturation in the 4,5-position. The Committee had evaluated one member of the group, (*E,E*)-2,4-hexadienoic acid (No. 1176, sorbic acid) in its capacity as an antimicrobial preservative at the seventeenth meeting (Annex 1, reference *32*), when an ADI of 0–25 mg/kg of body weight was established.

α,β-Unsaturated aldehydes are formed endogenously by lipid peroxidation of polyunsaturated fatty acids (PUFA) or can be consumed as naturally-occurring constituents of food, and are only consumed to a minor extent as added flavouring agents.

Twenty-one of the 26 flavouring agents in this group of flavouring agents have been reported to occur naturally in food. They have been detected in apples, grapes, broccoli, roast chicken, tea and beer.

Estimated daily per capita intake

The total annual volume of production of the 26 flavouring agents in this group is approximately 1000 kg in Europe and 1500 kg in the USA. Approximately 50–60% of the total annual volume of production of the aliphatic linear dienals in Europe and the USA is accounted for by 2-*trans*,4-*trans*-decadienal (No. 1190). The estimated combined per capita daily intake of the 12 dienals is approximately 40 µg in Europe and 120 µg in the USA, and the per capita daily intake of 2-*trans*,4-*trans*-decadienal is approximately 20 µg in Europe and 70 µg in the USA. The per capita intakes of all the other flavouring

Table 3
Summary of results of the safety evaluation of aliphatic, alicyclic, linear, α,β -unsaturated, di- and trienals and related alcohols, acids and esters used as flavouring agents[a,b]

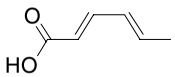
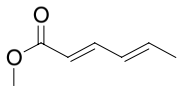
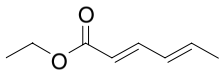
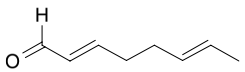
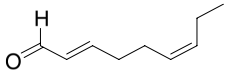
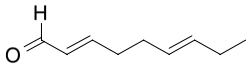
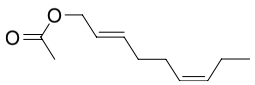
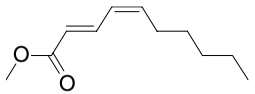
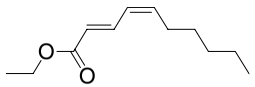
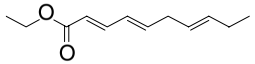
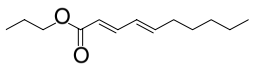
Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[c]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments	Conclusion based on current intake
Structural class I						
(*E*,*E*)-2,4-Hexadienoic acid	1176	110-44-1 	No Europe: ND USA: 6	NR	See note 3	See footnote d
Methyl sorbate	1177	689-89-4 	No Europe: 0.1 USA: ND	NR	See note 4	No safety concern
Ethyl sorbate	1178	2396-84-1 	No Europe: 59 USA: 3	NR	See note 4	No safety concern
2-*trans*,6-*trans*-Octadienal	1182	56767-18-1 	No Europe: 0.1 USA: 0.007	NR	See note1	No safety concern
2,6-Nonadien-1-ol	1184	7786-44-9 	No Europe: 2 USA: 1	NR	See note 2	No safety concern
Nona-2-*trans*-6-*cis*-dienal	1186	557-48-2 	No Europe: 7 USA: 24	NR	See note 1	No safety concern

Table 3 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[c]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments	Conclusion based on current intake
2-trans-6-*trans*-Nonadienal	1187	17587-33-6 	No Europe: ND USA: 0.007	NR	See note 1	No safety concern
(*E*,*Z*)-2,6-Nonadien-1-ol acetate	1188	68555-65-7 	No Europe: ND USA: 18	NR	See note 4	No safety concern
Methyl (*E*)-2-(*Z*)-4-decadienoate	1191	4493-42-9 	No Europe: ND USA: 1	NR	See note 4	No safety concern
Ethyl *trans*-2-*cis*-4-decadienoate	1192	3025-30-7 	No Europe: 34 USA: 3	NR	See note 4	No safety concern
Ethyl 2,4,7-decatrienoate	1193	78417-28-4 	No Europe: ND USA: 0.4	NR	See note 4	No safety concern
Propyl 2,4-decadienoate	1194	84788-08-9 	No Europe: 0.9 USA: ND	NR	See note 4	No safety concern

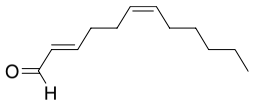
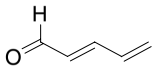
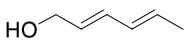
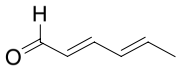
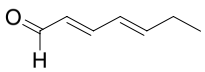
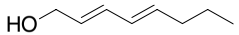
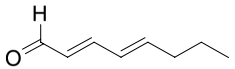
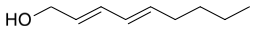
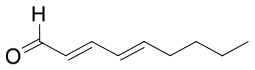
2-*trans*-6-*cis*-Dodecadienal	1197	21662-13-5 	No Europe: 0.6 USA: 0.02	NR	See note1	No safety concern
2,4-Pentadienal	1173	764-40-9 	No Europe: 0.1 USA: 0.2	Yes. The NOEL of 15 mg/kg bw per day for the related substance *trans,trans*-2,4-hexadienal is >1 million times the estimated daily intake of 2,4-pentadienal when used as a flavouring agent	See note 1	No safety concern
(*E,E*)-2,4-Hexadien-1-ol	1174	111-28-4 	No Europe: ND USA: 0.4	Yes. The NOEL of 15 mg/kg bw per day for the related substance *trans,trans*-2,4-hexadienal is >1 million times the estimated daily intake of (*E,E*)-2,4-hexadien-1-ol when used as a flavouring agent	See note 2	No safety concern
trans,trans-2,4-Hexadienal	1175	142-83-6 	No Europe: 1 USA: 0.1	Yes. The NOEL of 15 mg/kg bw per day is >100 000 times the estimated daily intake of *trans,trans*-2,4-hexadienal when used as a flavouring agent	See note 1	No safety concern
2,4-Heptadienal	1179	4313-03-5 	No Europe: 4 USA: 23	Yes. The NOEL of 15 mg/kg bw per day for the related substance *trans,trans*-2,4-hexadienal is >10 000 times the estimated daily intake of 2,4-heptadienal when used as a flavouring agent	See note1	No safety concern

Table 3 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[c]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments	Conclusion based on current intake
(*E,E*)-2,4-Octadien-1-ol	1180	18409-20-6 	No Europe: ND USA: 18	Yes. The NOEL of 15 mg/kg bw per day for the related substance *trans,trans*-2,4-hexadienal is >10 000 times the estimated daily intake of (*E,E*)-2,4-octadien-1-ol when used as a flavouring agent	See note 2	No safety concern
trans,trans-2,4-Octadienal	1181	30361-28-5 	No Europe: 0.7 USA: 0.007	Yes. The NOEL of 15 mg/kg bw per day for the related substance *trans,trans*-2,4-hexadienal is >1 million times the estimated daily intake of *trans,trans*-2,4-octadienal when used as a flavouring agent	See note1	No safety concern
2,4-Nonadien-1-ol	1183	62488-56-6 	No Europe: ND USA: 26	Yes. The NOEL of 33.9 mg/kg bw per day for the related substance 2-*trans*,4-*trans*-decadienal is >10 000 times the estimated daily intake of 2,4-nonadien-1-ol when used as a flavouring agent	See note 2	No safety concern
2,4-Nonadienal	1185	6750-03-4 	No Europe: 2 USA: 0.7	Yes. The NOEL of 33.9 mg/kg bw per day for the related substance 2-*trans*,4-*trans*-decadienal is >1 million times	See note 1	No safety concern

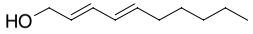
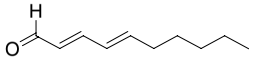
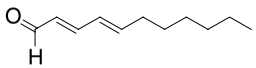
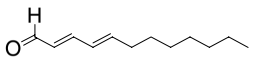
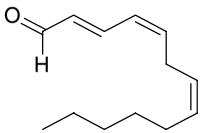
				the estimated daily intake of 2,4-nonadienal when used as a flavouring agent		
(*E,E*)-2,4-Decadien-1-ol	1189	18409-21-7 	No Europe: ND USA: 26	Yes. The NOEL of 33.9 mg/kg bw per day for the related substance 2-*trans*,4-*trans*-decadienal is >10 000 times the estimated daily intake of (*E,E*)-2,4-decadien-1-ol when used as a flavouring agent	See note 2	No safety concern
2-*trans*,4-*trans*-Decadienal	1190	25152-84-5 	No Europe: 22 USA: 70	Yes. The NOEL of 33.9 mg/kg bw per day for 2-*trans*,4-*trans*-decadienal is >10 000 times the estimated daily intake of 2-*trans*,4-*trans*-decadienal when used as a flavouring agent	See note 1	No safety concern
2,4-Undecadienal	1195	13162-46-4 	No Europe: 4 USA: 0.4	Yes. The NOEL of 33.9 mg/kg bw per day for the related substance 2-*trans*,4-*trans*-decadienal is >100 000 times the estimated daily intake of 2,4-undecadienal when used as a flavouring agent	See note 1	No safety concern
trans,trans-2,4-Dodecadienal	1196	21662-16-8 	No Europe: 0.7 USA: 0.1	Yes. The NOEL of 33.9 mg/kg bw per day for the related substance 2-*trans*,4-*trans*-decadienal is >1 million times the estimated daily intake of *trans,trans*-2,4-dodecadienal when used as a flavouring agent	See note 1	No safety concern

Table 3 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3* Does intake exceed the threshold for human intake?[c]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	Comments	Conclusion based on current intake
2-trans-4-*cis*-7-*cis*-Tridecatrienal	1198	13552-96-0 	No Europe: 0.3 USA: 0.009	Yes. The NOEL of 33 mg/kg bw per day is >1 million times the estimated daily intake of *2-trans*-4-*cis*-7-*cis*-tridecatrienal when used as a flavouring agent	See note 1	No safety concern

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

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

[b] Step 2: Thirteen flavouring agents (Nos 1176–1178, 1182, 1184, 1186–1188, 1191–1194, and 1197) in this group are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the decision-tree. The α,β-unsaturated dienals and related alcohole (Nos 1173–1175, 1179–1181, 1183, 1185, 1189, 1190, 1195, 1196, and 1198) in this group cannot be predicted to be metabolized o innocuous products. The evaluation of these 13 flavouring agents therefore proceeded via the B-side of the decision-tree.

[c] The threshold for human intake for structural class I is 1800 μg per day. All intake values are expressed in μg per day. The combined per capita intakes of flavouring agents in structural class I are 138 μg per day in Europe and 221 μg per day in the USA.

[d] An ADI of 0-25 mg/kg bw was established for (*E,E*)-2,4-hexadienoic acid by the Committee at its seventeenth meeting (Annex 1, reference *32*), and this was maintained at the present meeting. Use of the chemical as a flavouring agent is subsumed in the ADI.

Notes to Table 3:

1. Oxidized to acids, which may undergo β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle. Alternately, may undergo glutathione conjugation and excretion as mercapturic acid derivatives.
2. Oxidized to aldehydes and acids, which metabolize completely in the fatty acid β-oxidation pathway.
3. Undergo β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle.
4. Hydrolysed to corresponding alcohols and acids, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.

agents in the group are in the range of 0.007–24 μg/day, most values being at the lower end of this range. The daily per capita intake of each agent in Europe and in the USA is reported in Table 3.

Absorption, distribution, metabolism and elimination

In general, aliphatic esters are hydrolysed rapidly to their component alcohols and carboxylic acids by classes of enzymes known as carboxylesterases in the intestinal mucosa. Once hydrolysed, the resulting aliphatic alcohols and carboxylic acids are absorbed into the portal circulation. The unsaturated alcohols are oxidized successively to the corresponding aldehydes and carboxylic acids, which participate in fundamental biochemical pathways, including the fatty acid pathway and tricarboxylic acid cycle.

It is anticipated that humans will metabolize dienals and trienals by oxidation to the corresponding acids, which may undergo β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle. An alternate minor pathway may involve conjugation of the unsaturated aldehyde to glutathione, followed by excretion as the mercapturic acid derivative.

Under conditions of glutathione depletion and oxidative stress, and at high cellular concentrations, *α,β*-unsaturated aldehydes have been shown to form adducts with DNA nucleotides, to cause cytohistopathology, and to induce apoptosis. However, metabolic evidence indicates that low concentrations of *α,β*-unsaturated aldehydes are safely metabolized in the high-capacity β-oxidation pathway or via glutathione conjugation.

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 26 flavouring agents in this group, the Committee assigned all of them to structural class I.

Step 2. Thirteen flavouring agents (Nos 1176–1178, 1182, 1184, 1186–1188, 1191–1194, and 1197) in this group are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the decision-tree. The *α,β*-unsaturated 2,4-dienals and alcohol precursors (Nos 1173–1175, 1179–1181, 1183, 1185, 1189, 1190, 1195, 1196, and 1198) cannot be predicted to be metabolized to innocuous products and the evaluation of these 13 flavouring agents therefore proceeded via the B-side of the decision-tree.

Step A3. The estimated daily per capita intakes in Europe and the USA of the 13 flavouring agents in this group that are metabolized to innocuous products (Nos 1176–1178, 1182, 1184, 1186–1188, 1191–1194, and 1197) are below the threshold for concern for class I (i.e. 1800 µg/day). The Committee concluded that these substances would not be expected to be of safety concern at their currently estimated levels of intake as flavouring agents.

Step B3. The estimated daily per capita intakes in Europe and the USA of the remaining 13 flavouring agents in this group that cannot be predicted to be metabolized to innocuous products are also below the threshold of concern for structural class I (1800 µg/day). Accordingly, the evaluation of these 13 agents proceeded to step B4.

Step B4. The NOEL of 15 mg/kg of body weight per day for *trans*,*trans*-2,4-hexadienal (No. 1175) administered by gavage in a 14-week study in rats provides an adequate margin of safety (>100 000) in relation to the known levels of intake of this agent. This NOEL is also appropriate for the structurally related agents 2,4-pentadienal (No. 1173), 2,4-heptadienal (No. 1179), and *trans*,*trans*-2,4-octadienal (No. 1181), because these agents are all dienals which will undergo oxidation and subsequent metabolism via similar metabolic pathways. The NOEL for *trans*,*trans*-2,4-hexadienal is also appropriate for the structurally related (*E,E*)-2,4-hexadien-1-ol (No. 1174), and (*E,E*)-2,4-octadien-1-ol (No. 1180) because these alcohols will be oxidized to the corresponding aldehydes and subsequently undergo metabolism in a similar manner to the dienals.

The NOEL of 33.9 mg/kg of body weight per day for 2-*trans*,4-*trans*-decadienal (No. 1190), identified from a 14-week study in rats treated by gavage, provides an adequate margin of safety (>10 000) in relation to the known levels of intake of this agent. The NOEL for 2-*trans*,4-*trans*-decadienal is also appropriate for the structurally related substances 2,4-nonadien-1-ol (No. 1183), 2,4-nonadienal (No. 1185), (*E,E*)-2,4-decadien-1-ol (No. 1189), 2,4-undecadienal (No. 1195) and *trans,trans*-2,4-dodecadienal (No. 1196), because of their similar metabolic pathways.

For 2-*trans*-4-*cis*-7-*cis*-tridecatrienal (No. 1198), the NOEL of 33 mg/kg of body weight per day identified from a 4-week study in rats provides an adequate margin of safety (>1 000 000) in relation to the known levels of intake of this agent.

The Committee noted that 2,4-*trans*-hexadienal (No. 1175) induced forestomach hyperplasia and squamous cell tumours in rats and mice

of each sex. This is a common finding in USA National Toxicology Program bioassays in which a high concentration of an irritating material suspended in corn oil is delivered by gavage into the forestomach every day for 2 years.

Trans,trans-2,4-hexadienal gave positive results in some tests for genotoxicity in vitro, but was inactive in tests carried out in vivo. Thus, this substance may be genotoxic under some conditions, but this is not believed to be the basis for its effects in the rodent forestomach. There was evidence of injury to the forestomach epithelium attributable to exposure and this is believed to be the primary cause of the development of neoplasia. Mice and rats in the bioassays developed forestomach hyperplasia following corn oil gavage, and a low incidence of adenomas was observed in mice, reflecting the sensitivity of the forestomach to irritation. The forestomach was the only site of increased neoplasia in treated animals.

An IARC Working Group concluded that when evaluating the relevance for human cancer of the induction of forestomach tumours in rodents, the experimental conditions of exposure should be considered. The conditions of exposure during oral administration are unusual in that physical effects may cause high local concentrations of test substances in the forestomach and prolonged exposure of the epithelium. Agents that only produce tumours of the forestomach in rodents after prolonged treatment, through non-DNA-reactive mechanisms, may be of less concern to humans since human exposure to such agents would need to surpass time-integrated dose thresholds in order to elicit the carcinogenic response.

Therefore, the Committee concluded that the appearance of forestomach tumours in the 2-year bioassays in rodents in which *trans*,*trans*-2,4-hexadienal was administered at high concentration by gavage is of no relevance to humans.

Table 3 summarizes the evaluations of the 26 α,β-unsaturated flavouring agents in this group.

Consideration of combined intakes from use as flavouring agents

Although the flavouring agents evaluated in this group are not converted to a common metabolite, they are subject to conjugation with reduced glutathione (GSH). Accordingly, simultaneous consumption of the α,β-unsaturated aldehydes, at sufficiently high concentrations, could theoretically deplete concentrations of GSH , resulting in lipid peroxidation. However, under normal conditions, concentrations of intracellular replenishable GSH (approximately 1–10 mM) are

sufficient to detoxify the quantities of *α,β*-unsaturated aldehydes being ingested as flavouring agents. Additionally, since the α,β-unsaturated aldehydes provide similar flavouring characteristics, it is unlikely that all foods containing these flavouring agents would be consumed concurrently on a daily basis. Therefore, at the levels of *α,β*-unsaturated aldehydes used as flavouring agents, and in consideration of the constant replenishment of GSH by biosynthesis, the Committee considered that the combined intake of these flavouring agents does not present a safety concern.

The estimated current intake of (*E,E*)-2,4-hexadienoic acid (No. 1176, sorbic acid) (0.1 μg/kg of body weight per day) from its use as a flavouring agent is below the individual ADI (0–25 mg/kg of body weight) established previously by the Committee (Annex 1, reference *33*).

Consideration of secondary components

Ten members of this group of flavouring agents (Nos 1179, 1180, 1183, 1185, 1189–1192, 1196 and 1198) have minimum assay values of <95%. Information on the safety of the secondary components of these 10 compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less). In all cases, the secondary components were expected to share the same metabolic fate as the primary flavouring agents (Nos 1179, 1180, 1189, 1190 and 1196), or have metabolites that are substrates for the fatty acid cycle, which are subsequently excreted as carbon dioxide and water (Nos 1183, 1185, 1191, 1192, and 1198). One of the secondary components of No. 1185 (2,4-nonadien-1-ol) was evaluated at the present meeting, while two of the secondary components of No. 1190 (acetone and isopropanol) were evaluated at the fifty-first meeting. None of the secondary components was considered to present a safety concern at current levels of intake. The other secondary component of No. 1185 (2,4-nonen-1-ol) has not been evaluated previously; however, it is expected to be oxidized and completely metabolized in the fatty acid cycle. On this basis, 2,4-nonen-1-ol was considered not to present a safety concern at current intake levels.

Conclusions

The Committee retained the previously established ADI of 0–25 mg/kg of body weight for (*E,E*)-2,4-hexadienoic acid (No. 1176). The Committee concluded that none of the flavouring agents in this group would pose a safety concern at the currently estimated levels of intake. It was noted that other data on the toxicity and metabolism of

the flavouring agents in the group were consistent with the results of the safety evaluation. A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.3 *Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters*

The Committee evaluated a group of flavouring agents comprising 32 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters (see Table 4) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). The Committee had previously evaluated two members of this group; citronellol (No. 1219) and citral (No. 1225) were both evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), when conditional ADIs of 0–0.25 mg/kg of body weight and 0–1 mg/kg of body weight respectively, were allocated. Citronellol and citral were re-evaluated at the twenty-third meeting of the Committee (Annex 1, reference *50*) as part of a group of terpenoid flavouring agents, including geranyl acetate, linalool and linalyl acetate. A group ADI of 0–0.5 mg/kg of body weight, expressed as citral, was established for citral, geranyl acetate, citronellol, linalool, and linalyl acetate on the basis of their clearly-defined metabolism, rapid excretion, and low toxicity in short-term studies. The Committee maintained, however, that a long-term study was required for at least one member of this group. 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 alcohols and aliphatic acyclic linear and branched-chain carboxylic acids by the Procedure. Two-year studies of carcinogenicity had been conducted for a mixture of two of these esters, geranyl acetate and citronellyl acetate. The Committee concluded that there were no safety concerns for any of the 26 substances under the low levels of intake arising from their use as flavouring agents and maintained the group ADI for citral, geranyl acetate, citronellol, linalool, and linalyl acetate. Likewise, at its fifty-first meeting (Annex 1, reference *137*) when the Committee re-evaluated linalool and linalyl acetate, the group ADI was maintained.

Twenty-four of the 32 flavouring agents (Nos 1199–1202, 1204–1206, 1208–1210, 1212, 1213, 1215, 1217–1221, 1223–1225, 1227, 1228, 1230) have been reported to occur naturally in foods. The substances that occur naturally in the highest abundance are the monoterpene primary alcohols, aldehydes, and carboxylic acids. They have been detected in fruits such as raspberries, strawberries, bananas, kumquats, and myrtle berries, as well as in many alcoholic beverages,

including beer, wine, whisky, brandy and rum. They are most abundant, however, in citrus fruits and in many spices and spice oils, including ginger, coriander, cinnamon, mustard, chamomile, sage and thyme.

Estimated daily per capita intake

The total annual volume of production of the 32 flavouring agents in this group is approximately 66 000 kg in Europe and 66 000 kg in the USA. More than 95% of the total annual volume of production in Europe and the USA is accounted for by citronellol (No. 1219), citronellal (No. 1220), geraniol (No. 1223), nerol (No. 1224), and citral (No. 1225). Of these, citral accounts for approximately 73% of the total annual volume of production in Europe and 80% in the USA. The estimated daily per capita intakes of citral in Europe and the USA are 6849 µg and 6990 µg, respectively. The daily intakes per capita of all the other flavouring agents in the group are estimated to be in the range of 0.01–945 µg, most values being below 50 µg. The daily per capita intake of each agent in Europe and in the USA is reported in Table 4.

Absorption, distribution, metabolism, and elimination

The four esters in this group can be expected to be hydrolysed by esterases to the corresponding alcohols and carboxylic acids. Once formed, the latter substances, together with the other alcohols, acids and aldehydes in this group, are readily absorbed from the gastrointestinal tract. On the basis of the results of a study with citral, after absorption the substances can be expected to be distributed rapidly throughout the body, metabolized, and excreted as polar metabolites in the urine, faeces, and expired air. There is no evidence for accumulation in the body.

The substances in this group share common metabolic pathways. Shorter branched-chain aliphatic alcohols, aldehydes, and acids undergo β-oxidative cleavage to yield intermediates of the amino acid and/or fatty acid metabolic pathways. These intermediates are completely metabolized to CO_2 via the tricarboxylic acid cycle. As chain length and substitution increase, the alcohols and aldehydes undergo a combination of ω-, ω-1 and β-oxidation, and selective dehydrogenation and hydration to yield polar acidic metabolites. Therefore, all of the flavouring agents in this group will eventually be either completely oxidized or oxidized to polar metabolites which are excreted primarily in the urine.

Table 4
Summary of results of the safety evaluations of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters used as flavouring agents[a]

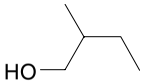
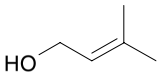
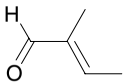
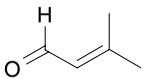
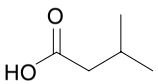
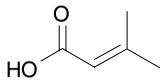
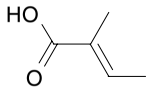
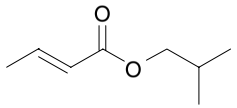
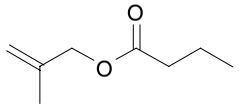
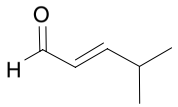
Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
(+/–) 2-Methyl-1-butanol	1199	137-32-6 	No Europe: ND USA: 35	NR	NR	See note 1	No safety concern
3-Methyl-2-buten-1-ol	1200	556-82-1 	No Europe: 5.4 USA: 3.8	NR	NR	See note 1	No safety concern
2-Methyl-2-butenal	1201	1115-11-3 	No Europe: 0.7 USA: 0.2	NR	NR	See note 1	No safety concern
3-Methyl-2-butenal	1202	107-86-8 	No Europe: 3.9 USA: 0.5	NR	NR	See note 1	No safety concern
Ammonium isovalerate (ammonium salt of isovaleric acid)	1203	7563-33-9 	No Europe: 18 USA: 16	NR	NR	See note 2	No safety concern

Table 4 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
3-Methylcrotonic acid	1204	541-47-9 	No Europe: 121 USA: 0.01	NR	NR	See note 2	No safety concern
trans-2-Methyl-2-butenoic acid	1205	80-59-1 	No Europe: 4.9 USA: 1.6	NR	NR	See note 2	No safety concern
Isobutyl 2-butenoate	1206	589-66-2 	No Europe: 0.5 USA: 45	NR	NR	See note 3	No safety concern
2-Methylallyl butyrate	1207	7149-29-3 	No Europe: ND USA: 0.2	NR	NR	See note 3	No safety concern
4-Methyl-2-pentenal	1208	5362-56-1 	No Europe: 0.3 USA: 0.2	NR	NR	See note 1	No safety concern

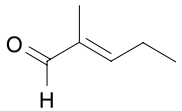
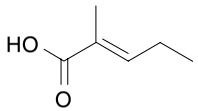
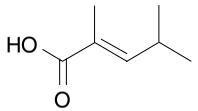
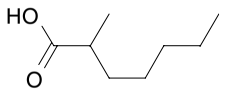
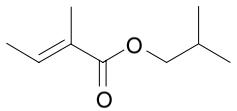
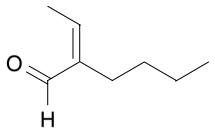
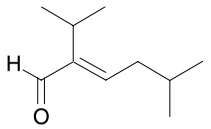
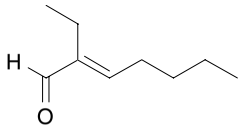
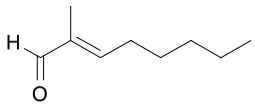
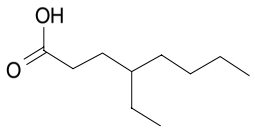
2-Methyl-2-pentenal	1209	623-36-9 	No Europe: 4 USA: 0.2	NR	NR	See note 1	No safety concern
2-Methyl-2-pentenoic acid	1210	3142-72-1 	No Europe: 42 USA: 20	NR	NR	See note 2	No safety concern
2,4-Dimethyl-2-pentenoic acid	1211	66634-97-7 	No Europe: 0.1 USA: 0.1	NR	NR	See note 2	No safety concern
2-Methylheptanoic acid	1212	1188-02-9 	No Europe: 17 USA: 6	NR	NR	See note 2	No safety concern
Isobutyl angelate	1213	7779-81-9 	No Europe: 0.1 USA: 0.1	NR	NR	See note 3	No safety concern
2-Butyl-2-butenal	1214	25409-08-9 	No Europe: ND USA: 0.01	NR	NR	See note 4	No safety concern

Table 4 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
2-Isopropyl-5-methyl-2-hexenal	1215	35158-25-9 	No Europe: 0.3 USA: 0.01	NR	NR	See note 4	No safety concern
2-Ethyl-2-heptenal	1216	10031-88-6 	No Europe: 0.01 USA: 0.1	NR	NR	See note 4	No safety concern
2-Methyl-2-octenal	1217	73757-27-4 	No Europe: ND USA: 7.9	NR	NR	See note 1	No safety concern
4-Ethyloctanoic acid	1218	16493-80-4 	No Europe: ND USA: 4	NR	NR	See note 2	No safety concern

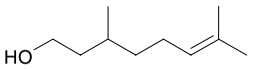
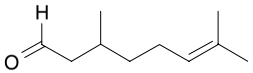
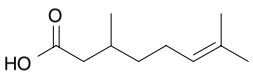
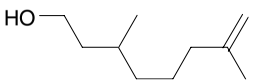
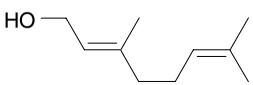
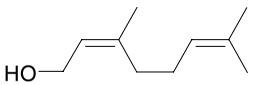
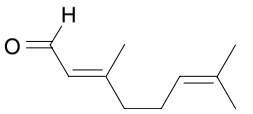
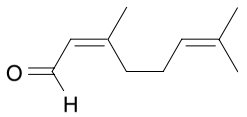
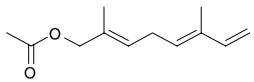
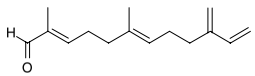
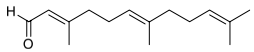
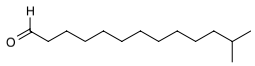
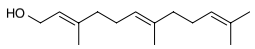
dl-Citronellol	1219	106-22-9 	No Europe: 370 USA: 0.5	NR	NR	See note 4	*
Citronellal	1220	106-23-0 	No Europe: 945 USA: 324	NR	NR	See note 4	No safety concern
3,7-Dimethyl-6-octenoic acid	1221	502-47-6 	No Europe: 3.1 USA: 0.2	NR	NR	See note 4	No safety concern
Rhodinol	1222	6812-78-8 	No Europe: 53 USA: 8.4	NR	NR	See note 4	No safety concern
Geraniol	1223	106-24-1 	No Europe: 640 USA: 315	NR	NR	See note 4	No safety concern
Nerol	1224	106-25-2 	No Europe: 290 USA: 171	NR	NR	See note 4	No safety concern
Citral (Mixture of the *trans* and *cis* isomers geranial and neral)	1225	5392-40-5 	Yes Europe: 6849 USA: 6990	No	Yes. The NOEL of 60 mg/kg of bodyweight per day for citral is >500 times the estimated daily	See note 4	*

Table 4 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
					intakes of 114 mg/kg of bodyweight in Europe and 117 μg/kg of bodyweight in the USA when used a flavouring agent		
8-Ocimenyl acetate	1226	197098-61-6 	No Europe: ND USA: 7.7	NR	NR	See note 4	No safety concern
2,6-Dimethyl-10-methylene-2,6,11-dodecatrienal	1227	60066-88-8 	No Europe: 5.1 USA: 0.5	NR	NR	See note 4	No safety concern
3,7,11-Trimethyl-2,6,10-dodecatrienal	1228	19317-11-4 	No Europe: ND USA: 0.2	NR	NR	See note 4	No safety concern
12-Methyltridecanal	1229	75853-49-5 	No Europe: ND USA: 0.5	NR	NR	See note 1	No safety concern

Farnesol	1230	4602-84-0 	No Europe: 9 USA: 2.6	NR	NR	See note 4	No safety concern

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

[a] All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[b] The threshold for human intake for structural class I is 1800 μg/day. All intake values are expressed in μg/day. The combined per capita intake of flavouring agents in structural class I is 9382 μg per day in Europe and 8732 μg per day in the USA.

* A group ADI of 0–0.5 mg/kg of body weight, expressed as citral, was established for citral, citronellol, geranyl acetate, linalool, and linalyl acetate by the Committee at its 23rd meeting (Annex 1, reference *50*), which was maintained at the present meeting. Use of citronellol and citral as flavouring agents is subsumed in the group ADI.

Notes to Table 4:

1. Primarily oxidized to corresponding carboxylic acid that may enter the β-oxidation pathway yielding shorter chain carboxylic acids that are subsequently metabolized to CO_2 via the tricarboxylic acid pathway.
2. Metabolized primarily via the β-oxidation pathway yielding shorter chain carboxylic acids that are subsequently metabolized to CO_2 via the tricarboxylic acid pathway.
3. Hydrolysed to the corresponding alcohol and carboxylic acid, then participates in the pathway cited in notes 1 and 2.
4. Oxidized to corresponding carboxylic acid. The acid may undergo partial β-oxidation, be excreted or undergo ω oxidation to yield polar polyoxygenated metabolites that are excreted free or conjugated primarily in the urine. If unsaturation is present, the polar polyoxygenated metabolites may also form hydrogenation or hydration 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 (see Fig. 1), the Committee assigned all of the flavouring agents in this group to structural class I.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes of 31 of the 32 flavouring agents are below the threshold of concern for structural class I (1800 μg). The Committee concluded that the safety of these 31 flavouring agents raises no concern at their currently estimated levels of intake as flavouring agents. One of the agents, citral (No. 1225), exceeds the threshold of concern for class I. The daily per capita intake of citral is 6849 μg in Europe and 6990 μg in the USA. Accordingly, the evaluation of citral proceeded to step A4.

Step A4. Citral is not endogenous in humans. The evaluation of citral therefore proceeded to step A5.

Step A5. The NOEL of 60 mg/kg of body weight per day for citral (No. 1225) from a 2-year study of carcinogenicity is approximately 500 times greater than the estimated intake of citral from its use as a flavouring agent in Europe (114 μg/kg of body weight per day) and in the USA (117 μg/kg of body weight per day). The Committee therefore concluded that citral would not pose a safety concern at the currently estimated level of intake.

Table 4 summarizes the evaluations of the 32 aliphatic branched-chain, saturated and unsaturated alcohols, aldehydes, acids, and related esters (Nos 1199–1230) in this group.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 32 of these flavouring agents were to be consumed concurrently on a daily basis, the estimated combined *per capita* intake would exceed the human intake threshold for structural class I (1800 μg per day). However, the agents in this group are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Consideration of secondary components

Seven members of this group of flavouring agents (Nos 1209, 1211, 1219–1223) have assay values of <95%. Information on the safety of the secondary components of these seven compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary compo-

nents for flavouring agents with minimum assay values of 95% or less). The secondary components of No. 1209 (propionaldehyde and propionic acid) were evaluated at the forty-ninth meeting and were considered not to present a safety concern at current levels of intake. The secondary component of No. 1211 (4-methyl-2-methylenevaleric acid) has not been evaluated previously; however, a structurally related substance (isovaleric acid) was evaluated at the forty-ninth meeting and considered not to present a safety concern at current levels of intake. Two of the secondary components of No. 1219 (geraniol and citronellal) were evaluated at the present meeting, while the remaining secondary component (citronellyl acetate) was evaluated at the fifty-ninth meeting; none of the secondary components was considered to present a safety concern at current intake levels. One of the secondary components of No. 1220 (eucalyptol) was evaluated at the present meeting, while three other secondary components (linalool, isopulegol, and citronellyl acetate) were evaluated at the fifty-first, fifty-fifth and fifty-ninth meetings. None of the secondary components of No. 1220 was considered to present a safety concern on the basis of current intake levels. One of the secondary components of No. 1221 (citronellal) was evaluated at the present meeting, and considered not to present a safety concern at current levels of intake. Some of the secondary components of Nos. 1221–1223 (citronellyl, neryl, and geranyl acetate esters) are expected to be hydrolysed to the corresponding terpene alcohols (citronellol, nerol, and geraniol), which were evaluated at the present meeting, and acetic acid, which was evaluated at the forty-ninth meeting. On this basis, none of the secondary components of Nos. 1221–1223 was considered to present a safety concern on the basis of current levels of intake.

Conclusions

The Committee maintained the previously established group ADI of 0–0.5 mg/kg of body weight, expressed as citral, for citral, citronellol, geranyl acetate, linalool, and linalyl acetate. The Committee noted that the estimated combined intake for citronellol (Table 4), citral (Table 4), geranyl acetate (Annex 1, reference *131*), linalool (Annex 1, reference *137*), and linalyl acetate (Annex 1, reference *137*) is approximately 0.20 mg/kg of body weight per day in Europe and 0.15 mg/kg of body weight per day in the USA, and therefore does not exceed the group ADI. It was also noted that even the total combined daily intake of all 32 flavouring agents under evaluation (approximately 0.15 mg/kg of body weight in Europe and the USA) is less than the group ADI. The Committee concluded that the safety of the flavouring agents in this group of aliphatic branched-chain

saturated and unsaturated alcohols, aldehydes, acids, and related esters would not raise concern at the currently estimated levels of intake. The Committee noted that all of the available data on toxicity and metabolism of the flavouring agents in the group were consistent with the results of the safety evaluation. A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.4 *Aliphatic and aromatic ethers*

The Committee evaluated a group of 29 aliphatic and aromatic flavouring agents (see Table 5) that included eucalyptol (No. 1234) and anisole (No. 1241) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). These agents have not been evaluated previously by the Committee. Benzyl butyl ether (No. 1253) and dibenzyl ether (No. 1256) were evaluated for specifications only at the twenty-fourth meeting (Annex 1, reference *53*).

Twenty-three of the 29 flavouring agents (Nos 1231–1239, 1241–1246, 1248–1255) have been reported to occur naturally in foods. They have been detected in fruits, vegetables, alcoholic beverages, cheese, oil and tea.

Estimated daily per capita intake

The total annual volume of production of the 29 flavouring agents in this group is approximately 11 000 kg in Europe and 19 000 kg in the USA. More than 90% of the total annual volume of production in Europe and >75% in the USA is accounted for by eucalyptol (No. 1234). The estimated daily per capita intake of eucalyptol in Europe and the USA is 1439 μg and 1954 μg, respectively. The daily per capita intakes of the other flavouring agents in the group range from 0.003–241 μg/day. The daily per capita intake of each agent in Europe and in the USA is reported in Table 5.

Absorption, distribution, metabolism and elimination

The aliphatic ethers in this group are either open-chain (Nos 1231–1232) or cyclic compounds (Nos 1233–1240). The open-chain aliphatic compounds can be expected to undergo *O*-dealkylation to yield the corresponding aldehyde and alcohol, followed by complete oxidation in the fatty acid pathway and tricarboxylic acid cycle. The alicyclic ethers can be expected to undergo either ring hydroxylation or side-chain oxidation followed by conjugation with glucuronic acid and excretion in the urine.

Most of the aromatic flavouring agents in this group have single benzene ring structures with an ether group and one or more simple saturated (Nos 1241–1250 and 1252–1254) or unsaturated (No. 1251)

Table 5
Summary of results of the safety evaluations of aliphatic and aromatic ethers used as flavouring agents[a]

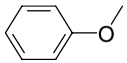
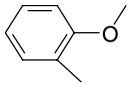
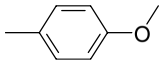
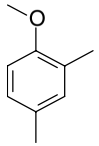
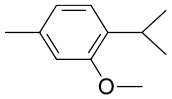
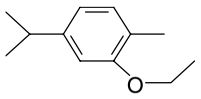
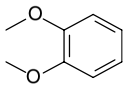
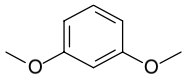
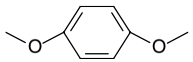
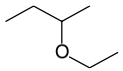
Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
Anisole	1241	100-66-3 	No Europe: 0.03 USA: 0.01	NR	NR	See note 1	No safety concern
o-Methylanisole	1242	578-58-5 	No Europe: 3 USA: 0.06	NR	NR	See note 1	No safety concern
p-Methylanisole	1243	104-93-8 	No Europe: 0.5 USA: 15	NR	NR	See note 2	No safety concern
2,4-Dimethylanisole	1245	6738-23-4 	No Europe: ND USA: 0.2	NR	NR	See note 3	No safety concern
1-Methyl-3-methoxy-4-isopropylbenzene	1246	1076-56-8 	No Europe: 2 USA: 0.1	NR	NR	See note 3	No safety concern

Table 5 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Carvacryl ethyl ether	1247	4732-13-2 	No Europe: 0.1 USA: 0.02	NR	NR	See note 3	No safety concern
1,2-Dimethoxybenzene	1248	91-16-7 	No Europe: ND USA: 20	NR	NR	See note 3	No safety concern
m-Dimethoxybenzene	1249	151-10-0 	No Europe: 5 USA: 2	NR	NR	See note 3	No safety concern
p-Dimethoxybenzene	1250	150-78-7 	No Europe: 18 USA: 7	NR	NR	See note 3	No safety concern
Structural class II							
sec-Butyl ethyl ether	1231	2679-87-0 	No Europe: 8 USA: 0.3	NR	NR	See note 4	No safety concern

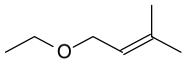
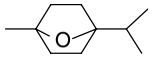
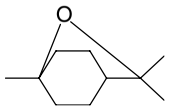
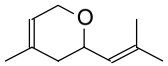
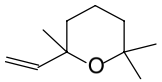
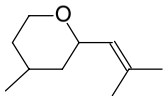
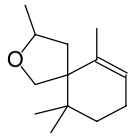
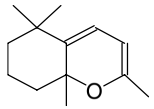
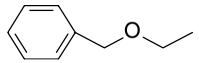
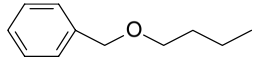
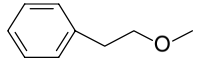
1-Ethoxy-3-methyl-2 butene	1232	22094-00-4 	No Europe: 0.9 USA: 2	NR	NR	See note 4	No safety concern
1,4-Cineole	1233	470-67-7 	No Europe: 5 USA: 146	NR	NR	See note 5	No safety concern
Eucalyptol	1234	470-82-6 	Yes Europe: 1439 USA: 1954	NO	Yes. The NOEL of >32mg/kg of body weight per day for eucalyptol is approximately 1000 times the estimated daily intakes of 24µg/kg of body weight in Europe and 33µg/kg of body weight in the USA when used as a flavouring agent	See note 5	No safety concern
Nerol oxide	1235	1786-08-9 	No Europe: 1 USA: 0.7	NR	NR	See note 5	No safety concern
2,2,6-Trimethyl-6-vinyltetrahydropyran	1236	7392-19-0 	No Europe: 0.01 USA: 8	NR	NR	See note 5	No safety concern
Tetrahydro-4-methyl-2-(2-methylpropen-1-yl) pyran	1237	16409-43-1 	No Europe: 4 USA: 0.2	NR	NR	See note 5	No safety concern

Table 5 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Theaspirane	1238	36431-72-8 	No Europe: 2 USA: 0.1	NR	NR	See note 5	No safety concern
Cycloionone	1239	5552-30-7 	No Europe: ND USA: 2	NR	NR	See note 5	No safety concern
Benzyl ethyl ether	1252	539-30-0 	No Europe: 0.003 USA: 2	NR	NR	See notes 3 and 6	No safety concern
Benzyl butyl ether	1253	588-67-0 	No Europe: ND USA: 0.02	NR	NR	See notes 3 and 6	No safety concern
Methyl phenethyl ether	1254	3558-60-9 	No Europe: 31 USA: 0.01	NR	NR	See notes 3 and 6	No safety concern

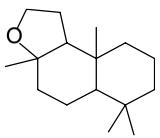
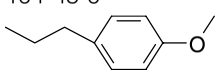
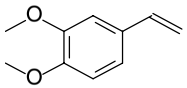
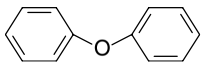
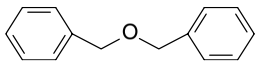
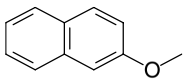
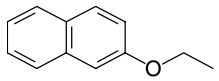
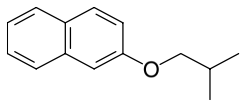
Structural class III							
1,5,5,9-Tetramethyl-13-oxatricyclo (8.3.0.0(4,9)) tridecane	1240	3738-00-9 	No Europe: 1 USA: 0.1	NR	NR	See note 5	No safety concern
p-Propylanisole	1244	104-45-0 	Yes Europe: 23 USA: 114	NO	Yes. The NOEL of 300 mg/kg of body weight for the related substance *p*-propenylanisole (trans anethole) is >100 000 times the daily intakes of *p*-propylanisole in Europe (0.4 µg/kg of body weight per day) and in the USA (2 µg/kg body weight) when used as a flavouring agent	See note 7	No safety concern
3,4-Dimethoxy-1-vinylbenzene	1251	6380-23-0 	No Europe: ND USA: 0.01	NR	NR	See notes 3 and 6	No safety concern
Diphenyl ether	1255	101-84-8 	No Europe: 14 USA: 5	NR	NR	See note 8	No safety concern

Table 5 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Dibenzyl ether	1256	103-50-4 	Yes Europe: 0.6 USA: 241	No	Yes. The NOEL of 196 mg/kg of bodyweight per day (females) and >620 mg/kg bw per day (males) for dibenzyl ether is >10 000 000 and >10 000 times the estimated daily intakes of 0.01 mg/kg of bodyweight in Europe and 4 mg/kg of bodyweight in the USA, respectively, when used as a flavouring agent	See notes 3 and 6	No safety concern
b-Naphthyl methyl ether	1257	93-04-9 	No Europe: ND USA: 0.01	NR	NR	See note 9	No safety concern

b-Naphthyl ethyl ether	1258	93-18-5 	No Europe: ND USA: 4	NR	NR	See note 9	No safety concern
b-Naphthyl isobutyl ether	1259	2173-57-1 	No Europe: 1 USA: 2	NR	NR	See note 9	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 flavouring agents in this group are expected to be metabolized to innocuous products.

[b] The threshold for human intake for structural classes I, II and III are 1800μg/day, 540μg/day and 90μg/day, respectively. All intake values are expressed in μg per day. The combined *per capita* intakes of flavouring agents in structural class I are 29μg per day in Europe and 44μg per day in the USA. The combined *per capita* intakes of flavouring agents in structural class II are 1491μg per days in Europe and 2115μg per day in the USA. The combined *per capita* intakes of flavouring agents in structural class III are 40μg per day in Europe and 386μg per day in the USA.

Notes to Table 5:

1. Metabolized primarily by *p*-hydroxylation with *O*-demethylation, and *o*-hydroxylation is the minor pathway
2. Metabolized primarily by *m*-hydroxylation with *O*-demethylation
3. Metabolized by *O*-demethylation
4. Metabolized by cytochrome P450-catalysed *O*-dealkylation to the corresponding alcohol and aldehyde, followed by complete oxidation in the fatty acid pathway and tricarboxylic acid cycle.
5. Oxidized by cytochrome P450 isoenzymes to polar metabolites, followed by conjugation with glucuronic acid and excretion in the urine
6. Metabolized by ring hydroxylation
7. Metabolized by *O*-demethylation, α and ω-1 oxidation of the side chain and side chain degradation
8. Metabolized by ring hydroxylation followed by conjugation with glucuronic acid and excretion
9. Excreted as a glucuronic acid conjugate with the methyl ether linkage intact

side-chains. Some have dual methoxy groups (Nos 1248–1251). Others in this group have two aromatic rings, which are either separate (Nos 1255 and 1256) or fused (Nos 1257–1259). These aromatic ethers can be expected to be metabolized by one or more of three pathways (ring hydroxylation, *O*-dealkylation, or side-chain oxidation), depending on the location of the substituents, and then conjugated with glucuronic acid, sulfate or glycine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the 29 flavouring agents in this group, the Committee assigned nine (Nos 1241–1243 and 1245–1250) to structural class I. Twelve flavouring agents (Nos 1231–1239, and 1252–1254) were assigned to structural class II and the remaining eight (Nos 1240, 1244, 1251, 1255–1259) were assigned to structural class III.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes of all nine of the flavouring agents in structural class I, 11 of the 12 agents in structural class II, and six of the eight agents in structural class III are below the threshold of concern (i.e. 1800 µg for class I, 540 µg for class II and 90 µg for class III). The Committee concluded that these 26 substances would not be expected to be of safety concern when used as flavouring agents at currently estimated levels of intake. Intake of one of the agents in structural class II, eucalyptol (No. 1234), and two agents in structural class III, *p*-propylanisole (No. 1244) and dibenzyl ether (No. 1256), exceed the thresholds of concern for class II and III. The daily intake of eucalyptol per capita has been reported to be 1439 µg in Europe and 1954 µg in the USA. The daily intake per capita of *p*-propylanisole is 23 µg in Europe and 114 µg in the USA. The daily intake per capita of dibenzyl ether is 0.6 µg in Europe and 241 µg in the USA. Accordingly, the evaluation of these agents proceeded to step A4.

Step A4. None of these three flavouring agents is endogenous in humans. The evaluation of these substances therefore proceeded to step A5.

Step A5. The NOEL of >32 mg/kg of body weight per day for eucalyptol (No. 1234) is approximately 1000 times greater than the estimated intake of eucalyptol from its use as a flavouring agent in Europe

(24 μg/kg of body weight per day) and in the USA (33 μg/kg of body weight per day)[1].

The NOEL of 300 mg/kg of body weight per day for *p*-propenylanisole, identified by the Committee (Annex 1, reference *138*), provides a margin of safety that is approximately 150 000 times greater than the highest estimated intake of *p*-propylanisole (No. 1244) from its use as a flavouring agent (0.4 μg/kg body weight per day in Europe and 2 μg/kg of body weight per day in the USA). The NOEL of 196 mg/kg of body weight per day for dibenzyl ether (No. 1256) provides a margin of safety that is 50 000 times greater than the highest estimated intake of dibenzyl ether from its use as a flavouring agent (0.01 μg/kg of body weight per day in Europe and 4 μg/kg of body weight per day in the USA). The Committee therefore concluded that the safety of these agents raises no concern at their currently estimated levels of use.

Table 5 summarizes the evaluations of the 29 aliphatic and aromatic ethers (Nos 1231–1259) in this group.

Consideration of combined intakes from use as flavouring agents

All 29 agents in this group are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Consideration of secondary components

Two members of this group, 1,4-cineole (No. 1233) and benzyl butyl ether (No. 1253), have a minimum assay value of <95%. Information on the safety of the secondary components of these three compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less). 1,8-Cineole (No. 1234), the secondary component in 1,4-cineole, was evaluated at the present meeting, while benzyl alcohol (No. 25), the secondary component in benzyl butyl ether, was evaluated at a previous meeting (Annex 1, reference *122*). The secondary components in these two flavouring agents were considered not to present a safety concern.

[1] The Committee was informed that certain products, such as lozenges/hard candies, may contain high levels of eucalyptol and noted that theoretical estimates of daily per capita intake of eucalyptol could be as great as 16 mg. Data on the frequency of use of such products were not available to the Committee, thus it was not possible to estimate chronic intake. The Committee noted, however, that even at an intake per capita of 16 mg per day, a margin of safety of approximately 120 existed between this intake and the NOEL of >32 mg/kg of body weight per day established in a chronic study conducted in rats.

Conclusions

The Committee concluded that none of the flavouring agents in this group of aliphatic and aromatic ethers would raise a safety concern at the currently estimated levels of intake. Other data on the toxicity and metabolism of these aromatic and aliphatic ethers were consistent with the results of the safety evaluation. A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.5 *Hydroxypropenylbenzenes*

The Committee evaluated a group of flavouring agents that included nine hydroxy- or alkoxy-substituted propenylbenzenes (see Table 6), commonly recognized as isoeugenol derivatives, by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). These agents have not been evaluated previously by the Committee.

Three of the nine flavouring agents (Nos 1260, 1265 and 1266) have been reported to occur naturally in foods. They have been detected in blueberries, mushrooms, ginger, raw fatty fish, and pork.

Estimated daily per capita intake

The total annual volume of production of the nine flavouring agents in this group is approximately 2000 kg in Europe and 4100 kg in the USA. More than 80% of the total annual volume of production in Europe is accounted for by isoeugenol (No. 1260) and isoeugenyl methyl ether (No. 1266), and > 65% of the total annual volume of production in the USA is accounted for by propenylguaethol (No. 1264). The estimated daily per capita intake of isoeugenol is approximately 120 µg in Europe and 40 µg in the USA. The estimated daily per capita intake of isoeugenyl methyl ether is approximately 130 µg in Europe and 130 µg in the USA. The estimated daily per capita intake of propenylguaethol is approximately 40 µg in Europe and 350 µg per day in the USA. The daily per capita intakes of the other flavouring agents in the group range from 0.009–11 µg, with most being <1 µg. The daily per capita intake of each agent in Europe and in the USA is reported in Table 6.

Absorption, distribution, metabolism and elimination

Six (Nos 1260–1265) of the nine flavouring agents contain a free phenolic OH group or are simple phenolic esters. The remaining three agents (Nos 1266–1268) are propenyl benzene derivatives that contain a methoxy, ethoxy, or benzoxy substituents on the para (*p*) position and a methoxy substituent on the meta (*m*) position.

Table 6
Summary of results of the safety evaluations of hydroxypropenylbenzenes[a]

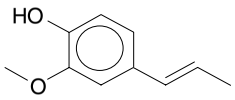
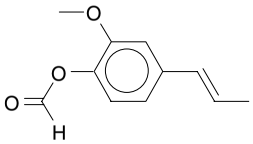
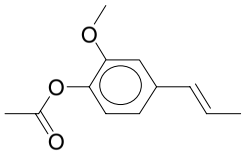
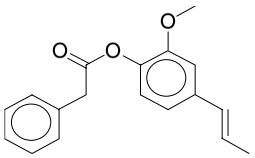
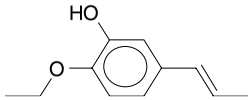
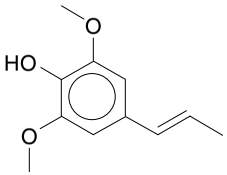
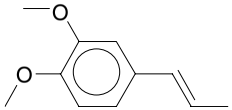
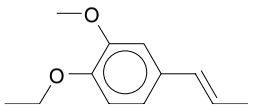
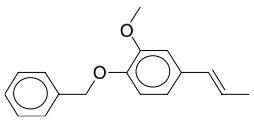
Flavouring agent	No.	CAS No. and structure	*Step A3*[b] Does intake exceed the threshold for human intake?	*Step A4* Is the substance or are its metabolites endogenous?	*Step A5* Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
Isoeugenol	1260	97-54-1 	No Europe: 117 USA: 43	NR	NR	See note 1	No safety concern
Isoeugenyl formate	1261	7774-96-1 	No Europe: ND USA: 0.2	NR	NR	See note 2	No safety concern
Isoeugenyl acetate	1262	93-29-8 	No Europe: 0.7 USA: 11	NR	NR	See note 3	No safety concern
Isoeugenyl phenylacetate	1263	120-24-1 	No Europe: ND USA: 0.3	NR	NR	See note 4	No safety concern

Table 6 *(continued)*

Flavouring agent	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 margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Propenylguaethol	1264	94-86-0 	No Europe: 44 USA: 354	NR	NR	See note 1	No safety concern
4-Propenyl-2,6-dimethoxyphenol **Structural class III**	1265	20675-95-0 	No Europe: ND USA: 2	NR	NR	See note 1	No safety concern
Isoeugenyl methyl ether	1266	93-16-3 	Yes Europe: 128 USA: 129	No	Yes; The NOEL of 6 mg/kg of body weight per day for isoeugenol methyl ether is >1000 times the estimated daily intakes of 2 μg/kg of bodyweight per day in Europe and the USA when used as a flavouring agent	See note 5	No safety concern

Isoeugenyl ethyl ether	1267	7784-67-0 	No Europe: ND USA: 0.009	NR	NR	See note 5	No safety concern
Isoeugenyl benzyl ether	1268	120-11-6 	No Europe: 1 USA: 1	NR	NR	See note 5	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 flavouring agents in this group are expected to be metabolized to innocuous products.

[b] The threshold for human intake for structural classes I and III are 1800μg/day and 90μg/day, respectively. All intake values are expressed in μg per day. The combined per capita intake of flavouring agents in structural class I is 162μg per day in Europe and 411μg per day in the USA. The combined per capita intake of flavouring agents in structural class III is 129μg per day in Europe and 130μg per day in the USA.

Notes to Table 6:

1. Detoxication primarily by conjugation of the phenolic OH group with sulfate or glucuronic acid and excretion mainly in the urine
2. Hydrolysed to isoeugenol and formic acid, which is oxidized to CO_2 and H_2O.
3. Hydrolysed to isoeugenol and acetic acid, which is absorbed from the gastrointestinal tract and acts as a precursor for synthesis of biomolecules.
4. Hydrolysed to isoeugenol and phenylacetic acid, which is endogenous in humans and excreted as the glutamine conjugate.
5. Detoxicated primarily by O-demethylation at the (*m*) or (*p*)-methoxy substituent to yield the corresponding phenol followed by excretion in the urine as the sulfate or glucuronic acid conjugate.

Isoeugenol derivatives containing a phenolic OH group (Nos 1260, 1264 and 1265) are rapidly absorbed from the gastrointestinal tract and are metabolized principally in the liver via conjugation of the phenolic hydroxy group with sulfate or glucuronic acid. The conjugates are subsequently excreted, primarily in the urine.

Esters of isoeugenol (Nos 1261–1263) are hydrolysed in vivo by carboxylesterases. Upon hydrolysis the product, isoeugenol, is conjugated and excreted while the component carboxylic acids are metabolized in well-recognized biochemical pathways.

The alkoxypropenylbenzene derivatives (Nos 1266–1268) in this group primarily undergo *O*-demethylation of either the (*m*) or (*p*)-methoxy substituent to yield the corresponding isoeugenol derivative that is then excreted as the sulfate or glucuronic acid conjugate (Annex 1, reference *137*).

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 flavouring agents, the Committee assigned six of the nine flavouring agents (Nos 1260–1265) to structural class I. The remaining three flavouring agents (Nos 1266–1268) were assigned to structural class III.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes of all six of the flavouring agents in structural class I and two of the three agents in structural class III are below the threshold of concern (i.e. 1800 µg for class I and 90 µg for class III). The Committee concluded that the safety of these eight flavouring agents raises no concern at their currently estimated levels of intake. One of the agents in structural class III, isoeugenyl methyl ether (No. 1266), exceeds the threshold of concern. The daily per capita intake of isoeugenyl methyl ether is 128 µg in Europe and 129 µg in the USA. Accordingly, the evaluation of isoeugenyl methyl ether proceeded to step A4.

Step A4. Isoeugenyl methyl ether is not endogenous in humans. The evaluation therefore proceeded to step A5.

Step A5. A NOEL of 100 mg/kg of body weight per day for isoeugenyl methylether (No. 1266) was identified from a 28-day study in rats fed diets containing isoeugenyl methylether. In another study of longer duration (13 weeks), no adverse effects were observed in rats at a

dietary intake of 6 mg isoeugenyl methyl ether/kg of body weight per day. The NOEL of 6 mg/kg of body weight per day was >1000 times the estimated intake of isoeugenyl methyl ether from its use as a flavouring agent in Europe and in the USA (2 µg/kg of body weight per day in each case). On the basis of these data, the Committee concluded that isoeugenyl methyl ether is not expected to be of safety concern at currently estimated levels of use.

Table 6 summarizes the evaluations of nine hydroxypropenylbenzenes (Nos 1260–1268).

Consideration of combined intakes from use as flavouring agents

All agents in this group are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Conclusions

The Committee concluded that the flavouring agents in this group of hydroxypropenylbenzenes would not be of safety concern at the currently estimated levels of intake. Other data on the toxicity and metabolism of these hydroxypropenylbenzenes were consistent with the results of the safety evaluation. An addendum to the monograph summarizing the safety data on this group of flavouring agents was prepared.

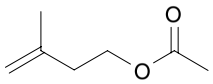
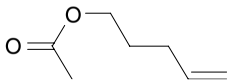
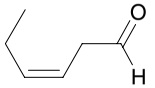
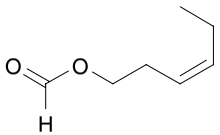
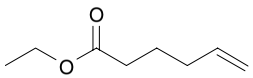
4.1.6 *Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters : additional compounds*

The Committee evaluated 20 flavouring agents that included linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters (see Table 7) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1).

The Committee had previously evaluated 42 other members of this chemical group of flavouring agents at the fifty-first meeting (Annex 1, reference *137*). It was concluded that 41 of the 42 substances in this group were of no safety concern at currently estimated levels of intake. The evaluation of one substance, ethyl 2-methyl-3,4-pentadienoate (No. 353) was deferred, pending review of a 90-day study of ethyl 2-methyl-3,4-pentadienoate in the diet.

Fourteen of the 20 agents evaluated at the present meeting are esters formed from linear or branched-chain unsaturated alcohols or carboxylic acids. The remaining six substances are linear unsaturated

Table 7
Summary of results of safety evaluations of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters[a,b]

Flavouring agent	No.	CAS No. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Isoprenyl acetate	1269	5205 07 2 	No USA: 11 Europe: 9	See note 1	No safety concern
4-Pentenyl acetate	1270	1576-85-8 	No USA: 4 Europe: 4	See note 1	No safety concern
3-Hexenal	1271	4440-65-7 	No USA: 53 Europe: 29	See note 2	No safety concern
3-Hexenyl formate	1272	2315 09 5 	No USA: 18 Europe: 14	See note 1	No safety concern
Ethyl 5-hexenoate	1273	54653-25-7 	No USA: 4 Europe: 4	See note 1	No safety concern

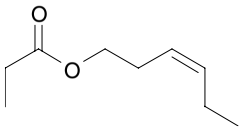
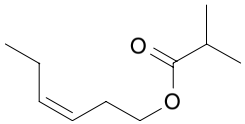
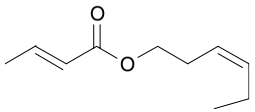
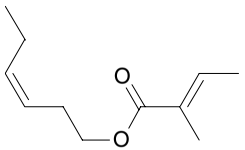
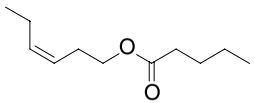
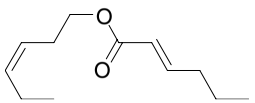
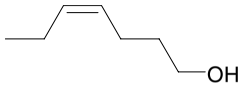
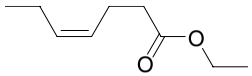
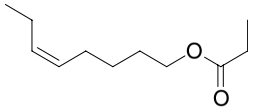
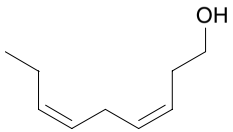
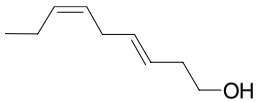
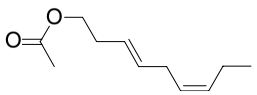
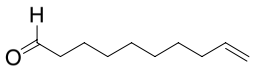
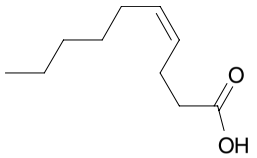
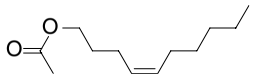
cis-3-Hexenyl propionate	1274	33467-74-2 	No USA: 18 Europe: 14	See note 1	No safety concern
cis-3-Hexenyl isobutyrate	1275	41519-23-7 	No USA: 18 Europe: 14	See note 1	No safety concern
(*Z*)-3-Hexenyl (*E*)-2-butenoate	1276	65405-80-3 	No USA: 0.2 Europe: 0.1	See note 1	No safety concern
cis-3-Hexenyl tiglate	1277	67883-79-8 	No USA: 70 Europe: ND	See note 1	No safety concern
cis-3-Hexenyl valerate	1278	35852-46-1 	No USA: 9 Europe: 7	See note 1	No safety concern
3-Hexenyl 2-hexenoate	1279	53398-87-1 	No USA: 0.1 Europe: 0.07	See note 1	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step A3*[c] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
(*Z*)-4-Hepten-1-ol	1280	6191-71-5 	No USA: 2 Europe: ND	See note 3	No safety concern
Ethyl-*cis*-4-heptenoate	1281	39924-27-1 	No USA: 4 Europe: 4	See note 1	No safety concern
(*Z*)-5-Octenyl propionate	1282	196109-18-9 	No USA: 4 Europe: ND	See note 1	No safety concern
(*Z,Z*)-3-6-Nonadien-1-ol	1283	53046-97-2 	No USA: 0.9 Europe: ND	See note 3	No safety concern
(*E*)-3-(*Z*)-6-Nonadien-1-ol	1284	56805-23-3 	No USA: 0.9 Europe: ND	See note 3	No safety concern

(*E*)-3-(*Z*)-6-Nonadien-1-ol acetate	1285	211232-05-6 	No USA: 18 Europe: ND	See note 1	No safety concern
9-Decenal	1286	39770-05-3 	No USA: 0.4 Europe: 0.7	See note 2	No safety concern
4-Decenoic acid	1287	26303-90-2 	No USA: 1 Europe: 2	See note 4	No safety concern
cis-4-Decenyl acetate	1288	67452-27-1 	No USA: 2 Europe: 2	See note 1	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported.

[a] Forty-two flavouring agents in this same congeneric group were previously evaluated by the Committee (Annex 1, reference *137*).

[b] *Step 2*: All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[c] The threshold for human intake for structural class I is 1800 μg/day. All intake values are expressed in μg/day. The combined per capita intake of the flavouring agents is 103 μg per day in Europe and 239 μg per day in the USA. The cumulative per capita intake for the amended group as a whole including the 42 agents in the original evaluation and the 20 additional substances is 5744 and 2760 μg per day in Europe and the USA, respectively.

Notes to Table 7:

1. The ester is expected to undergo hydrolysis to the corresponding primary alcohol and carboxylic acid. The alcohol is oxidized to the corresponding aldehyde and carboxylic acid, which is completely metabolized in the fatty acid and tricarboxylic acid pathways to carbon dioxide and water.
2. The aldehyde is oxidized to the corresponding carboxylic acid, which is subsequently oxidized in the fatty acid pathway and the tricarboxylic acid cycle.
3. The primary alcohol is oxidized to the corresponding aldehyde and carboxylic acid, which is completely metabolized in the fatty acid and tricarboxylic acid pathways to carbon dioxide and water.
4. The carboxylic acid is completely metabolized in the fatty acid and tricarboxylic acid pathways.

alcohols and aldehydes. None of these agents had been evaluated previously.

Fourteen of the 20 flavouring agents (Nos 1269, 1271-1278, 1281, 1283, 1284, 1286, and 1287) in this group have been reported to occur as natural components of foods. They have been detected in fruit, a variety of herbs and spices (e.g. chervil, coriander, thyme), peppermint oil, spearmint oil, chamomile oil, scotch whisky, black tea, beer, hop oil, olives, raw pork, roast beef and chicken (*5*).

Estimated daily per capita intake

The total annual volume of production of these 20 linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters is approximately 720 kg in Europe and 1400 kg in the USA (Table 8). Approximately 63% and 70% of the total annual volume of production in Europe and in the USA, respectively, is accounted for by four *cis*-3-hexenyl esters (*cis*-hexenyl isobutyrate, No. 1275; *cis*-hexenyl propionate, No. 1274; *cis*-3-hexenyl tiglate, No. 1277; and *cis*-hexenyl valerate, No. 1278) and by 3-hexenal (No. 1271). The daily per capita intake and poundage of each agent in Europe and in the USA are reported in Tables 7 and 8.

Absorption, distribution, metabolism and elimination

The aliphatic esters (Nos 1269, 1270, 1272-1279, 1281, 1282, 1285 and 1288) in this group can be expected to hydrolyse to the corresponding unsaturated aliphatic alcohol and carboxylic acid (*6–10*). Once formed, the linear and branched-chain unsaturated primary alcohols are rapidly absorbed from the gastrointestinal tract (*11*) and oxidized to their corresponding aldehydes. Long chain ($C > 8$) aldehydes (No. 1286) are readily absorbed as micelles, deposited in chylomicrons or low density lipoproteins, and transported to the liver via the lymphatic system (*12*). Once absorbed, aldehydes are oxidized to their corresponding unsaturated carboxylic acids.

After the action of 3-hydroxyacyl coenzyme A epimerase has converted *cis* isomers to *trans* isomers, and the double bond has been isomerized from the 3- to the 2-position by enoyl coenzyme A isomerase (*13*), linear unsaturated carboxylic acids enter the fatty acid pathway and are cleaved to yield acetyl or propionyl coenzyme A and subsequently completely metabolized to carbon dioxide and water in the tricarboxylic acid cycle.

Table 8

Annual volumes of production of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters used as flavouring agents in Europe and the USA[a]

Substance (No.)	Most recent annual volume (kg)[a]	Intake[b]		Intake of alcohol equivalents µg/kg bw per day[c]	Annual volume in naturally occurring foods (kg)[d]	Consumption ratio[e]
		µg/day	µg/kg bw per day			
Isoprenyl acetate (1269)						
Europe	60	9	0.1	0.07		
USA	60	11	0.2	0.13	+	NA
4-Pentenyl acetate (1270)						
Europe	25	4	0.06	0.04		
USA	25	4	0.07	0.05	–	NA
3-Hexenal (1271)						
Europe	200	29	0.5			
USA	300	53	0.9		+	NA
3-Hexenyl formate (1272)						
Europe	100	14	0.2	0.2		
USA	100	18	0.3	0.2	+	NA
Ethyl 5-hexenoate (1273)						
Europe	25	4	0.06	0.02		
USA	25	4	0.07	0.02	+	NA
cis-3-Hexenyl propionate (1274)						
Europe	100	14	0.2	0.1		
USA	100	18	0.3	0.2	0.4[f]	0.004
cis-3-Hexenyl isobutyrate (1275)						
Europe	100	14	0.2	0.1		
USA	100	18	0.3	0.2	6	0.1
(Z)-3-Hexenyl (E)-2-butenoate (1276)						
Europe	1	0.1	0.002	0.001		
USA	1	0.2	0.003	0.002	+	NA

Table 8 *(continued)*

Substance (No.)	Most recent annual volume (kg)[a]	Intake[b]		Intake of alcohol equivalents μg/kg bw per day[c]	Annual volume in naturally occurring foods (kg)[d]	Consumption ratio[e]
		μg/day	μg/kg bw per day			
Icis-3-Hexenyl tiglate (1277)						
Europe	ND	ND	ND			
USA	400	70	1	0.5	+	NA
cis-3-Hexenyl valerate (1278)						
Europe	50	7	0.1	0.05		
USA	50	9	0.1	0.05	8	0.2
3-Hexenyl 2-hexenoate (1279)						
Europe	0.5	0.07	0.001	0.0005		
USA	0.5	0.1	0.001	0.001	–	NA
***(Z)*-4-Hepten-1-ol (1280)**						
Europe	ND	ND	ND			
USA	9	2	0.03		–	NA
Ethyl cis-4-heptenoate (1281)						
Europe	25	4	0.06	0.02		
USA	25	4	0.07	0.02	+	NA
(Z)-5-Octenyl propionate (1282)						
Europe	ND	ND	ND			
USA	23	4	0.07	0.05	–	NA
(Z,Z)-3,6-Nonadien-1-ol (1283)						
Europe	ND	ND	ND			
USA	5	0.9	0.01		48.6	9.7
(E)-3-(Z)-6-Nonadien-1-ol (1284)						
Europe	ND	ND	ND			
USA	5	0.9	0.01		+	NA

(E)-3-(Z)-6-Nonadien-1-ol acetate (1285)						
Europe	ND	ND	ND			
USA	100	18	0.3	0.2	–	NA
9-Decenal (1286)						
Europe	5	0.7	0.01			
USA	2	0.4	0.006		+	NA
4-Decenoic acid (1287)						
Europe	10	1	0.02			
USA	10	2	0.03		5772	577.2
cis-4-Decenyl acetate (1288)						
Europe	15	2	0.04	0.03		
USA	10	2	0.03	0.02	–	NA
Total						
Europe	717	103				
USA	1351	239				

NA, not available; ND, no intake data reported; +, reported to occur naturally in foods (*5*), but no quantitative data; –, not reported to occur naturally in foods.

[a] The volumes cited are the anticipated annual volumes, which are the maximum amounts of flavouring agent estimated to be used annually in both Europe and the USA by the manufacturer at the time the material was proposed for flavour use (*15*).

[b] Intake (μg/person per day) was calculated as follows: [(annual volume, kg) x (1×10^9 μg/kg)/(population x survey correction factor × 365 days], where population (10%, "eaters only") = 32×10^6 for Europe and 26×10^6 for the USA. The correction factor = 0.6 for Europe and USA representing the assumption that only 60% of the annual flavour volume was reported (*15*). Intake (μg/kg bw per day) calculated as follows: [(μg/person per day)/body weight], where body weight = 60 kg. Slight variations may occur from rounding.

[c] Calculated as follows: (molecular weight alcohol/molecular weight ester) × daily per capita intake ("eaters only") ester

[d] Quantitative data for the USA reported by Stofberg and Grundschober (*16*)

[e] The consumption ratio is calculated as follows: (annual consumption in food, kg)/(most recently reported volume as a flavouring agent, kg)

[f] Engel and Tressl (1983)

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 20 flavouring agents in this group, the Committee assigned all of them (Nos 1269–1288) to structural class I (*14*).

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all agents in this group therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes of all 20 of the flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg). The Committee concluded that the safety of these 20 flavouring agents raises no concern when they are used at their currently estimated levels of intake.

Table 7 summarizes the evaluations of 20 linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters (Nos 1269–1288).

Consideration of combined intakes from use as flavouring agents

Seven (Nos 1272, 1274–1279) of the 20 substances are esters that will undergo hydrolysis to form *cis*-3-hexenol. Both *cis*-3-hexenol and 3-hexenal (No. 1271) will oxidize to a common metabolite, 3-hexenoic acid. The Committee concluded that under conditions of use the combined intake of these eight substances would not saturate the metabolic pathways leading to the common metabolite. In the unlikely event that all 20 agents considered here and the 42 agents considered previously were to be consumed concurrently on a daily basis, the estimated combined daily per capita intake would exceed the human intake threshold for class I (1800 μg). However, all 62 agents in this group are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Consideration of secondary components

Four members of this group of flavouring agents (Nos. *1271*, *1279*, *1282* and *1284*) have minimum assay values of <95%. Information on the safety of the secondary components of these four compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less). The secondary component of No. 1271 (*trans*-2-hexenal) has not been previously evaluated; however, based on a NOEL of 30 mg/kg of body weight per day from a 13-week study of oral administration in rats, *trans*-2-hexenal was considered not to present a

safety concern. One of the secondary components of No. 1279 (*cis*-3-hexenyl-*cis*-3-hexenoate) was evaluated at the fifty-first meeting, and was considered not to present a safety concern at current levels of intake. The remaining secondary components of No. 1279 (isomers 1, 2, and 3 of hexenyl hexenoate) are expected to undergo the same metabolic pathway as 3-hexenyl-3-hexenoate, which includes hydrolysis to hexenol and hexenoic acid; 3-hexen-1-ol and 3-hexenoic acid were evaluated at the fifty-first meeting. On this basis, the secondary components of No. 1279 were considered not to present a safety concern at current levels of intake. The secondary component of No. 1282 ((*E*)-5-octenyl propionate) is expected to undergo the same metabolic pathway as the primary compound, which includes hydrolysis to *cis*-5-octen-1-ol and propionic acid; both of which were evaluated previously at the forty-ninth and fifty-first meetings, and considered not to present a safety concern. Although the secondary component of No. 1284 ((*E,E*)-3,6-nonadien-1-ol) has not been evaluated previously, a structurally related compound ((*E,E*)-2,6-dodecadienal) was evaluated at the present meeting, and was considered not to present a safety concern based on current intake levels.

Conclusions

The Committee concluded that these 20 flavouring agents, which are additions to the group of linear and branched-chain aliphatic unsaturated primary alcohols and non-conjugated aldehydes, acids, and related esters evaluated previously, would not pose a safety concern at the currently estimated levels of intake. A monograph was published previously (Annex 1, reference *137*).

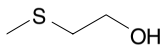
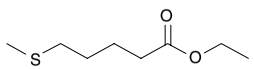
4.1.7 *Simple aliphatic and aromatic sulfides and thiols: additional compounds*

The Committee evaluated 12 flavouring agents that included simple aliphatic and aromatic sulfides and thiols (see Table 9) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1).

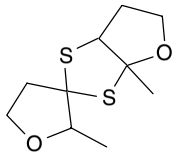
At its fifty-third meeting, the Committee had evaluated 137 other members of this chemical group of flavouring agents (Annex 1, reference *143*). The group was divided into 12 subgroups on the basis of the position of the sulfur atom in order to facilitate the assessment of the relevant data on metabolism and toxicity. All 137 substances in that group were concluded to be of no safety concern on the basis of currently estimated levels of intake.

Of the 12 additional flavouring agents considered here, six agents are thiols with oxidized side chains (subgroup v) (Nos 1289–1294) and

Table 9
Summary of results of the safety evaluations of simple aliphatic and aromatic sulfides and thiols[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substances or related substances?	Comments on predicted metabolism	Conclusion based on current intake
Subgroup ii — Acyclic sulfides with oxidized side chains						
Structural class I						
2-(Methylthio)ethanol	1297	5271-38-5 	No Europe: 1 USA: 0.9	Yes. The NOEL of 1.4 mg/kg of body weight per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is >10 000 times the estimated daily intake of 2-(methylthio)ethanol when used as a flavouring agent	See notes 7 and 2	No safety concern
Ethyl 5-(methylthio)valerate	1298	233665-98-0 	No Europe: 2 USA: 2	Yes. The NOEL of 1.4 mg/kg of body weight per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is >10 000 times the estimated daily intake of ethyl 5-(methylthio)valerate when used as a flavouring agent	See notes 5 and 7	No safety concern

Subgroup iii — Cyclic sulfides
Structural class III

spiro(2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane)	1296	38325-25-6 	No Europe: ND USA: 2	Yes. The NOEL of 25 mg/kg of body weight per day for spiro (2,4-dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane) is >100 000 times the estimated daily intake when used as a flavouring agent	See notes 10, 1 and 3	No safety concern

Subgroup v — Thiols with oxidized side chains
Structural class I

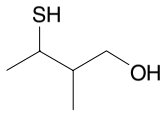
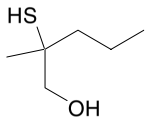
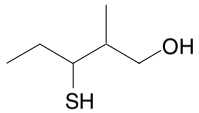
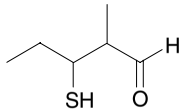
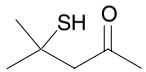
erythro- and *threo*-3-Mercapto-2-methylbutanol	1289		No Europe: 1 USA: 2	Yes. The NOEL of 0.7 mg/kg of body weight per day for the related substance 2-mercapto-3-butanol (No. 546) is >10 000 times the estimated daily intake of *erythro*- and *threo*-mercapto-2-methylbutan-1-ol when used as a flavouring agent	See notes 1 and 2	No safety concern
(±)2-Mercapto-2-methylpentan-1-ol	1290	258823-39-1 	No Europe: 3 USA: 4	Yes. The NOEL of 0.7 mg/kg of body weight per day for the related substance 2-mercapto-3-butanol (No. 546) is >10 000 times the estimated daily intake of (±)2-mercapto-2-methylpentan-1-ol when used as a flavouring agent	See notes 1 and 2	No safety concern

Table 9 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substances or related substances?	Comments on predicted metabolism	Conclusion based on current intake
3-Mercapto-2-methylpentan-1-ol (racemic)	1291	227456-27-1 	No Europe: 1 USA: 0.7	Yes. The NOEL of 0.7 mg/kg of body weight per day for the related substance 2-mercapto-3-butanol (No. 546) is >10 000 times the estimated daily intake of 3-mercapto-2-methylpentan-1-ol (racemic) when used as a flavouring agent	See notes 1 and 2	No safety concern
3-Mercapto-2-methylpentanal	1292	227456-28-2 	No Europe: 3 USA: 4	Yes. The NOEL of 0.7 mg/kg of body weight per day for the related substance 2-mercapto-3-butanol (No. 546) is >10 000 times the estimated daily intake of 3-mercapto-2-methylpentenal when used as a flavouring agent	See notes 1 and 4	No safety concern
4-Mercapto-4-methyl-2-pentanone	1293	19872-52-7 	No Europe: 0.01 USA: 0.02	Yes. The NOEL of 1.9 mg/kg of body weight per day for the related substance 3-mercapto-2-pentanone (No. 560) is >10 000 times the estimated daily intake of 4-mercapto-4-methyl-2-pentanone when used as a flavouring agent	See notes 1 and 3	No safety concern

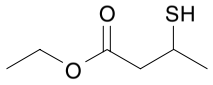
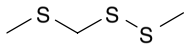
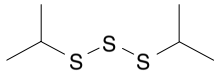
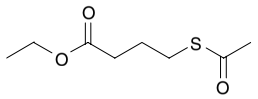
(±)Ethyl 3-mercaptobutyrate	1294	156472-94-5 	No Europe: 4 USA: 4	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >10 000 times the estimated daily intake of (±)ethyl 3-mercaptobutyrate when used as a flavouring agent	See notes 1 and 5	No safety concern
Subgroup vii — Simple disulfides **Structural class I**						
2,3,5-Trithiahexane	1299	42474-44-2 	No Europe: 0.03 USA: 0.04	Yes. The NOEL of 0.3 mg/kg of body weight per day for the related substance 3-methyl-1,2,4- trithiane (No. 574) is >10 000 times the estimated daily intake of 2,3,5-trithiahexane when used as a flavouring agent	See notes 7, 8 and 9	No safety concern
Subgroup ix — Trisulfides and polysulfides **Structural class I**						
Diisopropyl trisulfide	1300	5943-34-0 	No Europe: 0.006 USA: 0.007	Yes. The NOEL of 4.8 mg/kg of body weight per day for the related substance dipropyltrisulfide (No. 585) is >100 000 times the estimated daily intake of diisopropyl trisulfide when used as a flavouring agent	See notes 7, 8 and 9	No safety concern

Table 9 *(continued)*

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for substances or related substances?	Comments on predicted metabolism	Conclusion based on current intake
Subgroup xi — Thioesters						
Structural class I						
Ethyl 4-(acetylthio)butyrate	1295	104228-51-5 	No Europe: 4 USA: 4	Yes. The NOEL of 6.5 mg/kg of body weight per day for the related substance ethylthioacetate (No. 483) is >10 000 times the estimated daily intake of ethyl 4-(acetylthio)butyrate when used as a flavouring agent	See notes 1, 5 and 6	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported

[a] One hundred and thirty-seven (137) flavouring agents in this group were previously evaluated by JECFA. To facilitate the evaluations, the group was divided into 12 subgroups based on the position of the sulfur atom. The subgroup designations are indicated in the table.

[b] *Step 1*: Eleven flavouring agents are in structural class I and one (No. 1296) is in structural class III.

[c] *Step 2*: All of the agents in this group cannot be predicted to be metabolized to innocuous products.

[d] The threshold for human intake for structural class I, II and III are 1800, 540 and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intake of the 11 flavouring agents in structure class I is approximately 21 μg per day in Europe and 24 μg per day in the USA. The combined per capita intake of the remaining flavouring agent in structural class III is 2 μg per day in the USA. The cumulative per capita intake for the amended group as a whole including the 137 substances in the original evaluation and the 12 additional substances is 1181 and 1034 μg/person per day in Europe and the USA, respectively.

Notes to Table 9:

1. Sulfur is expected to be oxidized to sulfonic acid, undergo alkylation and conjugation followed by excretion.
2. The hydroxy group is expected to undergo oxidation to the carboxylic acid.
3. The ketone group is expected to be reduced to the alcohol, conjugated and subsequently excreted.
4. The aldehyde group is expected to be oxidized to the corresponding carboxylic acid, conjugated and subsequently excreted.
5. The ester is expected to undergo hydrolysis to the corresponding carboxylic acid and alcohol.
6. The thioester is expected to undergo hydrolysis to acetate and the corresponding thiol, which will be further oxidized.
7. The sulfur is expected to be oxidized to the sulfoxide and sulfone.
8. The di- or trisulfides are expected to be reduced to free thiols.
9. Free thiols may form mixed disulfides with glutathione or cysteine.
10. Thioketal will hydrolyse to liberate the corresponding ketone and dithiol.

contain an additional alcohol, aldehyde, ketone, or ester functional group. Two agents are acyclic sulfides with oxidized side-chains (subgroup ii) (Nos 1297 and 1298) in which an alcohol or ester functional group is present. The remaining four substances are a thioester (subgroup xi) (No. 1295), a disulfide (subgroup vii) (No. 1299), a trisulfide (subgroup ix) (No. 1300) and a cyclic sulfide (subgroup iii) (No. 1296). None of these agents has been evaluated previously.

Seven of the 12 flavouring agents in this group are naturally occurring components of food (Nos 1291–1294, 1297, 1299, 1300) and have been detected in onions, fruits, broccoli, cabbage, cauliflower, hop oil, wine, fish and cheese.

Estimated daily per capita intake

The total annual volume of production of the 12 simple aliphatic and aromatic sulfides and thiols is approximately 150 kg in Europe and in the USA. The daily per capita intake of each agent is reported in Table 9.

Absorption, distribution, metabolism and elimination

All of the sulfur-containing flavouring agents considered in this addendum are of low relative molecular mass and are sufficiently lipophilic to be absorbed. These flavouring agents can be expected to be metabolized through the various pathways described below and in the previous evaluation by the Committee (Annex 1, reference *143*).

Thiols with oxidized side-chains (Nos 1289–1294)

The metabolism of thiols with oxidized side-chains is predicted to involve a combination of pathways for simple thiols together with further oxidation or conjugation of the oxidized side-chain. Metabolic options for simple thiols include oxidation to form unstable sulfenic acids (RSOH) which are oxidized to sulfinic acids (RSO_2H), undergo methylation to yield methyl sulfides which then form sulfoxides and sulfones, react with endogenous thiols to form mixed disulfides, are conjugated with glucuronic acid, or undergo oxidation of the α-carbon which results in desulfuration and the formation of an aldehyde.

Acyclic sulfides with oxidized side-chains (Nos 1297, 1298)

The presence of oxygenated functional groups, such as an alcohol (No. 1297) or ester (No. 1298), provides additional sites for biotransformation of sulfides (thioethers), and the presence of these polar sites would result in increased renal excretion of these agents. The biotransformation of such oxygenated groups is well characterized and has been described for groups of flavouring agents evaluated

previously by the Committee (Annex 1, references *131*, *132*, *138*, *144*). Simultaneous metabolism of sulfur and oxygenated functional groups has been reported for various substrates. Sulfoxide formation usually predominates as the major metabolic detoxication pathway.

Cyclic sulfides (No. 1296)

Cyclic sulfides can be expected to undergo extensive *S*-oxidation by the cytochrome P450 superfamily to produce the corresponding sulfoxides.

Simple disulfides (No. 1299)

The reduction of xenobiotic disulfides is believed to be extensive and can be catalysed enzymatically, by glutathione reductase or thioltransferases, as well as chemically, by exchange with glutathione, thioredoxin, cysteine or other endogenous thiols. Reduction of non-cyclic disulfides (No. 1299) would result in the formation of thiols of low molecular mass, which are metabolized via the various pathways described above for simple thiols.

Trisulfides (No. 1300)

The trisulfide of glutathione is labile and readily converted to the disulfide, with the release of sulfur as hydrogen sulfide. Trisulfides are predicted to be converted rapidly to the corresponding disulfides with subsequent reduction to thiols, which would then be metabolized via the various pathways described above for simple thiols.

Thioesters (No. 1295)

Thioesters are hydrolysed by lipase and esterases; the rate of hydrolysis increases as the length of the carbon chain increases and decreases as the oxygenation of the carbon chain in the thiol moiety increases. After hydrolysis, the resulting alcohol and carboxylic acid would participate in the metabolic pathways described above for sulfides containing oxygenated functional groups.

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 these 12 flavouring agents, the Committee assigned 11 agents (Nos 1289–1295, 1297–1300) to structural class I. The remaining flavouring agent (No. 1296) was assigned to class III.

Step 2. At currently estimated levels of intake, none of the flavouring agents in this group is predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the B-side of the decision-tree.

Step B3. The estimated daily per capita intakes of the 11 flavouring agents in this group in structural class I are below the threshold of concern (i.e. 1800 µg). The estimated daily per capita intake for the one flavouring agent in structural class III is below the threshold of concern (i.e. 90 µg). Accordingly, the evaluation of all 12 agents in the group proceeded to step B4.

Step B4. For erythro- and threo-3-mercapto-2-methylbutanol (No. 1289), the NOEL of 0.7 mg/kg body weight per day for the structurally related substance 2-mercapto-3-butanol (No. 546) from a 92-day study in rats fed by gavage provides an adequate margin of safety (>10 000) in relation to known levels of intake of this agent. This NOEL is also appropriate for the structurally related agents (±)-2-mercapto-2-methylpentan-1-ol (No. 1290), 3-mercapto-2-methylpentan-1-ol (racemic) (No. 1291), 3-mercapto-2-methylpentanal (No. 1292), and (±)-ethyl 3-mercaptobutyrate (No. 1294), because they are all acyclic thiols with oxidized side-chains that are anticipated to undergo oxidation or hydrolysis and subsequent metabolism via similar metabolic pathways.

For 4-mercapto-4-methyl-2-pentanone (No. 1293), the NOEL of 1.9 mg/kg of body weight per day for the structurally related substance 3-mercapto-2-pentanone (No. 560) administered to rats by gavage in a 92-day study provides an adequate margin of safety (>10 000) in relation to known levels of intake of this agent.

For ethyl 4-(acetylthio)butyrate (1295), the NOEL of 6.5 mg/kg of body weight per day reported in a 13-week study in rats fed with the structurally related substance ethylthioacetate (No. 483) provides an adequate margin of safety (>10 000) in relation to known levels of intake of this agent.

For ethyl 2-(methylthio)ethanol (No. 1297), the NOEL of 1.4 mg/kg of body weight per day reported in a 13-week study in rats fed by gavage with the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) provides an adequate margin of safety (>10 000) in relation to known levels of intake of this agent. This NOEL is also appropriate for the structurally related agent ethyl 5-(methylthio)valerate (No. 1298) which is also an acyclic sulfide with an oxidized side-chain that is anticipated to undergo oxidation and subsequent metabolism via similar pathways.

For 2,3,5-trithiahexane (No. 1299), the NOEL of 0.3 mg/kg of body weight per day reported in a 13-week study in rats fed with the structurally related substance 3-methyl-1,2,4-trithiane (No. 574) provides an adequate margin of safety (>10 000) in relation to known levels of intake of this agent.

For diisopropyl trisulfide (No. 1300), the NOEL of 4.8 mg/kg of body weight per day reported in a 13-week study in rats fed by gavage with the structurally related substance dipropyltrisulfide (No. 585) provides an adequate margin of safety (>100 000) in relation to known levels of intake of this agent.

For spiro(2,4-dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane) (No. 1296), the NOEL of 25 mg/kg of body weight per day in the diet reported in a 13-week study in rats provides an adequate margin of safety (>100 000) in relation to known levels of intake of this agent.

Table 9 summarizes the evaluations of the 12 simple aliphatic and aromatic sulfides and thiols in this group

Consideration of combined intakes from use as flavouring agents

In the unlikely event that the 11 agents considered in this evaluation and the 97 agents considered previously in structural class I were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the daily per capita human intake threshold for class I (1800 μg). In the unlikely event that the one agent considered in this evaluation and the six agents considered previously in structural class III were to be consumed concurrently on a daily basis, the estimated combined daily per capita intake would not exceed the human intake threshold for class III (90 μg).

Consideration of secondary components

One member of this group of flavouring agents (No. 1293, 4-mercapto-4-methyl-2-pentanone) has a minimum assay value of <95%. Information on the safety of the secondary component of this compound is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less). The secondary component (4-methyl-3-penten-2-one) was evaluated at the fifty-ninth meeting, and was considered not to present a safety concern at current levels of intake.

Conclusion

The Committee concluded that these 12 flavouring agents, which are additions to the group of simple aliphatic and aromatic sulfides and thiols evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

An addendum to the monograph for this group (Annex 1, reference *143*) summarizing the safety data on these 12 additional members was prepared.

4.2 Revision of certain specifications for purity of flavouring agents

4.2.1 *Specifications for flavouring agents evaluated for the first time at the sixty-first meeting*

At its present meeting, the Committee reviewed 144 substances submitted for evaluation. New specifications were proposed for 139 flavouring agents, six of which (Nos 1203, 1218, 1263, 1273, 1291 and 1296) were designated "tentative", pending receipt of more data.

The flavouring agent (*E,E*)-2,4-hexadienoic acid (No. 1176, also known as sorbic acid) was evaluated previously as a food preservative and a specification was published. For such substances, the Committee has agreed that the material used for flavouring should comply with the existing food additive specifications. It is therefore unnecessary to maintain separate flavourings specifications. In the case of sorbic acid, the Committee noted that the existing specifications need to be updated, along with those for sorbate salts. Until this can be done, the Committee decided to maintain separate, tentative specifications. The Committee noted that specifications for other food additives which are used as flavouring agents in addition to other uses may also need revision at future meetings.

Specifications in the flavouring agents format were prepared for the six remaining substances (Nos 1171, 1172, 1219, 1225, 1253 and 1256), all of which have existing food additive specifications. Since these substances have no other functional uses than as flavouring agents, the Committee decided that revised specifications in the flavouring agents section should supersede those for food additives, and withdrew the food additive specifications. The substances in question are: benzyl butyl ether, citral, citronellol, dibenzyl ether, 3,4-dihydrocoumarin and 6-methylcoumarin.

4.2.2 *Revision of existing specifications for flavouring agents*

The existing specifications for 101 flavouring agents were reviewed. Revised specifications were adopted for all of these, of which 14 (Nos 53, 55, 68, 399, 471, 504, 557, 570, 605, 615, 628, 631.2, 632.2 and 633.2) were designated "tentative", pending receipt of further data.

5. Nutritional source of iron

5.1 Ferrous glycinate (processed with citric acid)

The call for data for the sixty-first meeting referred to this substance as ferrous bisglycinate. The Committee decided that this name did not

accurately describe the substance being evaluated and therefore agreed that this substance should be referred to as ferrous glycinate (processed with citric acid). Ferrous glycinate (processed with citric acid) is an iron (II) chelate with the amino acid glycine, and also contains citric acid. It is manufactured by the reaction of reduced iron with glycine, in the presence of citric acid. At chemical equilibrium, >97% of the ferrous ions are chelated. The resulting product is spray-dried without prior removal of the citric acid. The substance is highly hygroscopic and may contain water in variable amounts.

At its twenty-seventh meeting (Annex 1, reference *62*), the Committee allocated a provisional maximum tolerable daily intake of 0.8 mg/kg of body weight for iron from all sources, except for iron oxides used as food colouring agents, supplemental iron taken during pregnancy or lactation, and supplemental iron for specific clinical requirements. At its present meeting, the Committee was asked to comment on the safety of ferrous glycinate as a source of iron for dietary supplementation and as a fortificant for general use in food products.

Biological data. The Committee noted that ferrous glycinate is absorbed by the mucosal cells of the intestine, and is subsequently dissociated into its iron and glycine components within the intestinal mucosa. The available studies indicate that the absorption of iron from ferrous glycinate is regulated physiologically according to the body's iron status, in a manner similar to other non-haem iron compounds. The bioavailability of iron from ferrous glycinate is comparable to that of iron–EDTA (evaluated by the Committee at its forty-first and fifty-third meetings; Annex 1, references *107* and *143*) and is generally greater than that of ferrous sulphate. As is the case with other non-haem iron compounds, the nature of the food matrix may affect the bioavailability of the iron from ferrous glycinate.

In consideration of the potential for overuse of this product, the Committee noted the results of studies of dietary supplementation and fortification at doses of up to 60 mg iron per day, which confirmed the efficacy of ferrous glycinate in correcting iron status in individuals exhibiting iron deficiency, while showing no gastric side-effects. In iron-sufficient individuals, including children, iron absorption from ferrous glycinate is down-regulated according to iron status, and haemoglobin and serum ferritin concentrations are not significantly increased relative to pre-treatment or normal-range values at doses of up to 23 mg iron per day. The Committee therefore concluded that there was no evidence that the administration of iron in the form of

ferrous glycinate would result in increased body stores of iron after the nutritional requirement for iron had been satisfied.

The Committee reviewed a 90-day study of toxicity in rats fed diets containing ferrous glycinate. Despite the fact that a slight increase in iron deposition in the liver of rats of each sex occurred at high doses, no compound-related toxicological effects at doses of 100, 250 or 500 mg/kg of body weight per day were noted. The NOEL for this study was reported to be 500 mg ferrous glycinate/kg of body weight per day, corresponding to 100 mg iron/kg of body weight per day. This NOEL is 125-fold the provisional maximum tolerable daily intake of 0.8 mg/kg of body weight for iron from all sources.

Evaluation. On the basis of the available data on bioavailability, metabolism, and toxicity, and the studies in humans, the Committee concluded that ferrous glycinate was suitable for use as a source of iron for supplementation and fortification, provided that the total intake of iron did not exceed the provisional maximum tolerable daily intake of 0.8 mg/kg of body weight.

Products which are intended to provide a source of additional iron, including ferrous glycinate, should not be consumed by individuals with any type of iron storage disease, except under medical supervision.

The Committee did not receive information concerning estimated intakes for ferrous glycinate, either from its use in food or any possible use as an iron supplement. Information on levels of fortification in food, provided by the sponsor, suggest that intakes approaching the provisional maximum tolerable daily intake could not be attained without consuming extremely large amounts of foodstuffs fortified at the suggested levels.

A toxicological monograph, a chemical and technical assessment (CTA) and specifications were prepared. In preparing the specifications, the Committee was aware that food-grade ferrous glycinate (processed with citric acid) is commercially available, and is usually formulated with diluents and flow agents to facilitate the manufacture of iron-fortified food products.

6. Disinfectant for drinking-water

6.1 Sodium dichloroisocyanurate

Sodium dichloroisocyanurate (NaDCC) is the sodium salt of a chlorinated hydroxytriazine and is used as a source of free available chlo-

rine (in the form of hypochlorous acid, HOCl) for the disinfection of drinking-water. NaDCC can be manufactured either as the anhydrous salt or as the dihydrate. It has not been evaluated previously by the Committee. At its present meeting, the Committee considered the safety of NaDCC in relation to its possible use as a disinfectant for drinking-water in emergency situations, and for routine use in some water supplies. When NaDCC is added to water, it is rapidly hydrolysed to release free available chlorine, establishing a complex series of equilibria involving six chlorinated and four non-chlorinated isocyanurates. As free available chlorine is consumed by reaction with organic material in the water, chloroisocyanurates will rapidly dissociate and continue to release free chlorine. Conventional chlorination of drinking-water with elemental chlorine gives rise to a number of by-products as a result of the reaction of free available chlorine with natural organic matter. The safety of these by-products has been addressed by WHO, with the development of guidelines for drinking-water quality. The use of NaDCC as a source of free available chlorine is not expected to lead to greater production of such by-products than does the use of elemental chlorine.

A typical concentration of free available chlorine used for the treatment of drinking-water is 1.0 mg/l. As anhydrous NaDCC contains about 63% free available chlorine, 1.6 mg/l NaDCC (or 1.8 mg/l of the dihydrate) is equivalent to 1 mg/l free available chlorine. Drinking-water becomes increasingly unpalatable as concentrations of free chlorine increase above this level. However, to overcome initial chlorine demand, disinfection using NaDCC might require higher initial doses, but not greater than double these quantities (i.e. 3.2 mg/l), according to WHO estimates. The default upper-percentile drinking-water intake rates currently used by WHO are 2 litres per day for adults, 1 litre per day for a 10-kg child, and 0.75 litres per day for a 5-kg bottle-fed infant. WHO also recognizes that higher intake rates may occur in some tropical countries. These intakes include water consumed in the form of juices and other beverages containing tap water (e.g. coffee). Thus, the daily intake of the dissociation products of NaDCC from the consumption of water by adults, children and infants, assuming a maximum application of 3.2 mg NaDCC per litre, would be equivalent to 6.4, 3.2, and 2.4 mg/person per day, expressed as NaDCC, respectively. Given that 1 mole of NaDCC corresponds to 1 mole of cyanuric acid (the ultimate end-product of the application of NaDCC), ingestion of cyanuric acid is estimated to be 0.06 mg/kg of body weight for adults, 0.19 mg/kg of body weight for children, and 0.28 mg/kg of body weight for a bottle-fed infant.

In contact with saliva of about pH 7.0, chlorinated isocyanurates react extremely rapidly such that, at the concentrations required to deliver free available chlorine at the levels typically used in drinking-water, no detectable chlorinated isocyanurate remains. The material that reaches the gastrointestinal tract is, therefore, the unchlorinated cyanuric acid. The relevant toxicological studies cited refer to this compound.

In studies in which ^{14}C-labelled sodium cyanurate was administered in multiple doses of 5 mg/kg of body weight to rats, the sodium cyanurate was extensively absorbed and excreted unchanged in the urine, mainly within about 6 hours. Only 5% of the administered dose was detected in the faeces and the radiolabel was not exhaled as ^{14}C-carbon dioxide. In a similar study in the dog, between 2% and 13% of ^{14}C-labelled sodium cyanurate was excreted unchanged in the faeces and the remainder in the urine, mainly within 12 hours. In two human volunteers given a solution of cyanuric acid of unspecified concentration, greater than 98% of the cyanurate was recovered unchanged in the urine after 24 hours. The elimination half-life was 40–60 minutes in the rat, 1.5–2.0 hours in the dog and about 3 hours in humans.

Both NaDCC and sodium cyanurate have low acute oral toxicity.

In 13-week studies in mice given up to 5375 mg/l of sodium cyanurate (equivalent to 1500 mg/kg of body weight per day) in drinking-water, the only compound-related effect reported was the occurrence of bladder calculi in males receiving the highest dose. In a similar study in Charles River rats, 1 out of 28 males in the group receiving 1792 mg/l (equivalent to 145 mg/kg of body weight per day) and 7 out of 28 males in the group receiving the highest dose (equivalent to 495 mg/kg of body weight per day) showed epithelial hyperplasia of the bladder.

In a 2-year study, Charles River CD-1 rats were given sodium cyanurate in the drinking-water at doses estimated as 26, 77, 154 or 371 mg/kg of body weight, with control groups receiving drinking-water containing an equivalent amount of sodium hippurate, or untreated drinking-water. Survival was slightly lower in the group receiving the highest dose compared to the control group receiving untreated drinking-water, but not the control group receiving sodium hippurate. There was no substance-related increase in tumour incidence. Multiple lesions of the urinary tract (calculi and hyperplasia, bleeding and inflammation of the bladder epithelium, dilated and inflamed ureters and renal tubular nephrosis) and cardiac lesions (acute myocarditis, necrosis and vascular mineralization) were reported in males that died during the first year of the study and that were receiving a dose of 371 mg/kg. No toxicologically significant treatment related effects

were observed at 154 mg/kg of body weight, which was considered to be the NOEL in this study. In a similar 2-year study in which B6C3F_1 mice received doses of sodium cyanurate equivalent to 30, 110, 340 or 1523 mg/kg of body weight per day, survival was similar in all groups and there were no treatment-related changes in the incidence of tumours or other histopathological lesions.

There were no signs of toxicity in adult animals and no effects reported in the offspring of groups of Charles River COB and CD rats given sodium cyanurate at doses of 0, 200, 1000 or 5000 mg/kg of body weight per day by gavage on days 6–15 of gestation. In studies of pregnant rabbits, either Dutch belted or New Zealand White, in which 0, 50, 200 or 500 mg/kg of body weight per day of sodium cyanurate was administered by gavage on days 6–18 of gestation, a small reduction in body-weight gain was observed in the groups receiving the two highest doses on days 12–19 of gestation in New Zealand White rabbits only, but compensatory weight gains were made by the end of the study. An increased incidence of post-implantation loss, which was within the historical control range, was also observed in this strain in the group given a dose of 500 mg/kg. The Committee considered that these effects were not significant and there were no other effects that were considered to be related to treatment.

Three generations of Charles River CD rats were given doses estimated to be 26, 77 or 100 mg/kg of body weight sodium cyanurate in their drinking-water, with control groups receiving untreated drinking-water or sodium hippurate. There were no treatment-related effects on reproductive parameters in the P_0, F_1 and F_2 generations or on offspring of the F_1, F_2 or F_3 generations .

Sodium cyanurate was not genotoxic in four different tests.

The Committee concluded that studies of the toxicity of sodium cyanurate were appropriate for assessing the safety of sodium dichloroisocyanurate, because any residues of intact NaDCC in drinking-water would be rapidly converted to cyanuric acid on contact with saliva. Sodium cyanurate did not induce any genotoxic, carcinogenic or teratogenic effects.

The NOEL for sodium cyanurate derived from the 2-year study in rats was 154 mg/kg of body weight per day, equivalent to 220 mg anhydrous NaDCC/kg of body weight per day. With the application of an uncertainty factor of 100, a tolerable daily intake (TDI) of 0–2.0 mg anhydrous NaDCC/kg of body weight per day was determined by the Committee for intake from drinking-water treated with NaDCC for the purpose of disinfection.

A toxicological monograph and a chemical and technical assessment (CTA) were prepared and new specifications were established to cover both anhydrous NaDCC and the dihydrate.

7. Contaminants

7.1 Cadmium

7.1.1 *Introduction*

Cadmium was evaluated by the Committee at its sixteenth, thirty-third, forty-first and fifty-fifth meetings (Annex 1, references *30*, *83*, *107*, *149*). At its sixteenth meeting, the Committee allocated a provisional tolerable weekly intake (PTWI) of 400–500 μg of cadmium per person. At the three subsequent meetings, the Committee retained this PTWI, but expressed it in terms of the intake of cadmium per kg of body weight (7 μg/kg of body weight). At its fifty-fifth meeting, the Committee decided that the prevalences of renal tubular dysfunction that correspond to various dietary intakes of cadmium could serve as a reasonable basis for risk assessment, and concluded that the risk of excess renal tubular dysfunction in the population would be negligible below a urinary cadmium excretion of 2.5 μg/g of creatinine. The Committee noted, however, that these estimates are based on a model that is dependent on the values assumed for key parameters (e.g. dietary bioavailability, age dependency of the intake:excretion ratio). Although new information indicated that a proportion of the general population might be at an increased risk of tubular dysfunction at the current PTWI of 7 μg/kg of body weight, the Committee at the fifty-fifth meeting maintained the PTWI at this value because of lack of precision in the risk estimates. The Committee made several recommendations regarding the data that would be needed in order to reduce the uncertainty in the prevalence estimates. A considerable number of new studies addressed certain aspects of the issues identified in these recommendations and served as the basis for the Committee's deliberations at the present meeting.

7.1.2 *Observations in animals*

In the experimental animal species tested, the oral bioavailability of cadmium ranged from 0.5–3.0% on average. Experimental studies also identified various factors that can significantly influence the extent of cadmium absorption and retention from the diet, including sex, developmental stage, and nutritional status. Low dietary concentrations of protein and of essential minerals such as zinc, calcium, copper, and iron have been shown to promote cadmium absorption

while, in contrast, high or adequate dietary concentrations reduce cadmium absorption and retention. After absorption, cadmium is distributed mainly to the liver, with subsequent redistribution to the kidney in conjugated forms such as cadmium–metallothionein and cadmium–albumin.

Long-term oral exposure to cadmium resulted in a variety of progressive histopathological changes in the kidney, including proximal tubule epithelial cell damage, interstitial fibrosis, and glomerular basal cell damage with limited tubular cell regeneration. Biochemical indications of renal damage were seen in the form of low molecular weight proteinuria, glucosuria and aminoaciduria. Tubular dysfunction also caused the urinary excretion of cadmium to increase. Decreases in bone calcium concentrations and increased urinary excretion of calcium have also been associated with exposure to cadmium. Cadmium induced malignant transformation of animal and human cells in vitro.

Investigations into the ability of cadmium compounds to induce developmental effects in experimental animals have shown that decreased fetal weight, skeletal malformations and increased fetal mortality are common findings, usually in combination with indices of maternal toxicity. However, developmental neurobehavioural effects, including decreased locomotor and exploratory activity and certain electrophysiological changes, have been seen in the absence of any overt symptoms of maternal toxicity and appear to be a more sensitive indicator of toxicity.

A variety of immune system effects have been observed in experimental animals exposed to cadmium, including increased virus-induced mortality in mice co-exposed to non-lethal doses of cadmium and RNA viruses.

7.1.3 *Observations in humans*

A number of new epidemiological studies published since the fifty-fifth meeting have evaluated the relationship between exposure to cadmium and various health effects, particularly renal dysfunction, mortality, and calcium/bone metabolism.

Cadmium accumulates in the kidney and, because of its long half-life in humans, steady-state concentrations in the renal cortex are reached only after about 40 years.

Recent studies conducted in Japan, Europe, China, and the United States have attempted to refine estimates of the dose–effect/dose–response relationship between environmental exposure to cadmium

and renal dysfunction. In a Swedish study (the OSCAR study) involving >1000 individuals aged 16–80 years, an increase of nearly threefold in the prevalence of tubular proteinuria was observed in the group with urinary cadmium concentrations of 0.5–1 μg/g creatinine, compared to the group with a urinary cadmium concentration of <0.3 μg/g creatinine. Above a urinary cadmium concentration of 5 μg/g creatinine, the prevalence of tubular proteinuria was increased five fold. Two studies of populations with low concentrations of urinary cadmium (mean concentrations of 0.23 μg/g creatinine and 0.26 μg/g creatinine) found associations between markers of early kidney damage and urinary cadmium concentration. However, the findings of these two studies were inconsistent; although urinary β_2-microglobulin and *N*-acetyl-β-D-glucosaminidase (NAG) were measured as indices of tubular dysfunction in both studies, in one study, only β_2-microglobulin was associated with urinary cadmium concentration while in the other study, only NAG was associated with urinary cadmium concentration. In an environmental study, the prevalence of end-stage renal disease was found to be significantly, although modestly, related to the extent of environmental exposure to cadmium, as determined by area of residence. However, individual biomarkers of exposure were not measured in this study. In aggregate, the new data are consistent with the hypothesis that low-level environmental exposure to cadmium is associated with an increased prevalence of renal proximal tubular dysfunction, as assessed by biomarkers.

The epidemiological studies conducted in regions of Japan where levels of environmental cadmium vary identified several issues that complicate the interpretation of studies of renal function and low environmental exposure to cadmium. In some studies, a crude association between urinary cadmium and a biomarker of effect disappeared after adjusting for age. Simple adjustment for creatinine might be misleading if comparisons involve people differing in physique, physical activity, sex, age, and race. The appropriate concentrations of urinary biomarkers to use as cut-off values for identifying tubular proteinuria might also vary depending upon physiological or disease conditions. Finally, the long-term health implications of the changes in renal function observed at low concentrations of urinary cadmium are uncertain.

It is well-established that cadmium-induced low molecular weight proteinuria can progress to an acquired Fanconi syndrome (the continuous loss of calcium and phosphorus into urine) and/or the disturbance of vitamin D metabolism in the damaged kidneys. The latter

may eventually progress to Itai-itai disease, characterized by osteomalacia.

Some recent reports suggest that environmental exposure to cadmium, even at low concentrations, might alter calcium metabolism in bone tissue independently of renal effects, and might increase the risk of osteoporosis and bone demineralization. According to the results of the OSCAR study, the age- and sex-adjusted risk of having a reduced bone mineral density was increased two-fold among individuals with blood-cadmium concentrations of 0.6–1.1 µg/l and three-fold among individuals with blood-cadmium concentrations >1.1 µg/l. This association was corroborated by the results of two earlier studies, one in Belgium and one in Japan, although bone mineral density was correlated with age and body weight, and only weakly with urinary cadmium concentration. Two studies in Japan, one in which environmental exposure to cadmium was moderate and one in which it was high, showed no correlation between exposure to cadmium and bone mineral density or calcium excretion, after adjustment for age, body mass index, and menstrual status. The excretion of calcium was not correlated with exposure to cadmium, but with deterioration of renal tubular function, which was due mainly to ageing.

Bone metabolism is influenced by many factors, including age, estrogen status, physique, physical activity, nutritional status, ethnic group, and environmental factors such as sunlight. None of the studies adjusted for possible confounding by all of these factors. These studies were therefore considered by the Committee to be preliminary.

7.1.4 *Estimated dietary intake*

At its fifty-fifth meeting, the Committee evaluated the dietary intake of cadmium using data from a number of countries. At its present meeting, the Committee updated its review by adding new information from Australia, Croatia, France, Greece, Japan, Lithuania, Nigeria, Slovakia, Spain, and the European Union. The combined data showed that concentrations of cadmium range from about 0.01–0.05 mg/kg in most foods, although higher concentrations were found in nuts and oil seeds, molluscs, and offal (especially liver and kidney). Estimates of the mean national intake of cadmium ranged from 0.7–6.3 µg/kg of body weight per week. Mean dietary intakes derived from Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets (average *per capita* food consumption based on food balance sheets) and average concentrations of cadmium in these regions range from 2.8–4.2 µg/kg of body weight per week. These estimates consti-

tute approximately 40–60% of the current PTWI of 7 μg/kg of body weight. For some individuals, the total intake of cadmium might exceed the PTWI because total food consumption for high consumers is estimated to be about twice the mean. Regarding the major dietary sources of cadmium, the following foods contributed 10% or more to the PTWI in at least one of the GEMS/Food regions: rice, wheat, starchy roots/tubers, and molluscs. Vegetables (excluding leafy vegetables) contribute >5% to the PTWI in two regions.

7.1.5 *Evaluation*

The Committee considered an extensive amount of new information, particularly from a series of Japanese environmental epidemiological studies, that addressed issues identified as research needs at its fifty-fifth meeting. The Committee reaffirmed its conclusion that renal tubular dysfunction is the critical health outcome with regard to the toxicity of cadmium. Although the sensitive biomarkers used by some recent studies conducted in Japan, Europe and the USA indicated that changes in renal function and bone/calcium metabolism are observed at urinary cadmium concentrations of <2.5 μg/g creatinine, the Committee noted that appreciable uncertainty remains regarding the long-term health significance of these changes. In addition, the Committee noted inconsistencies between studies regarding the specific biomarkers of renal function that were most commonly associated with urinary cadmium concentrations. Although recent studies suggested that increased concentrations of cadmium biomarkers are associated with health effects such as diabetes, hypertension, pancreatic cancer, fetal growth, and neurotoxicity, the Committee concluded that these data were not, at this time, sufficiently robust to serve as a basis for the evaluation. The Committee reaffirmed its conclusion that an excess prevalence of renal tubular dysfunction would not be expected to occur if urinary cadmium concentration remains <2.5 μg/g creatinine, even under a range of plausible assumptions about the relationship between the amount of bioavailable cadmium in the diet and the urinary excretion of cadmium. Uncertainty remains about how these assumptions affect the predicted excess prevalence of renal tubular dysfunction at concentrations of urinary cadmium of >2.5 μg/g creatinine. The Committee concluded that the new data which became available since its fifty-fifth meeting do not provide a sufficient basis for revising the PTWI, and therefore maintained the current PTWI of 7 μg/kg body weight. No excess prevalence of renal tubular dysfunction would be predicted to occur at the current PTWI under the most appropriate assumptions about the fractional bioavailability of cadmium and the percentage of the absorbed cad-

mium that is excreted in urine. The Committee noted that two issues being considered by the Joint FAO/WHO Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food were of particular relevance to the present evaluation: the dose–response assessment of biomarkers of effect and their relationship to disease outcome, and the possible specification of longer tolerable intake periods (e.g. PTMI) for contaminants with longer biological half-lives. The Committee recommended that the evaluation of cadmium should be revisited when this project has been completed.

7.2 Methylmercury

7.2.1 *Introduction*

Methylmercury was evaluated by the Committee at its sixteenth, twenty-second, thirty-third and fifty-third meetings (Annex 1, references *30*, *47*, *83*, *144*). At the last meeting, the Committee reaffirmed the previously established provisional tolerable weekly intake (PTWI) of 200 µg methylmercury (3.3 µg/kg of body weight) for the general population, but noted that fetuses and infants might be more sensitive than adults to its toxic effects. The Committee concluded that data from studies undertaken in the Seychelles and the Faroe Islands, which were evaluated at its fifty-third meeting, did not provide consistent evidence concerning neurodevelopmental effects in children of women whose methylmercury intakes had resulted in hair-mercury burdens of 20 mg/kg of body weight and below. Adverse effects on neurodevelopment were reported in the study in the Faroe Islands, but not in that in the Seychelles; however, different methods for assessing neurobehavioural effects had been used in the two cohorts. The Committee recommended that methylmercury be re-evaluated at a subsequent meeting when the results of the analysis of neurodevelopmental effects in the Seychelles cohort after 8 years and other relevant data had become available. The Committee noted that fish make an important nutritional contribution to the diet, especially in certain regions, and recommended that nutritional benefits be weighed against the possibility of adverse effects when limits were being considered for methylmercury concentrations in fish or for fish consumption, nutritional benefits should be weighed against the possibility of adverse effects. Studies published since the fifty-third meeting were considered at the present meeting.

7.2.2 *Observations in animals*

In its previous assessment, the Committee reviewed many experimental results which indicated that the developing nervous

system, particularly in non-human primates, is a sensitive target for methylmercury.

In all experimental animal species evaluated, methylmercury was readily absorbed (up to 95%) after oral exposure. Methylmercury crossed both the blood–brain barrier and the placenta effectively, resulting in higher concentrations of mercury in the brain of the fetus than of the mother. Methylmercury is eliminated mainly via the bile and faeces, neonatal animals having a lower excretory capacity than adults. Experimental evidence indicates a possible protective effect of selenium against some toxic effects of methylmercury, but the results are conflicting.

Ataxia, paralysis, loss of coordination, and hind limb crossing are common neurological signs of exposure to methylmercury in rodents. Changes in behaviour, decreased activity, and deficiencies in learning and memory have also been observed. In rodents, neurotoxic effects attributable to methylmercury usually become evident at doses that also affect other organ systems. The neurotoxic effects observed in non-human primates were consistent with the symptoms of Minamata disease, the syndrome observed in humans poisoned with methylmercury via consumption of contaminated seafood. The nature and severity of symptoms depend on dose and duration of exposure, and developmental stage. Exposure of neuroepithelial cells to methylmercury in vitro resulted in disruption of intracellular calcium homeostasis, induction of reactive oxygen species and oxidative DNA damage, and inhibition of axonal morphogenesis and cell cycle progression.

Treatment of pregnant female rodents with methylmercury induced abortion, increased the frequency of fetal resorption and malformations, and reduced offspring viability. Methylmercury also affected the rodent immune system, reducing mast cell function and, at high oral doses, decreasing spleen and thymus cell viability.

7.2.3 *Observations in humans*

At its fifty-third meeting, the Committee noted that methylmercury is toxic to the nervous system, kidney, liver and reproductive organs. At its present meeting, the Committee confirmed that neurotoxicity is the most sensitive endpoint. In humans, the indices of neurotoxicity include neuronal loss, ataxia, visual disturbances, impaired hearing, paralysis and death. Both the central and peripheral nervous systems show signs of damage.

Information about the neurotoxicity caused by chronic fetal exposure to low doses of methylmercury has come primarily from epidemio-

logical studies of populations in which fish consumption is frequent. The results of the neurodevelopmental assessments of 8-year-old children in the Seychelles study cohort were consistent with those obtained in this cohort previously, and provide no evidence for an inverse association between maternal exposure to methylmercury and neurodevelopmental effects in the children. Many of the neuropsychological test instruments included in the battery were the same as those used in the study in the Faroe Islands and which had been observed to be associated with biomarkers of prenatal exposure to methylmercury in 7-year-old children. Further analyses of the results of assessments of the Seychellois children at the age of 5.5 years have been published; these present alternative statistical approaches, adjustment for additional potential confounding factors, and more detailed evaluation of specific test scores. The results of these analyses do not alter the conclusion that in these populations with frequent fish consumption, no adverse effects have been detected that are attributable to prenatal exposure to methylmercury.

No new data were available from the main Faroe Islands study. Additional analyses of the assessments of the cohort at 7 years of age were carried out to explore the possibility of age- and test-dependent variation on susceptibility to methylmercury. Analyses were also conducted to determine whether the methylmercury-associated neuropsychological deficits observed in this cohort were attributable to episodes of higher exposure to methylmercury during pregnancy (associated with consumption of whale-meat), residual confounding due to concomitant exposure to polychlorinated biphenyls (PCBs), and effects on children's visual function. The results did not support a role for any of these factors in the positive associations observed in this study.

In a second smaller cohort (182 infants) assembled in the Faroe Islands, prenatal exposure to methylmercury was found to be inversely related to newborn neurological status and to postnatal growth at 18 months of age. The association was still present after adjustment for exposure to 28 PCB congeners and 18 organochlorine pesticides or their metabolites.

A few new epidemiological studies of neurodevelopment have been reported, although these were cross-sectional rather than prospective in design, and involved much smaller sample sizes than either the Seychelles or Faroe Islands studies, and, in most cases, exposure to higher concentrations of methylmercury. A cross-sectional study of neurotoxic effects in adults reported significant mercury-associated neurobehavioural deficits in persons whose current hair-mercury

concentration was <15 mg/kg. Because of the cross-sectional design of this study and because an adult's hair-mercury concentration does not accurately reflect concentrations during the critical exposure period for neurodevelopment, the Committee considered that these results could not form the basis of a dose–response assessment.

Additional epidemiological studies have addressed issues such as reproductive toxicity, immunotoxicity, cardiotoxicity, and general medical status. With regard to reproductive toxicity, a methylmercury-associated decrease in the ratio of male : female births in the area of Minamata City during the period of peak pollution was reported, but the ratio subsequently returned to control levels. In a case–control study, higher blood mercury concentrations were found in infertile than fertile couples. With respect to cardiotoxicity, in a cohort study, hair-mercury concentrations of ≥2 mg/kg were associated with a doubling of the risk of suffering an acute myocardial infarction and, over a 4-year follow-up interval, with an increased risk for atherosclerotic disease. The results of two large case–control studies of mercury exposure and coronary heart disease were conflicting, one study reporting significantly higher concentrations of mercury in the toenails of cases than of controls, whereas the other reported similar concentrations in the two groups. In the latter study, half the participants were dentists and had concentrations of toenail-mercury that were twice as high as those of non-dentists, suggesting that much of their exposure had been to metallic mercury rather than to methylmercury. In another study, high fish consumption, the primary route of exposure to methylmercury, was associated with an increased risk of stroke, but no biomarkers of mercury exposure were measured. The Committee determined that the available evidence for the potential cardiotoxicity of methylmercury was not conclusive, but noted that further studies were needed. With regard to general health status, the prevalence rates of liver disease, renal disease, and diabetes mellitus were not significantly increased in persons living near Minamata Bay, although the frequencies of many neurological and neuromuscular symptoms were higher.

7.2.4 *Dose-response assessments*

The Committee concluded that neurotoxic effects resulting from exposure to methylmercury *in utero* were the most sensitive health outcome. A number of dose–response assessments have been conducted using the results of the three major epidemiological studies of fetal neurotoxicity, conducted in the Faroes Islands, the Seychelles, and New Zealand. These assessments were made on the basis of evaluations of children at 7 years of age in the Faroes Islands study,

5.5 years of age in the Seychelles Islands study, and 6 years of age in the New Zealand study. A comprehensive dose–response assessment on the basis of the evaluations of the children in the Seychelles study at 8 years of age has not yet been reported, but the study results were similar to those obtained at 5.5 years of age. Mercury in maternal hair and/or cord blood served as the primary biomarkers of exposure to methylmercury *in utero* in the studies in the Faroe Islands and the Seychelles. After consideration of numerous publications, the Committee confirmed the validity of these biomarkers for both short-term (blood) and longer-term (hair) intake of methylmercury.

The maternal hair-mercury concentration corresponding to a NOEL for neurobehavioural effects was identified for the study in the Seychelles, and a mathematical analysis of the concentration–response relationship was used to determine a benchmark-dose lower-confidence limit (BMDL) for the studies in the Faroes Islands and New Zealand. The Committee noted that the maternal hair-mercury concentration of one child (out of 237) in the study in New Zealand was 86 mg/kg, more than four times the next highest concentration in the study sample and had a heavy impact on the BMDLs. The inclusion of this observation produced BMDLs of 17–24 mg/kg, while omitting it produced BMDLs of 7.4–10 mg/kg. Because of uncertainty about which set of BMDLs was most valid, the Committee decided to base the evaluation only on the results of the studies in the Faroe Islands and the Seychelles (see Table 10). The Committee noted, however, that the inclusion of the results of the study in New Zealand did not materially alter its evaluation.

The Committee used the average from the two studies, 14 mg/kg maternal hair-mercury, as an estimate of the concentration of methylmercury in maternal hair that reflects exposures that would have no appreciable adverse effect on the offspring in these two study populations.

Calculation of steady-state ingestion of methylmercury (μg/kg of body weight per day) from a maternal hair-mercury concentration comprises two steps: conversion of the concentration of methylmercury in maternal hair to that in maternal blood, and conversion of the concentration of mercury in maternal blood into maternal intake.

The mean ratio of the concentrations of methylmercury in hair to those in blood was determined in a number of studies, using samples from various study groups and with a variety of analytical methods, and was usually in the range of 140–370. The Committee used a value of 250 to represent the overall average ratio. The concentration of methylmercury in maternal blood that would be expected to have no

Table 10
Estimated maternal hair-mercury concentrations at no-observed-effect level (NOEL) and bench-mark-dose lower-confidence limit (BMDL) for neurotoxicity associated with exposure to methylmercury *in utero*

Study	*N*	NOEL/BMDL (mg/kg maternal hair)	Reference (*17–22*)
Faroes Islands	917	12.0	Budtz-Jorgensen et al. (1999, 2000, 2001); US National Research Council (2000); Rice et al. (2003)
Seychelles	711	15.3	US ATSDR (1999)
Average for two studies	Not applicable	14.0	Not applicable

appreciable adverse effects on the offspring was calculated to be 0.056 mg/l, determined by dividing a maternal hair-mercury concentration of 14 mg/kg by the hair : blood ratio of 250.

In humans, the steady state concentration of mercury in blood can be related to average daily intake using a one-compartment model that incorporates refinements (*20*) to the original WHO (1990) formula (*24*), as follows:

$$d = \frac{C \times b \times V}{A \times f \times b\,w}$$

where C = mercury concentration in blood (μg/l)
b = elimination rate constant (0.014 per day^{-1})
V = blood volume (9% of body weight for a pregnant female)
A = fraction of the dose absorbed (0.95)
f = the absorbed fraction distributed to the blood (0.05)
bw = body weight (65 kg for a pregnant female)
d = dose (μg/kg of body weight per day)

The Committee used values appropriate to conversion during pregnancy, as the fetal period is considered to be the most vulnerable stage of life. Despite an elimination half-life for methylmercury of approximately 2 months, the maternal body burden at term is determined largely by intakes during the second and third trimesters of pregnancy.

Using this equation, the Committee determined that a steady-state daily ingestion of methylmercury at 1.5 μg/kg of body weight per day would result in a maternal blood-mercury concentration that would

have no appreciable adverse effects on offspring in these two study populations.

7.2.5 *Estimated dietary intake*

At its fifty-third meeting, the Committee re-evaluated the safety of methylmercury-contaminated foods, and fish in particular. The re-evaluation included consideration of information on potential intake submitted by numerous national bodies. For most populations, fish is the only significant source of methylmercury in food. Generally, concentrations of methylmercury are <0.4 mg/kg, but fish at the highest trophic levels may contain concentrations >5 mg/kg. Older and larger predatory fish species and certain marine mammals contain the highest concentrations of methylmercury.

At its current meeting, the Committee updated its evaluations of national intakes, adding intake information submitted by Australia, France, Japan, New Zealand, and Slovakia, and use of biomarkers of exposure for methylmercury. The Committee also evaluated information published between 1997 and 2003 on concentrations of mercury and methylmercury in various fish species, as well as analyses of methylmercury intake by populations consuming large amounts of fish (>100 g per person per day). The Committee noted that overall methylmercury concentrations in fish species were similar to those considered at the fifty-third meeting and therefore concluded that the analyses of exposure conducted at the fifty-third meeting remained current. These estimates range from 0.3–1.5 µg/kg of body weight per week for the five regional GEMS/Food diets and from 0.1–2.0 µg/kg of body weight per week for numerous national diets.

7.2.6 *Evaluation*

The Committee evaluated new information which had become available since methylmercury was considered at the fifty-third meeting. This information included the results of studies performed in laboratory animals and humans, and epidemiological studies of the possible effects of prenatal exposure to methylmercury on child neurodevelopment. Neurodevelopment was considered to be the most sensitive health outcome, and life *in utero* the most sensitive period of exposure.

The calculations made in the dose–response assessment are based on average values for each parameter, and did not allow for interindividual variability in either the hair : blood ratio or in the elimination rate constant in the equation shown above. Potential human variability was taken into account by the application of adjust-

ment or uncertainty factors. In choosing the factors to apply to this intake estimate, the Committee considered the following:

- Neurodevelopment is a sensitive health outcome, and life *in utero* is the critical period for the occurrence of neurodevelopmental toxicity as a result of exposure to methylmercury. As the two study samples represent diverse populations, no uncertainty factor is needed to account for variation in vulnerability among subgroups.
- The available data on the hair:blood ratio show both interstudy and intersubject variability. No population-specific hair:blood ratios are available for the populations of the Faroe Islands or the Seychelles. Most of the published means are within a range of 140–370. Few data were available to the Committee on the range of individual hair:blood ratios, although the ratios reported for humans in a limited number of studies were in the range of 137–585, including any analytical errors. The ratio of the overall average (250) to the highest mean found (370) was 1.5 (370/250), while the ratio to the highest individual value (585) was 2.3 (585/250). The Committee concluded that the available data on the distribution of individual ratios were not adequate to allow derivation of a chemical-specific adjustment factor, and decided to apply a factor of 2 to the overall average of 250 to allow for the likely interindividual variation, which is indicated by the differences in study means and by the limited individual data.
- Interindividual variation in pharmacokinetics should be taken into account when converting the steady-state concentration of mercury in maternal blood to an estimated daily intake. As limited data were available which were specific to the study populations used in this assessment, the Committee recommended the use of a combined uncertainty factor of $3.2(10^{0.5})$ (*23*) to account for the total human interindividual variation for dose reconstruction (converting maternal blood concentration to a steady-state dietary intake).

A steady-state intake of methylmercury of 1.5 µg/kg of body weight per day was estimated to represent the exposure that would be expected to have no appreciable adverse effects on children. A total uncertainty factor of 6.4 (2×3.2) was applied to this figure to derive a PTWI of 1.6 µg/kg of body weight. This PTWI is considered sufficient to protect developing fetuses, the most sensitive subgroup of the population.

Pending reduction in the uncertainty associated with various aspects of the derivation of the steady-state intake from maternal hair-mercury concentrations, the Committee concluded that the

uncertainty factor could be refined and possibly reduced. The Committee also reaffirmed its position that fish are an important part of a balanced, nutritious diet and that this should be appropriately considered in public health decisions to set limits for methylmercury concentrations in fish. The Committee considered whether a provisional tolerable monthly intake PTMI rather than a PTWI for methylmercury should be established, but deferred its decision pending the outcome of the Joint FAO/WHO Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food.

At its sixteenth meeting (Annex 1, reference *30*), the Committee established a PTWI for total mercury of 300 µg/person, of which no more than 200 µg should be present as methylmercury. This PTWI of 3.3 µg/kg of body weight for methylmercury was confirmed at subsequent meetings. Since the PTWI for methylmercury was revised at the current meeting, the Committee recommended that the PTWI for total mercury be revised.

8. Future work

- The Committee recommended that the General Method for the determination of residual solvents included in the *Guide to Specifications* (Annex 1, reference *100*) be updated to reflect more modern technology, for example, head space chromatography and gas chromatography with capillary columns.
- The Committee recommended a revision of the guidelines for the preparation of monographs for flavouring agents, to accommodate compounds with high intake evaluated by the "B-side" of the Procedure for the Safety Evaluation of Flavouring Agents.
- The Committee recommended that the evaluation of cadmium should be revisited after completion of the Project to Update the Principles and Methods for the Risk Assessment of Chemicals in Food.
- The Committee recommended that the PTWI for total mercury should be reviewed in light of the new PTWI established for methylmercury.

9. Recommendations

1. The Committee recognized that quillaia extracts differ from each other in their chemical composition and recommended that the Codex Committee on Food Additives and Contaminants consider

adopting individual International Numbering System (INS) numbers for each type of extract.

2. The Committee noted that several substances assigned high priority by the Codex Committee on Food Additives and Contaminants for review by the Expert Committee have a carcinogenic potential. The Committee recommended that these substances should not be evaluated by JECFA before the International Programme on Chemical Safety (IPCS) Harmonization Project has reached a conclusion on how to assess the dose–response relationship for such substances.

3. At its fifty-ninth meeting, the Committee recognized the need for a working definition of the term "flavouring agent". At the present meeting, the Committee noted that a range of regulatory definitions exist and that such a definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission.

4. 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 the meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.

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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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12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
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 Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
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 Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
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Annex 2

Acceptable daily intakes, other toxicological information and information on specifications

Food additives evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI in mg/kg of body weight) and other toxicological recommendations
α-Amylase from *Bacillus licheniformis* containing a genetically engineered α-amylase gene from *B. licheniformis*	N	ADI "not specified"[d]
Annatto extract (solvent-extracted bixin) — "Annatto B"[b]	R, T	0–7 (temporary); for preparations containing not less than 85% pigment (as bixin, of which not more than 2.5% is is norbixin)[c]
Annatto extract (solvent-extracted norbixin) — "Annatto C"	R, T	0–0.4 (temporary); for preparations containing not less than 85% pigment (as norbixin)[c]
Annatto extract (oil-processed bixin suspension) — "Annatto D"	R, T	No ADI established, since no data on toxicity were available
Annatto extract (aqueous-processed bixin) — "Annatto E"	R, T	0–4 (temporary); for a preparations containing not less than 25% pigment (as bixin, of which not more than 7% is norbixin)[c]
Annatto extract (alkali-processed norbixin) — "Annatto F"	R, T	0–0.4 (temporary); for a preparation containing not less than 35% pigment (as norbixin)[c]
Annatto extract (alkali-processed norbixin, not acid-precipitated) — "Annatto G"	R, T	No ADI established, since no data on toxicity were available
Curcumin	R	0–3
Diacetyltartaric and fatty acid esters of glycerol	—	0–50
D-Tagatose	R	0–125 (temporary)
Laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*	N	ADI "not specified"[d]
Mixed xylanase, β-glucanase enzyme preparation, produced by a strain of *Humicola insolens*	N	ADI "not specified"[d]

Food additives evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI in mg/kg of body weight) and other toxicological recommendations
Neotame	N	0–2
Polyvinyl alcohol	N	0–50
Quillaia extract (type 1)[e]	R	0–5
Quillaia extract (type 2)[e]	N	No ADI established due to limited information on the qualitative and quantitative composition
Xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*	N	ADI "not specified"[d]

[a] N: new specifications prepared; R: existing specifications revised; T: tentative specifications.
[b] To ensure clarity of the text, the Committee adopted for this report the designations B, C, D, E, F, G, as employed in the submitted information, to refer to the different extracts under evaluation.
[c] The ADI is established for the extract as tested biologically and specified. It is not expressed in relation to content of bixin and/or norbixin.
[d] 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 effects 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 the reasons stated in the 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.
[e] Quillaia extract (type 1): saponin content of 20–26%; quillaia extract (type 2): saponin content of 75–90%.

Food additives considered for specifications only

Food additive	Specifications[a]
β-Carotene from *Blakeslea trispora*	R
Magnesium silicate	R
Monomagnesium phosphate	S, T
Natamycin	R
Sucrose esters of fatty acids	R
Talc	R
Trisodium diphosphate	R, T

[a] R: existing specifications revised; S: specifications maintained (revision considered but not required); T: the existing specifications are tentative, information required.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

A. Alicyclic, alicyclic-fused and aromatic-fused ring lactones

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
4-Hydroxy-4-methyl-5-hexenoic acid γ-lactone	1157	N	No safety concern
(+/–) 3-Methyl-g-decalactone	1158	N	No safety concern
4-Hydroxy-4-methyl-7-*cis*-decenoic acid γ-lactone	1159	N	No safety concern
Tuberose lactone	1160	N	No safety concern
Dihydromintlactone	1161	N	No safety concern
Mintlactone	1162	N	No safety concern
Dehydromenthofurolactone	1163	N	No safety concern
(+/–)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid γ-lactone	1164	N	No safety concern
Sclareolide	1165	N	No safety concern
Octahydrocoumarin	1166	N	No safety concern
2-(4-Methyl-2-hydroxyphenyl)propionic acid γ-lactone	1167	N	No safety concern
3-Propylidenephthalide	1168	N	No safety concern
3-*n*-Butylphthalide	1169	N	No safety concern
3-Butylidenephthalide	1170	N	No safety concern
Dihydrocoumarin	1171	R	No safety concern
6-Methylcoumarin	1172	N	No safety concern

[a] N: new specifications prepared; R: revised specifications.

B. Aliphatic di- and trienals and related alcohols, acids, and esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
2,4-Pentadienal	1173	N	No safety concern
(*E*,*E*)-2,4-Hexadien-1-ol	1174	N	No safety concern
trans,*trans*-2,4-Hexadienal	1175	N	No safety concern
(*E*,*E*)-2,4-Hexadienoic acid	1176	N	See footnote[b]
Methyl sorbate	1177	N	No safety concern
Ethyl sorbate	1178	N	No safety concern
2,4-Heptadienal	1179	N	No safety concern
(*E*,*E*)-2,4-Octadien-1-ol	1180	N	No safety concern
trans,*trans*-2,4-Octadienal	1181	N	No safety concern
2-*trans*,6-*trans*-Octadienal	1182	N	No safety concern
2,4-Nonadien-1-ol	1183	N	No safety concern
2,6-Nonadien-1-ol	1184	N	No safety concern
2,4-Nonadienal	1185	N	No safety concern
Nona-2-*trans*-6-*cis*-dienal	1186	N	No safety concern
2-*trans*-6-*trans*-Nonadienal	1187	N	No safety concern

B. Aliphatic di- and trienals and related alcohols, acids, and esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
(*E*,*Z*)-2,6-Nonadien-1-ol acetate	1188	N	No safety concern
(*E*,*E*)-2,4-Decadien-1-ol	1189	N	No safety concern
2-*trans*,4-*trans*-Decadienal	1190	N	No safety concern
Methyl (*E*)-2-(*Z*)-4-decadienoate	1191	N	No safety concern
Ethyl *trans*-2-*cis*-4-decadienoate	1192	N	No safety concern
Ethyl 2,4,7-decatrienoate	1193	N	No safety concern
Propyl 2,4-decadienoate	1194	N	No safety concern
2,4-Undecadienal	1195	N	No safety concern
trans,*trans*-2,4-Dodecadienal	1196	N	No safety concern
2-*trans*-6-*cis*-Dodecadienal	1197	N	No safety concern
2-*trans*-4-*cis*-7-*cis*-Tridecatrienal	1198	N	No safety concern

[a] N, new specifications prepared.
[b] An ADI of 0–25 mg/kg of body weight was established at the seventeenth meeting. The ADI was maintained and the use of the chemical as a flavouring agent subsumed in the ADI. R: existing specifications revised.

C. Aliphatic branched-chain unsaturated alcohols, aldehydes, acids, and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
(+/–) 2-Methyl-1-butanol	1199	N	No safety concern
3-Methyl-2-buten-1-ol	1200	N	No safety concern
2-Methyl-2-butenal	1201	N	No safety concern
3-Methyl-2-butenal	1202	N	No safety concern
Ammonium isovalerate	1203	N	No safety concern
3-Methylcrotonic acid	1204	N	No safety concern
trans-2-Methyl-2-butenoic acid	1205	N, T	No safety concern
Isobutyl 2-butenoate	1206	N	No safety concern
2-Methylallyl butyrate	1207	N	No safety concern
4-Methyl-2-pentenal	1208	N	No safety concern
2-Methyl-2-pentenal	1209	N	No safety concern
2-Methyl-2-pentenoic acid	1210	N	No safety concern
2,4-Dimethyl-2-pentenoic acid	1211	N	No safety concern
2-Methylheptanoic acid	1212	N	No safety concern
Isobutyl angelate	1213	N	No safety concern
2-Butyl-2-butenal	1214	N	No safety concern
2-Isopropyl-5-methyl-2-hexenal	1215	N	No safety concern
2-Ethyl-2-heptenal	1216	N	No safety concern
2-Methyl-2-octenal	1217	N	No safety concern
4-Ethyloctanoic acid	1218	N, T	No safety concern
dl-Citronellol	1219	R	See footnote[b]
Citronellal	1220	N	No safety concern
3,7-Dimethyl-6-octenoic acid	1221	N	No safety concern
Rhodinol	1222	N	No safety concern

C. Aliphatic branched-chain unsaturated alcohols, aldehydes, acids, and related esters

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Geraniol	1223	N	No safety concern
Nerol	1224	N	No safety concern
Citral	1225	R	See footnote[b]
8-Ocimenyl acetate	1226	N	No safety concern
2,6-Dimethyl-10-methylene-2,6,11-dodecatrienal	1227	N	No safety concern
3,7,11-Trimethyl-2,6,10-dodecatrienal	1228	N	No safety concern
12-Methyltridecanal	1229	N	No safety concern
Farnesol	1230	N	No safety concern

[a] N: new specifications prepared; R: existing specifications revised; T: the existing, new or revised specifications are tentative and new information is required.

[b] A group ADI of 0–0.5 mg/kg of body weight expressed as citral, was established for citral, citronellol, geranyl acetate, linalool and linalyl acetate at the twenty-third meeting. The ADI was maintained and the use of the chemical as a flavouring agent subsumed in the ADI.

D. Aliphatic and aromatic ethers

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
sec-Butyl ethyl ether	1231	N	No safety concern
1-Ethoxy-3-methyl-2-butene	1232	N	No safety concern
1,4-Cineole	1233	N	No safety concern
Eucalyptol	1234	N	No safety concern
Nerol oxide	1235	N	No safety concern
2,2,6-Trimethyl-6-vinyltetrahydropyran	1236	N	No safety concern
Tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran	1237	N	No safety concern
Theaspirane	1238	N	No safety concern
Cycloionone	1239	N	No safety concern
1,5,5,9-Tetramethyl-13-oxatricyclo (8.3.0.0(4,9))tridecane	1240	N	No safety concern
Anisole	1241	N	No safety concern
o-Methylanisole	1242	N	No safety concern
p-Methylanisole	1243	N	No safety concern
p-Propylanisole	1244	N	No safety concern
2,4-Dimethylanisole	1245	N	No safety concern
1-Methyl-3-methoxy-4-isopropylbenzene	1246	N	No safety concern
Carvacryl ethyl ether	1247	N	No safety concern
1,2-Dimethoxybenzene	1248	N	No safety concern
m-Dimethoxybenzene	1249	N	No safety concern
p-Dimethoxybenzene	1250	N	No safety concern
3,4-Dimethoxy-1-vinylbenzene	1251	N	No safety concern
Benzyl ethyl ether	1252	N	No safety concern
Benzyl butyl ether	1253	R	No safety concern

D. Aliphatic and aromatic ethers

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Methyl phenethyl ether	1254	N	No safety concern
Diphenyl ether	1255	N	No safety concern
Dibenzyl ether	1256	R	No safety concern
β-Naphthyl methyl ether	1257	N	No safety concern
β-Naphthyl ethyl ether	1258	N	No safety concern
β-Naphthyl isobutyl ether	1259	N	No safety concern

[a] N, new specifications prepared; R, existing specifications revised

E. Hydroxypropenylbenzenes

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Isoeugenol	1260	N	No safety concern
Isoeugenyl formate	1261	N	No safety concern
Isoeugenyl acetate	1262	N	No safety concern
Isoeugenyl phenylacetate	1263	N, T	No safety concern
Propenylguaethol	1264	N	No safety concern
4-Propenyl-2,6-dimethoxyphenol	1265	N	No safety concern
Isoeugenyl methyl ether	1266	N	No safety concern
Isoeugenyl ethyl ether	1267	N	No safety concern
Isoeugenyl benzyl ether	1268	N	No safety concern

[a] N, new specifications prepared; T, T, the existing, new or revised specifications are tentative and new information is required.

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

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Isoprenyl acetate	1269	N	No safety concern
4-Pentenyl acetate	1270	N	No safety concern
3-Hexenal	1271	N	No safety concern
3-Hexenyl formate	1272	N	No safety concern
Ethyl 5-hexenoate	1273	N,T	No safety concern
cis-3-Hexenyl propionate	1274	N	No safety concern
cis-3-Hexenyl isobutyrate	1275	N	No safety concern
(*Z*)-3-Hexenyl (*E*)-2-butenoate	1276	N	No safety concern
cis-3-Hexenyl tiglate	1277	N	No safety concern
cis-3-Hexenyl valerate	1278	N	No safety concern
3-Hexenyl 2-hexenoate	1279	N	No safety concern
(*Z*)-4-Hepten-1-ol	1280	N	No safety concern
Ethyl *cis*-4-heptenoate	1281	N	No safety concern
(*Z*)-5-Octenyl propionate	1282	N	No safety concern

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

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
(*Z*,*Z*)-3,6-Nonadien-1-ol	1283	N	No safety concern
(*E*)-3,(*Z*)-6-Nonadien-1-ol	1284	N	No safety concern
(*E*,*Z*)-3,6-Nonadien-1-ol acetate	1285	N	No safety concern
9-Decenal	1286	N	No safety concern
4-Decenoic acid	1287	N	No safety concern
cis-4-Decenyl acetate	1288	N	No safety concern

[a] N, new specifications prepared; T, the existing, new or revised specifications are tentative and new information is required.

G. Simple aliphatic and aromatic sulfides and thiols

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
erythro and *threo*-3-Mercapto-2-methylbutan-1-ol	1289	N	No safety concern
(+/–)2-Mercapto-2-methylpentan-1-ol	1290	N	No safety concern
3-Mercapto-2-methylpentan-1-ol (racemic)	1291	N, T	No safety concern
3-Mercapto-2-methylpentanal	1292	N	No safety concern
4-Mercapto-4-methyl-2-pentanone	1293	N	No safety concern
(+/–) Ethyl 3-mercaptobutyrate	1294	N	No safety concern
Ethyl 4-(acetylthio)butyrate	1295	N	No safety concern
spiro(2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane)	1296	N, T	No safety concern
2-(Methylthio)ethanol	1297	N	No safety concern
Ethyl 5-(methylthio)valerate	1298	N	No safety concern
2,3,5-Trithiahexane	1299	N	No safety concern
Diisopropyl trisulfide	1300	N	No safety concern

[a] N, new specifications prepared; T, the existing, new or revised specifications are tentative and new information is required.

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
42	Isoamyl formate	R
53	Citronellyl formate	R,T
54	Geranyl formate	R
55	Neryl formate	R,T
56	Rhodinyl formate	R
57	Citronellyl acetate	R
60	Rhodinyl acetate	R

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
61	Citronellyl propionate	R
62	Geranyl propionate	R
65	Citronellyl butyrate	R
66	Geranyl butyrate	R
68	Rhodinyl butyrate	R,T
71	Citronellyl isobutyrate	R
73	Neryl isobutyrate	R
95	Heptanal	R
98	Octanal	R
101	Nonanal	R
104	Decanal	R
107	Undecanal	R
110	Lauric aldehyde	R
112	Myristaldehyde	R
117	Propyl formate	R
119	n-Amyl formate	R
124	Isobutyl formate	R
170	n-Amyl heptanoate	R
180	Methyl laurate	R
205	Methyl 2-methylbutyrate	R
212	2-Methylbutyl 2-methylbutyrate	R
237	6-Hydroxy-3,7-dimethyloctanoic acid lactone	R
244	3-Heptyldihydro-5-methyl-2(3*H*)-furanone	R
272	3,7-Dimethyl-1-octanol	R
302	2,6-Dimethyl-4-heptanone	R
303	2,6-Dimethyl-4-heptanol	R
322	*cis*-5-Octen-1-ol	R
323	*cis*-5-Octenal	R
325	*cis*-6-Nonenal	R
329	9-Undecenal	R
332	Linoleic and linolenic acid (mixture)	R
337	Methyl *cis*-4-octenoate	R
338	Ethyl *cis*-4-octenoate	R
346	Methyl linoleate & Methyl linolenate (mixture)	R
348	2,6-Dimethyl-6-hepten-1-ol	R
349	2,6-Dimethyl-5-heptenal	R
358	Linalyl formate	R
360	Linalyl propionate	R
384	β-Damascone	R
385	α-Damascone	R
396	Dehydrodihydroionone	R
397	Dehydrodihydroionol	R
399	Methyl-β-ionone	R,T
409	3-Hydroxy-2-pentanone	R
410	2,3-Pentadione	R
417	2,3-Undecadione	R
419	Ethylcyclo-pentenolone	R
422	3-Ethyl-2-hydroxy-4-methylcyclopent-2-en-1-one	R

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
423	5-Ethyl-2-hydroxy-3-methylcyclopent-2-en-1-one	R
435	Piperitone	R
443	l-Menthol ethylene glycol carbonate	R
465	2-Methylthioacetaldehyde	R
468	4-(Methylthio)butanal	R
631.2	3-Methyl-2-oxobutanoic acid, sodium salt	R,T
632.2	3-Methyl-2-oxopentanoic acid, sodium salt	R,T
633.2	4-Methyl-2-oxopentanoic acid, sodium salt	R,T
668	Linalyl cinnamate	R
669	Terpinyl cinnamate	R
704	*p*-Tolyl laurate	R
470	2-(Methylthio)methyl-2-butenal	R
471	2,8-Dithianon-4-ene-4-carboxaldehyde	R,T
473	Methylthiomethyl butyrate	R
479	Methylthiomethyl hexanoate	R
480	Ethyl 3-(methylthio)butyrate	R
488	S-Methyl 4-methylpentanethioate	R
489	S-Methyl hexanethioate	R
495	1-Methylthio-2-propanone	R
502	Di(butan-3-one-1-yl) sulfide	R
504	S-Methyl benzothioate	R,T
519	2-Ethylhexanethiol	R
548	4-Methoxy-2-methyl-2-butanethiol	R
556	3-Mercaptohexyl hexanoate	R
557	1-Mercapto-2-propanone	R,T
559	2-Keto-4-butanethiol	R
568	Allyl methyl disulfide	R
569	Methyl 1-propenyl disulfide	R
570	Propenyl propyl disulfide	R,T
571	Methyl 3-methyl-1-butenyl disulfide	R
583	Methyl ethyl trisulfide	R
586	Allyl methyl trisulfide	R
590	Methyl 2-hydroxy-4-methylpentanoate	R
592	Citronelloxyacetaldehyde	R
603	Ethyl 2,4-dioxohexanoate	R
604	3-(Hydroxymethyl)-2-heptanone	R
605	1,3-Nonanediol acetate (mixed esters)	R,T
615	Butyl ethyl malonate	R,T
625	Dibutyl sebacate	R
628	Ethyl aconitate (mixed esters)	R,T
735	2-Phenylphenol	R
737	2,3,6-Trimethylphenol	R
918	Glyceryl monostearate	R
923	Glycerol 5-hydroxydecanoate	R
924	Glycerol 5-hydroxydodecanoate	R
937	Pyruvaldehyde	R

[a] R, existing specifications revised; S, existing specifications were maintained; T, the existing, new, or revised specifications are tentative and new information is required.

Evaluation of a water-treatment agent

Agent	Specifications[a]	Tolerable daily intake (TDI) and other toxicological recommendations
Sodium dichloroisocyanurate (NaDCC)	N	0–2.0 mg/kg of body weight for anhydrous NaDCC; applicable for intake from drinking-water treated with NaDCC for the purpose of disinfection

[a] N, new specifications prepared

Evaluation of a nutritional source of iron

Source	Specifications[a]	Toxicological recommendation
Ferrous glycinate (processed with citric acid)	N	Suitable for use as a source of iron for supplementation and fortification, providing that the total intake of iron does not exceed the provisional maximum tolerable daily intake of 0.8 mg/kg of body weight per day

[a] N, new specifications prepared

Contaminants

Contaminant	Tolerable intake and other toxicological recommendations
Cadmium	Provisional tolerable weekly intake (PTWI) of 7 μg/kg of body weight (maintained)
Methylmercury	Provisional tolerable weekly intake (PTWI) of 1.6 μg/kg of body weight

Annex 3

Further information required or desired

Annatto extracts

The Committee requested additional information to clarify the role that non-pigment components of the extract play in the expression of the qualitative and quantitative differences in toxicity of the various extracts. In addition, the Committee requested data on the reproductive toxicity of an extract, such as Annatto F, that contains norbixin.

Monomagnesium phosphate, trisodium diphosphate

Information on the method for loss on drying for the hydrates is necessary in order to express the assay on the dry basis for the above additives. Requested by end of 2004.

D-Tagatose

The Committee requested information on the histological examination of the adrenals, kidneys and testes of the rats from the 2-year study, by 2006.

Annex 4

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of 95% or less

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
A. Alicyclic, alicyclic-fused and aromatic-fused ring lactones				
1158	(+/–) 3-Methyl-,,-decalactone	94% (sum of *cis* and *trans* isomers)	1–2% heptan-1-ol	The Committee has evaluated heptan-1-ol (No. 94) and concluded it was of no safety concern at current levels of intake.
1160	Tuberose lactone	45%	28–35% ,,-Dodecalactone; 22–30% 2(3*H*)-Furanone, dihydro-5-(2-octenyl)-(*Z*)	The Committee has evaluated ,,-dodecalactone (No. 235) and 2(3*H*)-furanone, dihydro-5-(2-octenyl)-(*Z*) (No. 249) and concluded they were of no safety concern at current levels of intake.
1164	(+/-)-(2,6,6,-Trimethyl-2-hydroxycyclohexylidene)acetic acid ,,-lactone	90%	3.5–4.5% 2,9-Dimethyl 3,8-decanedione; 3.5–4.5% 4-Hydroxy-5,6-oxo ,-ionone	2,9-Dimethyl-3,8-decadione has not been evaluated by the Committee However, the NOEL for another diketone, 3,4-hexandione (No. 413) was >17 mg/kg bw per day in a 90-day study in rats (Posternak et al., 1969). A NOEL of 10 mg/kg bw per day was reported for the structurally related substance, ,-ionone (No. 389), in a 90-day study in rats (Gaunt et al., 1983). Another 90-day study reported NOELs of 11 and 13 mg/kg bw per day for males and females, respectively (Oser et al., 1965).

B. Aliphatic, alicyclic, linear •,,-unsaturated, di- and trienals and related alcohols, acids and esters

1179	(*E*,*E*)-2,4-Heptadienal	92%	2–4% (*E*,*Z*)-2,4-isomer; 2–4% 2,4-heptadienoic acid	Both secondary components are expected to share the same metabolic fate as the primary material. The (*E,Z*) isomer is expected to be converted to the (*E,E*) form by the action of 3-hydroxy acyl CoA epimerase and oxidized to 2,4-heptadienoic acid by aldehyd dehydrogenase (ALDH) (Feldman and Weiner, 1972). 2,4-Heptadienoic acid is a substrate of the fatty acid cycle and is metabolized and excreted primarily as carbon dioxide and water (Nelson and Cox, 2000). A 98-day study for the structurally related material 2,4-hexadienoic showed a NOEL of 15 and 60 mg/kg bw for male and female rats, respectively (NTP, 2001b).
1180	(*E*,*E*)-2,4-Octadien-1-ol	94%	2–4% (*E*,*Z*)-2,4-isomer	The (*E,Z*) isomer is expected to share the same metabolic fate as the (*E,E*) isomer: conversion to the corresponding carboxylic acid by alcohol dehydrogenase (ADH) (Pietruzko et al., 1973) and ALDH (Feldman and Weiner, 1972) and entry in to fatty acid cycle where it is metabolized and excreted primarily as carbon dioxide and water (Nelson and Cox, 2000). A 90 day study for the structurally related material 2,4-hexadienal showed a NOEL of 15 and 60 mg/kg bw per day for male and female rats, respectively (NTP, 2001a).

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
				A-90 day study for the structurally related material (*E*,*E*)-2,4-decadienal established a NOEL of 100 mg/kg bw per day (NTP, 1997).
1183	2,4-Nonadien-1-ol	92%	4–5% 2-nonen-1-ol	2-Nonen-1-ol is scheduled to be evaluated by the Committee in 2004. It is expected to be oxidized to the corresponding acid and metabolized in the fatty acid cycle and excreted primarily as carbon dioxide and water (see Nos. 1179 and 1180 above).
1185	2,4-Nonadienal	89%	5–6% 2,4-nonadien-1-ol; 1–2% 2-nonen-1-ol	2,4-Nonadien-1-ol (No. 1183) has been evaluated by the Committee. It is expected to be oxidized and completely metabolized in the fatty acid cycle (see No. 1179 and 1180 above). 2-Nonen-1-ol, see No. 1183 above.
1189	(*E*,*E*)-2,4-Decadien-1-ol	92%	3–5% (*E*,*Z*) isomer	The *(E*,*Z*) isomer is expected to share the same metabolic fate as the (*E*,*E*) isomer. The alcohol is converted to the corresponding carboxylic acid by ADH and ALDH and then enters the fatty acid cycle where it is metabolized and excreted primarily as carbon dioxide and water (see Nos 1179 and 1180 above).

1190	2-*trans*,4-*trans*-Decadienal	89%	3–4% mixture of *cis cis*, *cis trans*, and *trans cis* 2,4-decadienals; 3–4% acetone plus trace of isopropanol; 0.5% unknown .	The (*Z*,*Z*), (*Z*,*E*) and (*E*,*Z*) isomers are expected to share the same metabolic fate as the (*E*,*E*) isomer. The aldehyde is converted to the corresponding carboxylic acid by ALDH and then enters the fatty acid cycle where it is metabolized and excreted primarily as carbon dioxide and water (See Nos 1179 and 1180 above). The NOEL for (*E*,*E*)-2,4-decadienal was 100 mg/kg bw per day (NTP, 1997) and 33.9 mg/kg bw per day (Damske et al., 1980) in separate 90-day studies. Acetone (No. 139) and isopropanol (No. 277) have been evaluated by the Committee. Both substances were concluded to be of no safety concern at current intake levels.
1191	Methyl (*E*)-2-(*Z*)-4-decadienoate	93%	5–7% (*E*,*E*) isomer	Readily hydrolysed to methanol and (*E*,*E*)-2,4-decadienoic acid which is a substrate for the fatty acid cycle (See No. 1180 above). Separate 90-day studies on the related substance (*E*,*E*)-2,4-decadienal established NOELs of 100 mg/kg bw per day (NTP, 1997) and 33.9 mg/kg bw per day (Damske et al., 1980). Methanol is oxidized in vivo to formic acid. The Committee has evaluated formic acid (No. 79) and concluded that it was of no safety concern at current intake levels. A NOEL of

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
				>400 mg/kg bw per day for formic acid was established in a 2-year rat study (Malorny, 1969).
1192	Ethyl *trans*-2-*cis*-4-decadienoate	90%	5–10% ethyl *trans*-2, *trans*-4-decadienoate	Readily hydrolysed to ethanol and (*E*,*E*)-2,4-decadienoic acid which is a substrate for the fatty acid cycle (see No. 1180 above). Separate 90-day studies on the related substance (*E*,*E*)-2,4-decadienal established NOELs of 100 mg/kg bw per day (NTP, 1997) and 33.9 mg/kg bw per day (Damske et al., 1980). Ethanol is oxidized in vivo to acetic acid. The Committee has evaluated acetic acid (No. 81) and concluded that it was of no safety concern at present intake levels. In a 63-day study in rats, the NOEL for acetic acid was 350 mg/kg bw per day (Pardoe, 1952).
1196	*trans*,*trans*-2,4-Dodecadienal	85%	11–12% 2-*trans*-4-*cis* isomer	The (*E*,*Z*) isomer is expected to share the same metabolic fate as the (*E*,*E*) isomer. The alcohol is converted to the corresponding carboxylic acid by ADH and ALDH and then enters the fatty acid cycle where it is metabolized and excreted primarily as carbon dioxide and water (see Nos 1179 and 1180 above).

				NOELS for the related substance (*E,E*)-2,4-decadienal were 100 mg/kg bw per day (NTP, 1997) and 33.9 mg/kg bw per day (Damske et al., 1980) in separate 90-day studies.
1198	2-*trans*-4-*cis*-7-*cis*-Tridecatrienal	71%	14% 4-*cis*-7-*cis*-tridecadienol; 6% 3-*cis*-7-*cis*-tridecadienol; 5% 2-*trans*-7-*cis*-tridecadienal; 3% 2-*trans*-4-*trans*-7-*cis*-tridecatrienal	All secondary materials are expected to be oxidized to the corresponding acids and enter the fatty acid cycle where they will be metabolized and excreted primarily as carbon dioxide and water (See Nos 1179 and 1180 above). NOELS for the related substance (*E,E*)-2,4-decadienal were 100 mg/kg bw per day (NTP, 1997) and 33.9 mg/kg bw per day (Damske et al., 1980) in separate 90-day studies.
C. Aliphatic branched-chain, saturated and unsaturated alcohols, aldehydes, acids, and related esters				
1209	2-Methyl-2-pentenal	92%	1.5–2.5% propionaldehyde; 3.5–4.5% propionic acid	Propionaldehyde (No. 83) and propionic acid (No. 84) have been evaluated by the Committee. It was concluded that both substances were of no safety concern at current intake levels.
1211	2,4-Dimethyl-2-pentenoic acid	92% (sum of isomers)	5–7% 4-methyl-2-methylenevaleric acid	4-Methyl-2-methylenevaleric acid has not been evaluated previously. A 90-day study of oral administration in rats established a NOEL of >2500 mg/kg bw per day for the structurally related material, isovaleric acid (No. 259) (Amoore, 1978).

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
1219	dl-Citronellol	90% (of total alcohols as $C_{10}H_{20}O$)	5–8% di-unsaturated and saturated C_{10} terpene alcohols; 1% citronellyl acetate; 1% citronellal	Geraniol, a terpene alcohol, exhibited NOELs of >1000 and >100 mg/kg bw per day in 16- and 28-week studies in rats, respectively (Hagan et al., 1967). A NOEL of 2000 mg/kg bw per day was reported when rats were fed a mixture of 71% geranyl acetate and 29% citronellyl acetate for two years (NTP, 1987b). This corresponds to an estimated daily dose of 580 mg/kg bw for citronellyl acetate. In a 2-year study, citral, a structurally related material to citronellal, exhibited a NOEL of 100 mg/kg bw per day in male and female rats (NTP, 2001b).
1220	Citronellal	85% of aldehydes as $C_{10}H_{18}O$	12–14% mixture of terpenoid materials: mainly 1,8-cineole, 2-Isopropylidene-5-methylcyclohexanol, linalool, citronellyl acetate and other naturally occurring terpenes	1,8-Cineole (eucalyptol, No. 1234) has been evaluated by the Committee. In a 28-day rat study, 1,8-cineole exhibited NOELs of 300 and 1,200 mg/kg bw per day for males and females, respectively (NTP, 1987a). In an 80-week mouse study a NOEL of 32 mg/kg bw per day was reported (1979). Roe et al., The Committee has evaluated 2-isopropylidene-5-methylcyclohexanol (isopulegol, No. 755) and concluded that it was not of safety concern at current intake levels. In a 14-day oral rat study isopulegol was reported to

				have a NOEL of 250 mg/kg bw per day (Imaizumi et al., 1985). Linalool (No. 356) has been evaluated by the Committee. It was concluded that linalool was not a safety concern at current intake levels. In an 84-day study in rats, linalool exhibited a NOEL of >50 mg/kg bw per day (Oser,1967). A NOEL of 2000 mg/kg bw per day was reported when rats were fed a mixture of 71% geranyl acetate (No. 58) and 29% citronellyl acetate (No. 57) for 2 years (NTP, 1987b). This corresponds to an estimated daily dose of 580 mg/kg bw for citronellyl acetate.
1221	3,7-Dimethyl-6-octenoic acid	90%	5–8% citronellal, citronellyl, neryl, and geranyl acetate esters and other naturally occurring terpenes	Citronellal (No. 1220) has been evaluated by the Committee. In a 2-year study, the structurally related material citral, exhibited a NOEL of 100 mg/kg bw per day in male and female rats (NTP, 2001b). The naturally occurring terpenoid esters are expected to hydrolyse in vitro to the acetic acid and the corresponding terpene alcohols citronellol, nerol, and geraniol. See No. 1220 above for geranyl and citronellyl acetate. Neryl acetate, being the *cis* isomer of geranyl acetate is expected to follow similar metabolic pathways and exhibit similar toxicologic potential.

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
1222	Rhodinol	82% (of total alcohols as $C_{10}H_{20}O$)	15–17% terpenoid esters: mainly citronellyl, neryl, and geranyl acetate esters and other naturally occurring terpenes	See No. 1221 above.
1223	Geraniol	88% (of total alcohols as $C_{10}H_{18}O$)	8–10% terpene esters: mainly citronellyl, neryl, and geranyl acetate esters and other naturally occurring terpenes	See No. 1221 above.
D. Aliphatic and aromatic ethers				
1233	1,4-Cineole	75%	20–25% 1,8-cineole	1,8-Cineole (eucalyptol, No. 1234) has been evaluated by the Committee. In a 28-day rat study 1,8-cineole exhibited NOELs of 300 and 1200 mg/kg bw per day for males and females, respectively (NTP, 1987a). In an 80-week mouse study a NOEL of 32 mg/kg bw per day was reported (Roe et al., 1979).
1253	Benzyl butyl ether	93%	2–5% benzyl alcohol	Benzyl alcohol (No. 25) has been evaluated by the Committee, which concluded that it was not a safety concern at current intake levels. A 13-week and a 2-year study in rats established NOELs of 100 and >200 mg benzyl alcohol/kg bw per day, respectively (NTP, 1989).

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

1271	3-Hexenal	80% (total of *cis* and *trans* isomers)	18–20% *trans*-2-Hexenal	*trans*-2-Hexenal is scheduled to be evaluated by the Committee in 2004. A 13-week study of oral administration in rats established a NOEL of 30 mg/kg bw per day for this material (Gaunt, 1971).
1279	3-Hexenyl 2-hexenoate	86%	6–8% 3-Hexenyl-3-hexenoate; 4–6%, 0–1%, and 0–0.5% of isomers 1, 2, and 3 of hexenyl hexenoate, respectively	*cis*-3-Hexenyl-*cis*-3-hexenoate (No. 336) has been evaluated by the Committee, which concluded that it was not a safety concern at current intake levels. 3-Hexenyl-3-hexenoate and its isomers are expected to hydrolyse in vivo to mono unsaturated hexenol and mono unsaturated hexenoic acid. Regardless of the position of unsaturation, the resulting alcohol is oxidized to the corresponding acid that participates in normal fatty acid metabolism. The Committee has evaluated 3-hexen-1-ol (No. 315) and 3-hexenoic acid (No. 317) and concluded that they are of no safety concern at current intake levels. A NOEL of 120–150 mg/kg bw per day was reported for *cis*-3-hexen-1-ol in a 98-day study of oral administration (Gaunt et al., 1969). A NOEL of >400 mg/kg bw per day was reported for 10-undecenoic acid (No. 331), a material that is structurally related to 3-hexenoic acid, in a 6-month study in rats (Tislow et al., 1950).

No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
1282	(*Z*)-5-Octenyl propionate	93%	2–3% (*E*)-5-Octenyl propionate; 0.5–1% (*Z*)-5-Octenol	Like the *Z* isomer, the *E* isomer is expected to hydrolyse in vivo to 5-octenol and propionic acid. The Committee has evaluated *cis*-5-octen-1-ol (No. 322) and concluded that it was of no safety concern at current intake levels. The Committee has evaluated propionic acid (No. 84) and concluded that it was of no safety concern at current intake levels.
1284	(*E*)-3,(*Z*)-6-Nonadien-1-ol	92%	6% (*E*,*E*) isomer	In a 28-day study in rats, a NOEL* of 2.06 mg/kg bw per day was reported for the structurally related material (*E*,*Z*)-2,6-dodecadienal (No. 1197) (Edwards, 1973). *material was administered as part of a mixture.
G. Simple aliphatic and aromatic sulfides and thiols				
1293	4-Mercapto-4-methyl-2-pentanone	48–50%	48–50% 4-methyl-3-penten-2-one	The Committee has evaluated 4-methyl-3-penten-2-one (No. 1131) and concluded that it posed no safety concern at current intake levels. A 14-day study in rats established a NOEL of >10 mg/kg bw per day for the structurally related substance 5-methyl-5-hexen-2-one (No. 1119) (Gill & van Miller, 1987).

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