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

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

Safety evaluation of certain food additives and contaminants. WHO Food Additive Series, No. 54, in preparation. Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, *Addendum 13*. FAO Food and Nutrition Paper, No. 52, Add. 13, 2004, in preparation.

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 Organisation 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 (JECFA) met in Geneva from 8 to 17 June 2004. The meeting was opened by Dr Margaret Chan, Director of Protection of the Human Environment (PHE), World Health Organization (WHO), on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). She thanked the participants for their invaluable contribution to the work of the Committee.

Dr Chan noted that the work of the Committee plays an important role in the improvement of food safety on a global basis, particularly in developing countries or regions, and that WHO and FAO were committed to strengthening this system. Dr Chan indicated that increased financial resources were to be devoted to the JECFA programme, both by WHO and by FAO.

In this context Dr Chan made reference to a workshop, held in Geneva in early 2004, which had led to a number of recommendations on how to improve the provision of scientific advice by FAO/WHO to Codex and Member States. She noted that FAO and WHO were committed to the implementation of these recommendations and were jointly developing procedural guidelines with a focus on improving transparency, timeliness and consistency. Much of the experience gained through this Expert Committee will facilitate future improvements.

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-two previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the sixtieth meeting (Annex 1, reference *163*).

The tasks before the Committee were:

— to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);

— to undertake toxicological evaluations of certain food additives and flavouring agents (sections 3 and 4, and Annex 2);

— to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4, and Annex 2); and

— to undertake a toxicological evaluation of a natural constituent, glycyrrhizinic acid (section 5).

2.1 Modification of the agenda

Monosodium glutamate, thaumatin and thaumatin B were removed from the agenda because data necessary for their evaluation or re-evaluation as flavouring agents were not available. The evaluation of magnesium sulfate was removed from the agenda because the intended use and use levels were not identified to the Committee; however the available data were sufficient to establish tentative specifications for the compound (see section 3).

The natural flavouring complexes bois de rose oil, lemongrass oil and cardamom seed oil were removed from the agenda because discussions on the procedural framework necessary for their evaluation remained to be completed (see general consideration 2.3). The evaluation of these complexes was deferred to a future meeting. For the other two natural flavouring complexes listed in the call for data, cardamom extract and cardamom oleoresin, no data were available to the Committee.

The group of aliphatic and aromatic hydrocarbons used as flavouring agents was divided into two separate groups, aliphatic and alicyclic hydrocarbons, and aromatic hydrocarbons.

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 WHO Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as the principles elaborated at subsequent meetings of the Committee (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116*, *122*, *131*, *137*, *143*, *149*, *152*, *154*, *160*, *166*, including the present one. WHO 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.

2.3 The safety evaluation of flavouring agents

2.3.1 *Estimating intake of flavouring agents*

At its fifty-fifth meeting (Annex 1, reference *149*), the Committee considered the use of the per capita × 10 method for estimating the

intake of flavouring agents according to the Procedure for the Safety Evaluation of Flavouring Agents, as well as alternative procedures (Annex 1, reference *149*). While the Committee concluded that use of this method was appropriate, it acknowledged that it may, in some cases, result in an underestimate of the intake of persons with high levels of consumption of specific foods. At its forty-ninth meeting (Annex 1, reference *131*), the Committee also recognized that further consideration may be required in certain cases where there is conflicting information on intake. At its present meeting, the Committee reaffirmed these conclusions.

The Committee recognized that the estimates of current intake that it uses in evaluating the safety of flavouring agents according to the Procedure are difficult to reconcile with reported maximum use levels for some flavouring agents in different food groups. To help understand the basis for the apparent discrepancy in the information available to the Committee, the Committee requested that industry provide precise data on the use levels of flavouring agents that may be used in food products that are not widely distributed and that may be eaten on a regular basis by specific population groups in specific regions of the world.

The Committee anticipates that estimating the intake of flavouring agents, especially those with particularly low or particularly high production volumes, will be considered in detail at the forthcoming Joint FAO/WHO workshop on exposure assessment to be held in 2004.

Combined exposure

The Committee also recognized that the current procedure to estimate the combined intake for all congeners of one congeneric group of flavouring substances reflects an unlikely situation in which the same individuals are consumers of all the substances. Nevertheless, this results in conservative estimates that allow evaluations to be completed. The Committee therefore recommended the establishment of a working group to develop a more adequate approach, to be discussed at the next meeting of the Committee.

2.3.2 *Flavour complexes derived from natural sources*

At its present meeting, the Committee further considered a possible approach to the safety assessment of complex flavours derived from natural sources (usually from plant material), such as essential oils, oleoresins and solvent extracts. After considering the available data on three of the five flavour complexes originally included on the agenda — derived from essential oils of lemongrass, cardamom seed and rosewood — the Committee defined the information that would

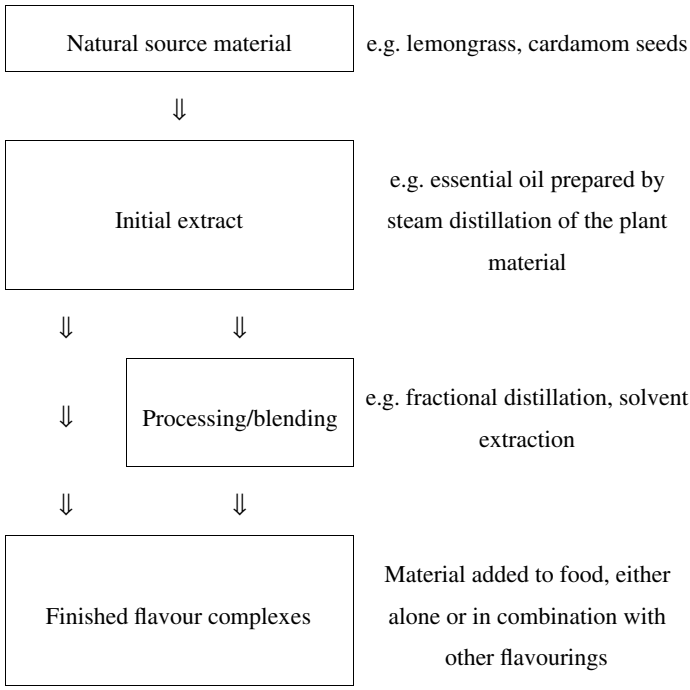
be required in order to test the application of the revised Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*), which it had previously adopted for the safety evaluation of chemically-defined flavourings.

Background

Although these flavourings are typically named after the initial extract prepared from the source material, it is common practice for the initial extracts to be processed and refined in a variety of ways, to produce a range of flavour complexes with the specific properties desired for particular food applications. These processes might include distillation, concentration, solvent extraction and blending of extracts from different batches. Processing is generally carried out by flavour companies or, in certain cases, by food manufacturers who use the finished flavours. The progression from source material to finished flavour is illustrated in Figure 1.

The initial extracts are typically prepared from the plant material close to the point of production. Their composition may vary considerably at this level owing to a variety of factors, such as climate,

Figure 1
Progression from source material to finished flavour



geography, genotype and maturity of the source material. The flavour producer aims to supply flavour complexes with consistent technical and olfactory properties. This is primarily achieved by processing and blending to meet a target composition that is monitored by chemical analysis.

Use of the scheme for evaluation of finished flavour complexes is dependent upon:

— information on the composition of the material that is added to food (and hence on the elaboration of a reliable specification that covers the range of finished flavour complexes that may be derived from the initial extracts);

— existing safety evaluations of the individual components and congeneric groups;

— estimates of intake of the finished flavour complexes, and hence of the individual components.

Although the finished flavour complexes are entirely derived from the original extract, using only physical processes such as those described above, their composition is likely to differ quantitatively from that of the initial extracts prepared directly from the source material.

Compositional data necessary to support the safety evaluation of a finished flavour complex

General considerations

The safety evaluations of finished flavour complexes derived from natural sources would be based on the revised Procedure, with particular consideration of the major components and of congeneric groups. The analytical data should be adequate to apply the revised Procedure.

Intake should be taken into account in determining the extent to which chemical characterization and identification of individual components is necessary, beyond that which is necessary to define the flavour characteristics. In applying the Revised Procedure for the Safety Evaluation of Flavouring Agents, the estimated intake of the individual agent is compared with appropriate thresholds of toxicological concern, to determine whether or not the intake represents a safety concern. The same numerical thresholds can be applied to the intakes of individual identified components and of combinations of components, such as occur in congeneric groups, that are present in finished flavour complexes derived from natural sources. The same intake thresholds can also be used as a basis for establishing analytical requirements, as described below.

The human intake thresholds of toxicological concern are of two types: thresholds of 1800, 540 and 90 µg/person per day, which are applied for structural classes I, II and III, and a general threshold of 1.5 µg/person per day, which is applicable to all structural classes. The thresholds for classes I, II and III are based on the lower 5th percentile no-observed-effect level (NOEL) for the structural class, from toxicological studies in animals, divided by the usual 100-fold safety (uncertainty) factor. The general threshold (step B5 of the Procedure) is a pragmatic value based on an estimate of the human intake associated with a lifetime risk of cancer of less than 1 in a million, calculated by linear-extrapolation from animal studies (as described by the Committee at its forty-sixth meeting; Annex 1, reference *122*). Because of the assumptions used in the derivation of this threshold, it is considered to be sufficiently conservative to cover all types of toxicity. The Committee considered that these thresholds can provide the basis for a pragmatic approach to the development of limits of sensitivity for analytical methods, when linked to reliable and validated estimates of intake, which should be derived from long-term average poundage (disappearance data).

Consideration of individual components

Identified components: On the basis of step B5 of the Procedure, the Committee concluded that there would be no significant safety concern if the intake for an identified component in a finished flavour complex derived from natural sources were <1.5 µg/person per day. This threshold can be used to establish a general limit for analytical characterization for components in a finished flavour complex as described in (b) below, based on the estimated intake of the complex. For example, if the estimated daily intake of the finished flavour complex were 150 µg/person per day, then there would be no safety concern for any component present at <1%. Similarly, if the estimated daily intake of the finished flavour complex were 15 µg/person per day, then there would be no safety concern for any component present at <10%. For high-volume finished flavour complexes, the limit for analytical characterization would be set at 0.1–0.5% (see (b) below). Because the threshold is based on lifetime carcinogenicity data, the percentage should be the average value of the available analyses, and not the highest single value.

Unidentified components: The chromatographic analysis of a finished flavour complex is likely to reveal the presence of a large number of unidentified minor components. Previously the Committee has not considered the general threshold of 1.5 µg/person per day for unidentified components. The Committee recognized that application

of the general threshold to an unidentified component could not provide the same reassurance of safety as for structurally defined compounds, but considered that it could be incorporated into a pragmatic approach to establishing analytical requirements for finished flavour complexes derived from natural sources. This threshold combined with the estimated intake of the complex can be used to define a limit for the percentage of a chromatographic peak above which structural characterization would be necessary. For example, if the estimated daily intake of the finished flavour complex were 150 µg/person per day, then chemical characterization would be required for any component present at >1%, so that safety evaluation of the component could be undertaken.

Product descriptions and specifications: A key part of the safety assessment would be the preparation of appropriate specifications covering the relevant finished flavour complexes. As with all food additive evaluations, the purpose of specifications for flavour complexes is to identify the material, to ensure that it meets the criteria for safe use, and to encourage good manufacturing practice. Specifications should reflect the materials used throughout the world and should take account of existing specifications drawn up at national or international level, as described in WHO Environmental Health Criteria, No. 70 (*2*).

The Committee noted the existence of internationally agreed specifications prepared by the International Organization for Standardization (ISO) for more than 100 essential oils obtained by steam distillation of plant materials. Essential oils and derived products are numerically the largest group of flavour complexes. ISO standards describe the oils and define the acceptable ranges for various parameters, including the methods for measuring these values. Many of these standards include ranges for the key chemical components, accompanied by typical gas chromatograms that can be used to confirm the identity of the oils. The Committee concluded that it is necessary to take these standards into account when setting specifications for food flavourings, particularly when selecting the parameters to be included and the associated analytical methods.

In order to develop specifications for flavour complexes added to food, and to provide the data necessary for the safety evaluation to proceed, the Committee requires a full description of the range of source materials and processing conditions. Sponsors should also provide the results of appropriate analyses carried out on samples of representative flavour complexes, accompanied by details of the analytical methods (including validation of the methods) and a full

description of each sample, including the source materials and production processes. Sponsors should also address the possible presence of undesirable compounds associated with the source material (or species with which it might be confused) and should provide sufficient information to differentiate the flavour complexes from other products with similar properties.

Standard information in the specifications for finished flavour complexes would include: descriptions of the source material(s), the derivation of the initial extract, and any subsequent processing stages; a physical description of the flavour complexes; information on solubility; and (for liquid products) specific gravity, refractive index and optical rotation.

Specifications developed by the Committee will include the following information on composition, which is essential for the safety evaluation to proceed:

(a) upper and lower concentrations of major characterizing components, including all key constituents identified in relevant ISO standards and any other components considered to be critical for the organoleptic properties of the flavouring.

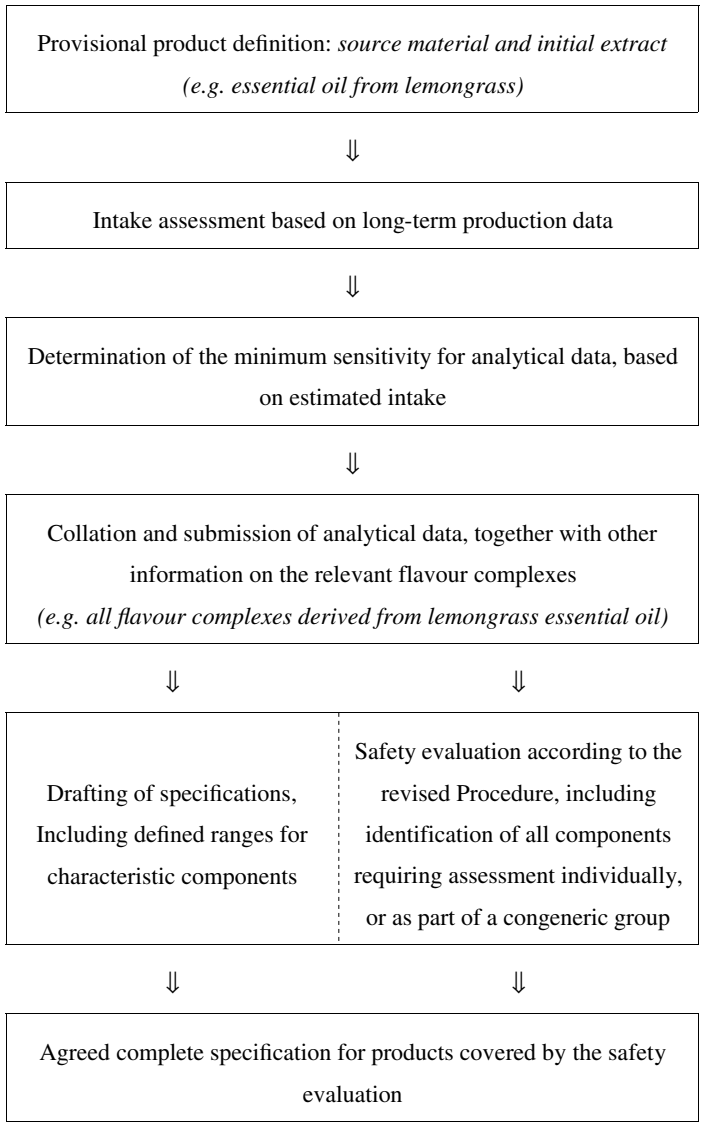
(b) a list of other components that may be present at or above a given concentration; the concentration will depend on the intake and the relevant threshold of toxicological concern (see above) in the revised Procedure for the Safety Evaluation of Flavouring Agents. Components present in the flavour complex at levels above 0.1–0.5% whose estimated intake exceeds 1.5 µg/day should be characterized if their estimated intake exceeds 1.5 µg/day. The need for more detailed characterization would be determined on a case-by-case basis, depending on the nature of the starting material.

(c) upper limits for any other relevant components, including likely impurities and contaminants or potentially toxic components, such as inherent toxins associated with any part of the source species or with related species with which it might be confused.

The overall scheme for evaluating finished flavour complexes is summarized in Figure 2.

The Committee requested data, in line with the above proposals, on examples of flavour complexes with a range of different constituents and representing different estimated intakes, in order to develop appropriate specifications and to evaluate the application of the revised Procedure to this type of flavouring agent. In particular, in the

Figure 2
Overall scheme for evaluating finished flavour complexes



first detailed consideration of finished flavour complexes, quantitative data should be provided on the composition of representative samples of the selected flavour complexes, allowing the identification of all components present in the flavour complexes at concentrations of >0.1% and with an estimated intake of ≥1.5 µg/day.

2.4 Evaluation of dietary nutrients and other ingredients

The Committee evaluated the safety of several substances that were claimed to have nutritional or health benefits. It was observed that there was increased interest from Member States in having the Committee evaluate such substances. The Committee noted that whether such products meet appropriate definitions as nutrients or are worthy of health, nutrient, or other claims was outside its remit. Therefore, the Committee only evaluated the safety of these ingredients. Moreover, the Committee expressed the view that the safety evaluation of these ingredients should not be interpreted to mean that the Committee endorses the use of these substances for their claimed nutritional or health benefits.

2.5 Principles governing the establishment and revision of specifications

2.5.1 *Determination of carotenoids*

The Committee recognized that there was an increasing number of specifications for the analysis of members of the family of carotenoid compounds. Each specification prescribes the use of a different instrumental method of analysis. The Committee decided that it would be advantageous to consolidate and minimize the number of methods for the analysis of members of the carotenoid family and to publish them in FAO Food and Nutrition Paper, No. 5.

2.5.2 *Revision of heavy metals and arsenic specifications*

At its fifty-third meeting (Annex 1, reference *143*), the Committee agreed to implement the decision taken at its forty-ninth and fifty-first meetings, namely, to review and replace the limit test for heavy metals and arsenic with, as appropriate, limits for the individual elements of concern in all existing specifications established by the Committee. In order to accomplish this, the Committee decided to review the existing specifications on the basis of functional use (e.g. antioxidant, preservative), and set a target of 5 years for completion of the task.

At its fifty-fifth and subsequent four meetings (Annex 1, references *149*, *152*, *154*, *157*, and *161*), the Committee reviewed all the specifications that had not been modified during previous meetings.

The principles adopted by the Committee in its reviews were as follows:

— After removing the "heavy metals (as lead)" specification, a maximum concentration of 2 mg/kg for lead and 1 mg/kg for cadmium and mercury would be established, except where there were data

to support higher or lower maximum concentrations, or there were issues related to consumer exposure.

— A limit for arsenic would only be included when the source from which the additive was prepared or the nature of the manufacturing method for the additive indicated that arsenic was likely to be a contaminant.

At the present meeting of the Committee, specifications for the remaining 84 food additives were reviewed for heavy metals and arsenic levels, and the specifications for these elements only were revised accordingly.

2.6 Core Standing Committee for JECFA

According to current procedure, JECFA is not a standing Committee. Members are selected for each meeting on the basis of their expertise and according to the compounds scheduled for evaluation. The Committee as such is only in existence for the duration of the meeting until the adoption of the report.

In order to improve current working procedures and to facilitate the work of the Committee as well as of the Secretariats, the Joint Secretaries proposed the establishment of a core JECFA Committee as a standing Committee for the period of 3 years. Chairs (one FAO expert and one WHO expert), rapporteurs (one from FAO and one from WHO) as well as four Members (two from FAO and two from WHO) would be appointed by the Secretariats, according to WHO and FAO rules established for Expert Committees. The appointment of the Core JECFA Committee would be published on the JECFA websites.

The role of this standing committee would be to ensure the continuity of the work of the Committee. Further responsibilities would be to assist the Secretariats in the following tasks: finalization of the agenda and formulation of appropriate call for data, identification of appropriate experts, and assignment of experts to specific compounds for each meeting. In addition, on agreement with the Secretariats, the Core Members could represent JECFA at specific meetings.

For each meeting, additional Members would be appointed according to existing procedures to cover all necessary expertise and to work with the Core Standing Committee in the evaluation of scheduled substances. All Members of the Committee at the meeting would have the same rights and responsibilities.

2.7 Provision of scientific advice by FAO and WHO

The Committee was informed about the advances on the consultative process carried out by FAO and WHO to enhance the procedures followed by both organizations for the provision of scientific advice to the Codex Alimentarius Commission and Member countries. In particular, reference was made to the Joint FAO/WHO Workshop on the Provision of Scientific Advice to Codex and Member Countries held from 27 to 29 January 2004, which resulted in a set of recommendations on essential principles, definitions and scope governing the provision of scientific advice, management issues, and procedures and mechanism to be improved. The report of the Workshop was available on the web sites of FAO and WHO[1].

The Committee noted that implementation of the recommendations would have a direct impact on the work of the Committee and that increased participation of experts from developing countries would require specific actions, for example, training on the operation of the Committee.

The Committee was informed that comments received by FAO and WHO from their Member countries and international nongovernmental organizations with observer status in Codex on the workshop recommendations would be presented at the Twenty-seventh Session of the Codex Alimentarius Commission, and that procedural guidelines on provision of scientific advice would be prepared and made public to increase transparency of the overall system. FAO/WHO would complete the consultative process and continue the implementation of the workshop recommendations, depending on availability of resources.

2.8 IPCS Project on Dose–Response Modelling

The Committee was informed of the development of the Project on Dose–Response Modelling organized by the International Programme on Chemical Safety. The goal of this project is a state-of-the art review of dose–response modelling and its application in risk assessment, also harmonizing environmental and human health risk assessment. The outcome will be published in the WHO Environmental Health Criteria series.

The Committee recognized the importance of this project with regard to chemical contaminants in food, and endorsed the effort and urged its continuing support.

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[1] FAO web site: http://www.fao.org/es/esn/proscad/index_en.stm; WHO web site: http://www.who.int/foodsafety/codex/consult/en/

2.9 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 its present evaluations:

— dose–response modelling of end-points, 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.

3. Specific food additives (other than flavouring agents)

The Committee evaluated four food additives and one mixture of components for the first time and re-evaluated nine food additives. 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 *Benzoyl peroxide*

Explanation

Benzoyl peroxide is used as a bleaching agent in flour, in milk for production of cheeses and in whey from the manufacture of cheeses in which annatto and carotenoid pigments are present. At its current meeting, the Committee evaluated the safety of benzoyl peroxide used as a bleaching agent in whey at a maximum concentration of 100 mg per kg.

At its seventh meeting (Annex 1, reference *7*), the Committee evaluated benzoyl peroxide used as a bleaching agent in flour, and concluded that treatment of flour with benzoyl peroxide at concentrations of up to 40 mg per kg of flour was acceptable. At that meeting, the Committee noted that when benzoyl peroxide is used as a bleaching agent in flour, it reacts with oxidizable constituents of the flour and is almost totally converted to benzoic acid; any remaining traces of benzoyl peroxide are further reduced during the baking process and converted into benzoic acid. On this basis, the issues requiring consideration for use of benzoyl peroxide as a bleaching agent were determined to be the presence of small amounts of benzoic acid in bread and bakery products, the possible effects of oxidative treatment on the nutritional value of flour, and the possible formation of harmful substances or anti-metabolites.

At the fifty-fifth meeting of the Committee (Annex 1 reference *149*), the evaluation of the nutritional and toxicological implications of treatment of foods with benzoyl peroxide with respect to potential effects on proteins, vitamins, antioxidants and physiologically important lipids was postponed, owing to lack of information.

Benzoyl peroxide is manufactured by the reaction of benzoyl chloride, sodium hydroxide and hydrogen peroxide. During cheese-making or whey-drying, nearly all (>91%) benzoyl peroxide is converted to benzoic acid.

Concerning residues of benzoic acid, at the forty-first meeting of the Committee (Annex 1, reference *107*) a group acceptable dietary intake (ADI) of 0–5 mg/kg of body weight (bw) for benzoic acid and its calcium, potassium and sodium salts, benzyl acetate, benzyl alcohol, benzaldehyde and benzyl benzoate was established, and this was maintained by the Committee at its forty-sixth meeting (Annex 1, reference *122*). At its fifty-fifth meeting (Annex 1, reference *149*), the Committee noted that the intake of benzoic acid from foods treated with benzoyl peroxide should be considered together with intake from other dietary sources of benzoates in the group ADI of 0–5 mg/kg bw.

Toxicological data

Almost all the benzoyl peroxide used in food processing is converted to benzoic acid during heat treatment or storage. While traces of benzoyl peroxide may be present in the processed food, most, if not all, of the benzoyl peroxide ingested will be degraded to benzoic acid in the intestine. It is likely that any benzoyl peroxide absorbed will be metabolized to benzoic acid in the liver. Finally, benzoic acid will be

excreted in the urine, either as benzoate or as a conjugate with glycine. On this basis, the major issues to be considered when benzoyl peroxide is used as a bleaching agent in whey are the presence of small amounts of benzoic acid residues and the potential nutritional effects on whey.

During the metabolism of benzoyl peroxide, superoxide anion radicals may be produced. The low concentration of radicals formed will not, however, saturate superoxide dismutase and do not pose a safety concern.

Clinical studies have shown that benzoyl peroxide can be a severe dermal irritant, and is a dermal sensitizing agent in humans. The short-term studies of toxicity that are available are of limited quality. Benzoyl peroxide did not cause significant toxicity in rats or mice after repeated intraperitoneal injection. Benzoyl peroxide has been shown to cause single-strand breaks in DNA and to disrupt intercellular communication in vitro. However, it was not mutagenic and did not bind covalently to DNA. Benzoyl peroxide was not carcinogenic after subcutaneous or after dermal application. Benzoyl peroxide was shown to be a promoter in assays for initiation–promotion in mice treated dermally.

In a long-term study of carcinogenicity, the incidence of tumours did not increase in rats and mice receiving diets containing benzoyl peroxide. These and additional, although limited, data indicate that it is unlikely that treatment of food with benzoyl peroxide will have an effect on the nutritional value of whey, or result in the formation of harmful substances.

Epidemiological and clinical studies did not find an association between the incidence of skin cancer in industrial workers or acne patients and exposure to benzoyl peroxide. Adverse effects were usually limited to dermal irritation and sensitization reactions.

Intake

In the FAO food balance sheet for the year 2000, it was reported that 89 million metric tonnes of whey are annually produced in the world. Estimates based on the production figures in the FAOSTAT 2000 food balance sheet tables suggest that <15% of the world"s whey production would be subject to this bleaching process. The worldwide consumption per capita of whey (both bleached and unbleached) was 0.8 kg per year, and the highest consumption per capita was 15.4 kg per year in the USA. This results in a total daily exposure to benzoic acid of 0.01 mg/kg bw (for a 60 kg person), assuming complete conversion of benzoyl peroxide.

Evaluation

The Committee considered the acceptability of small amounts of benzoic acid residues added to the diet by the consumption of food products containing bleached whey.

Assuming that 15% of cheese whey were bleached, the intake of benzoic acid per capita was estimated to be 0.01 mg/kg bw per day. The Committee concluded that this was a minor contribution to the total dietary intake of benzoic acid for which a group ADI was established at the forty-first meeting and that treatment of whey with benzoyl peroxide at a maximum concentration of 100 mg/kg did not pose a safety concern.

The Committee restated its conclusion from the fifty-first meeting (Annex 1, reference *122*) that it was possible that the intake of benzoic acid from all dietary sources by some consumers could exceed the ADI, and concluded that more precise intake data were required to estimate the number of such consumers and the magnitude and duration of intakes that are greater than the ADI.

A toxicological monograph was prepared. The existing specifications for benzoyl peroxide were revised to expand the definition and description of the substance and to amend the functional use. A Chemical and Technical Assessment[1] was prepared.

3.1.2 *α-Cyclodextrin*

Explanation

α-Cyclodextrin (synonyms: cyclohexaamylose, cyclomaltohexaose, α-Schardinger dextrin) is a non-reducing cyclic saccharide comprising six glucose units linked by α-1,4 bonds. α-Cyclodextrin was evaluated by the Committee at its fifty-seventh meeting (Annex 1, reference *154*). The Committee concluded that, on the basis of the results of available studies with α-cyclodextrin and with the structurally related compounds β-cyclodextrin (seven glucose units) and γ-cyclodextrin (eight glucose units), for which ADIs had been allocated, there was sufficient information to allocate an ADI "not specified" for α-cyclodextrin.

[1] Considering the recommendations of the Committee at its fifty-ninth meeting, the Secretariat has adapted the format and structure of the Technical Data Sheet and renamed it the Chemical and Technical Assessment with the intention of making this document publicly available. The Chemical and Technical Assessment reflects and emphasizes the role that chemical characterization plays in risk assessment for food additives. The document is prepared by an expert assigned before the meeting and is intended to provide to the Committee the basic information related to identity, purity and use of the food additive, as related to its risk assessment.

At its fifty-seventh meeting, the Committee evaluated α-cyclodextrin on the basis of known uses under good manufacturing practice as a carrier and stabilizer for flavours, colours, and sweeteners, as a water-solubilizer for fatty acids and certain vitamins, as a flavour modifier in soya milk, and as an absorbent in confectionery. The annular (or doughnut-shaped) structure of α-cyclodextrin provides a hydrophobic cavity that allows the formation of inclusion complexes with a variety of non-polar organic molecules of appropriate size, while the hydrophilic nature of the outer surface of the cyclic structure causes such complexes to be soluble in water. α-Cyclodextrin is produced by the action of cyclodextrin glucosyltransferase and may contain residues of 1-decanol, which is used in the purification process.

At its present meeting, the Committee evaluated α-cyclodextrin for use as a food ingredient, suggested by the manufacturer to be a dietary fibre. It is stressed that the Committee only evaluated the safety of the estimated intake of α-cyclodextrin resulting from the proposed use levels. The Committee did not assess the efficacy of α-cyclodextrin used as a dietary fibre.

Specifications for α-cyclodextrin established at the fifty-seventh meeting were not considered by the Committee at its present meeting.

Toxicological data

Only small quantities (1% or less of the administered dose) of intact α-cyclodextrin are absorbed from the small intestine. Absorbed α-cyclodextrin is rapidly excreted in the urine. α-Cyclodextrin, like β-cyclodextrin, is not digested in the gastrointestinal tract but is fermented to short-chain fatty acids by the intestinal microflora. These fatty acids are absorbed, oxidized, and eliminated largely as exhaled carbon dioxide.

α-Cyclodextrin is not hydrolysed by human salivary and pancreatic amylases in vitro. Indirect proof that α-cyclodextrin is not digested in humans is drawn from experiments showing that the intake of 25 g of α-cyclodextrin does not lead to an increase in blood concentrations of glucose and insulin.

The results of short-term (28- and 90-day) studies of toxicity indicate that α-cyclodextrin has low oral toxicity in rats and dogs. After administration of α-cyclodextrin at a very high dietary concentration (20% in the diet, corresponding to a dose of 13.9 g/kg bw per day in rats and 10.4 g/kg bw per day in dogs), caecal enlargement and associated changes were seen in both species. This effect is likely to result from the presence of a high concentration of an osmotically active substance in the large intestine.

Studies on embryotoxicity and teratogenicity in mice, rats, and rabbits fed diets containing α-cyclodextrin at a concentration of up to 20% (corresponding to a dose of 49.3 g/kg bw per day in mice, 20 g/kg bw per day in rats, and 5.9–7.5 g/kg bw per day in rabbits) did not indicate any adverse effects.

α-Cyclodextrin is neither an irritant nor a sensitizer after dermal application.

α-Cyclodextrin showed no effects in assays for genotoxicity in vitro and in vivo. No long-term studies of toxicity, carcinogenicity, or reproductive toxicity have been conducted with α-cyclodextrin, but the Committee reiterated its conclusion from the fifty-seventh meeting (Annex 1, reference *154*), stating that such studies were not required for the evaluation, in view of the known fate of this compound in the gastrointestinal tract.

It is possible that the potential interaction of α-cyclodextrin with lipophilic nutrients might impair their absorption. Although this has not been studied specifically for α-cyclodextrin, such an effect was considered unlikely by analogy to the results of studies with β-cyclodextrin. Complexes between fat-soluble vitamins and β-cyclodextrin have been shown to have a greater bioavailability than uncomplexed forms. In this context, α-cyclodextrin is known to enhance the solubility of retinol acetate and vitamin K1 in water, but does not form complexes with vitamin D and vitamin E.

It is also considered unlikely that the consumption of large amounts of α-cyclodextrin would impair the absorption of minerals, since it is known that the ingestion of resistant starch does not significantly affect the absorption or retention of calcium, phosphorus, magnesium or zinc. Moreover, α-cyclodextrin is of low viscosity, and its chemical structure lacks anionic or cationic groups.

A few studies in human volunteers indicate that flatulence, bloating, nausea and soft stools may occur in some individuals upon ingestion of high doses of α-cyclodextrin. This is a well-known phenomenon for carbohydrates of low digestibility, particularly if ingested in liquid form on an empty stomach. It is partly caused by an influx of water in the small intestine (achieving isotonicity) and partly by the ensuing fermentation process in the more distal parts of the gut. Mild abdominal discomfort occurred in four out of twelve overnight-fasted subjects given a single dose of 25 g of α-cyclodextrin in water, while no effects were reported after administration of 10 g of α-cyclodextrin in water together with white bread. In studies with other carbohydrates of low digestibility, such as inulin, fructooligosaccharides,

polydextrose, resistant (malto)dextrins and other oligosaccharides, abdominal complaints were reported after a single dose of ≥20 g in adults, and children of school age tolerated supplementation of the diet with fructooligosaccharides at a single dose of 3–9 g.

Evaluation of potential impurities

The enzyme cyclodextrin-glycosyl transferase, which is used in the production of α-cyclodextrin, is derived from a non-toxinogenic micro-organism. The enzyme is completely removed from α-cyclodextrin during purification and is therefore of no safety concern. 1-Decanol, which is used as complexant for the precipitation of α-cyclodextrin, may be present in the final product at a concentration of <20 mg/kg. For example, an assumed intake of α-cyclodextrin of 65 g/person per day would correspond to an intake of 1-decanol of <1.3 mg/person per day. This is not a safety concern because 1-decanol is rapidly oxidized in the intestinal mucosa to the corresponding fatty acid, which then undergoes β-oxidation.

Intake

At its fifty-seventh meeting (Annex 1, reference *154*), the Committee estimated the potential intake of α-cyclodextrin from known food uses. The predicted mean intake of α-cyclodextrin by consumers, based on individual dietary records for the USA and maximum proposed levels of use in a variety of foods, was 1.7 g/person per day. For consumers at the 90th percentile of intake, the predicted daily intake of α-cyclodextrin was 3 g.

The intended use levels from the proposed new use of α-cyclodextrin as an ingredient in a number of food products range from a maximum of 10 g/kg in non-alcoholic beverages to a maximum of 100 g/kg in bakery products.

Assuming that α-cyclodextrin would be added to all possible food categories at the maximum proposed use levels, and using the WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme (GEMS/Food) database "European diet" food consumption figures, the Committee calculated a total intake of α-cyclodextrin of 65 g/person per day. This estimate is very conservative since it is unlikely that α-cyclodextrin would be consumed simultaneously from all sources on a regular basis.

An intake assessment based on a national 1-day recall survey was provided by Australia and New Zealand. It was assumed that α-cyclodextrin would be present at the highest proposed concentrations in all foods for which use was intended. The average dietary intake

from intended uses was estimated to be 16 g/person per day and the 95th percentile of intake was estimated to reach 37 g.

In order to estimate the potential intake of α-cyclodextrin in a single eating occasion, the Committee used the GEMS/Food "large portion" database, which contains the highest 97.5th percentile consumption figures (eaters only) reported from national surveys. The highest estimated potential ingestion of α-cyclodextrin per eating occasion is between 19 and 38 g for bread only, depending on the proposed use level.

Evaluation

At its present meeting, the Committee evaluated the safety of α-cyclodextrin based on its known use as food additive and on its proposed use as food ingredient.

A very conservative assessment of international exposure to α-cyclodextrin suggested that intakes could reach 65 g/person per day, while more realistic estimates at a national level suggested that intakes were likely to be 30 to 50% of this value. α-cyclodextrin has been tested in various studies in animals, and no toxicity was observed at the highest doses tested, which were 10–100 times higher than the different estimates of potential intake by humans.

With respect to the previously evaluated use of α-cyclodextrin as a food additive and the present consideration of α-cyclodextrin as a food ingredient, the Committee concluded that there were no safety concerns at the proposed use levels and resulting predicted consumption.

The fact that the ingestion of ≥20 g of α-cyclodextrin on a single eating occasion may cause gastrointestinal effects in humans should be taken into account when considering appropriate levels of use.

The previously established ADI "not specified" for the food additive uses of α-cyclodextrin as a carrier and stabilizer for flavours, colours, and sweeteners, as a water-solubilizer for fatty acids and certain vitamins, as a flavour modifier in soya milk, and as an absorbent in confectionery was retained.

A addendum to the toxicological monograph was prepared. The existing specifications for α-cyclodextrin established at the fifty-seventh meeting were not considered by the Committee at its present meeting.

3.1.3 *Hexose oxidase from* Chondrus crispus *expressed in* Hansenula polymorpha

Explanation

The enzyme preparation under evaluation contains the active enzyme hexose oxidase, which has not been previously evaluated by the Committee. Hexose oxidase is an enzyme that catalyses the oxidation of C6 sugars to their corresponding lactones, with the concomitant formation of hydrogen peroxide. The hexose oxidase is produced from a nonpathogenic and nontoxigenic genetically modified strain of the yeast *Hansenula polymorpha* containing the hexose oxidase gene derived from the red alga *Chondrus crispus*. It can be used as a processing aid in the production of a range of foods at doses of 150–200 hexose oxidase units (HOXU)/kg of food (typical) or 500–2500 HOXU/kg of food (maximum). The commercial preparation contains 0.2 mg of total organic solids per HOXU. The technological functions of hexose oxidase are dough strengthening, curd formation, oxygen scavenging, and decreasing the formation of the products of the Maillard reaction.

Genetic modification

The gene encoding hexose oxidase was derived from the red alga *Chondrus crispus*, which is not known to be pathogenic or toxigenic. *C. crispus* has a long history of use in food in Asia and is one of the sources of carageenan, which has been evaluated as a food additive (Annex 1, references *32*, *137*). A synthetic gene was constructed, based on the hexose oxidase cDNA from *C. crispus*, that was adapted for expression in yeast. The synthetic gene encodes a hexose oxidase with the same amino acid sequence as that of the native *C. crispus* enzyme. The synthetic gene was combined with regulatory sequences, promoter and terminator, derived from *H. polymorpha*, and inserted into the commonly-used plasmid pBR322. The *URA3* gene from *Saccharomyces cerevisiae* (Baker's yeast) and the *HARS1* sequence from *H. polymorpha* were also inserted into the plasmid. The *URA3* gene serves as a selectable marker to identify cells containing the transformation vector. The native pBR322 plasmid contains genes encoding proteins that confer resistance to ampicillin and tetracycline. These genes were removed during the construction of the transformation vector.

In order to develop the *H. polymorpha* production strain, the wild-type strain ATCC 34438 was subjected to chemical mutagenesis. A strain requiring uracil for growth (uracil auxotroph) was used as a host strain. The strain was transformed with the hexose oxidase transformation vector. The transformed strain was further improved using ultraviolet mutagenesis and used as the hexose oxidase

production strain. All the introduced DNA is well-characterized and would not result in the production of any toxic or undesirable substances. The production strain is stable with respect to the introduced DNA.

Product characterization

Hexose oxidase is produced by submerged fermentation of a pure culture of the *H. polymorpha* production strain. The enzyme is produced intracellularly and, upon cell disruption with lauryl trimethyl ammonium bromide (LTAB), is released into the fermentation broth and is subsequently separated from the yeast cells and subjected to ultrafiltration and diafiltration to obtain concentrated hexose oxidase. It is then spray-dried onto a suitable food-grade carrier, such as wheat starch, to form microgranules that are off-white to brownish in colour. Small amounts of LTAB may be present in the final product. The enzyme is typically denatured during heat treatment, and thus no longer active in the final food product as eaten. The enzyme preparation conforms to the *General specifications for enzyme preparations in food processing* (Annex 1, reference *156*).

Toxicological data

Toxicological studies were performed with water-soluble turbid liquid enzyme test concentrates, designated Ferm sample I, Ferm sample II, HOX-TOX-3-99, HOX-TOX-1 and HOX-TOX-4. These enzyme preparations were not acutely toxic when tested in rats, nor irritating to the skin or eye of rabbits, nor mutagenic in an assay for mutations in bacteria in vitro nor clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro. In a 2-week range-finding study in rats treated with HOX-TOX-1 by gavage and in a 13-week study in rats treated by gavage with HOX-TOX-3-99 (containing not only hexose oxidase but also LTAB), no significant treatment-related effects were seen up to and including the highest dose of 5000 HOXU/kg bw per day (equivalent to total organic solids of 955 mg/kg bw per day). This highest dose, which also represents an exposure to LTAB at 11.3 mg/kg bw per day, is therefore considered to be the NOEL. No toxicological data on LTAB only were available. The closely-related quaternary ammonium compound cetyl trimethyl ammonium bromide (CTAB) was not mutagenic in an assay for mutations in bacteria in vitro. In a 1-year study of toxicity with CTAB in rats, the only effect observed was reduced body-weight gain; the NOEL was 20 mg/kg bw per day.

Neither *H. polymorpha* nor *C. crispus* have been associated with allergenicity.

Intake

A conservative estimate of the intake of hexose oxidase when used at maximum dosage in the production of all potential food categories is 4 mg of total organic solids (or 22 HOXU)/kg bw per day. When this intake is compared with the NOEL of 5000 HOXU (equivalent to 955 mg of total organic solids)/kg bw per day, the highest dose tested in the 13-week study of oral toxicity, the margin of safety exceeds 200. The concomitant intake of LTAB present at maximum concentrations of residue in all potential food categories was estimated to be 2.7 µg/kg bw per day. When this intake is compared with the NOEL for LTAB of 11.3 mg/kg bw per day in the 13-week study of oral toxicity and with the NOEL for the closely-related substance CTAB of 20 mg/kg bw per day in a 1-year study of toxicity in rats, the margin of safety is at least 4000.

Evaluation

The Committee allocated an ADI "not specified" to hexose oxidase from the recombinant strain of *Hansenula polymorpha* when used in the applications specified and in accordance with good manufacturing practice. The Committee concluded that the presence of LTAB at the concentrations observed in the enzyme preparation would not pose a safety concern to consumers.

A toxicological monograph was prepared. New specifications and a Chemical and Technical Assessment were prepared for the commercial enzyme preparation.

3.1.4 *Lutein from* Tagetes erecta *L.*

Explanation

Lutein ((all-*E*,3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol), a naturally occurring xanthophyll pigment, is an oxygenated carotenoid that has no pro-vitamin A activity. It occurs, with the isomeric xanthophyll, zeaxanthin, in many foods, particularly vegetables and fruits. It is used as a food colour and nutrient supplement in a wide range of applications at concentrations ranging from 2 to 330 mg/kg.

Xanthophylls obtained from *Tagetes erecta* L. (marigold) petals were considered by the Committee at its thirty-first meeting (Annex 1, reference *77*). At that time, tentative specifications were prepared, but no toxicological data were available and no evaluation was made. *Tagetes* extract, containing low levels of xanthophylls was again considered by the Committee at its fifty-fifth and fifthy-seventh meetings (Annex 1, references *149*, *154*) and the revised tentative specifications (Annex 1, reference *151*) were superseded by full specifications (Annex 1, reference *156*). At the present meeting,

information was received on preparations with a high lutein content (>80%), which have been used in a number of toxicological studies. These studies were reviewed in the safety assessment and allocation of an ADI for this product.

Chemical and technical considerations

Lutein is a purified extract from marigold (*Tagetes erecta* L.) oleoresin. The final product, after saponification and crystallization, contains lutein as the major component, and a smaller proportion of zeaxanthin. Because the composition of the substance under evaluation at the present meeting was substantially different from that of the crude preparations containing lutein esters considered previously, the Committee prepared new specifications for lutein.

Toxicological data

In rats, peak concentrations of radioactivity in the plasma and tissues occurred about 4 h after a single oral dose of [^{14}C]lutein. Most of the radiolabel was eliminated via the faeces within about 2 days; very low urinary and biliary excretion indicated that there was poor absorption from the intestinal tract. Based on faecal excretion data, the absorption of lutein was about 30–40% when administered to rats in the diet as beadlets containing vitamin E (the beadlet formulation was used to enhance the stability of lutein). Ten-fold increases in dose in the range of 2–200 mg/kg bw, resulted in 2- to 3-fold increases in plasma concentrations, indicating reduced absorption at higher doses. Steady-state plasma concentrations of lutein were reached by about 3 days after the start of dietary administration of lutein to rats, indicating that the half-life is about 1 day.

In humans, peak plasma or serum concentrations of lutein occurred at 11–16 h after administration of a single oral dose. During daily supplementation with 20 mg of lutein per day, steady-state plasma concentrations were reached within about 30 days. This is consistent with an elimination half-life of about 5–7 days.

The food matrix, including its fibre and lipid contents, and the concentrations of other carotenoids in the diet may influence the extent of absorption of carotenoid compounds. The relative absorption of lutein from a mixed vegetable diet was lower than from a diet containing pure lutein. A mixed preparation of lutein and zeaxanthin did not influence the absorption of β-carotene.

Lutein has an oral median lethal dose (LD_{50}) of >2000 mg/kg bw in rats. In a 13-week study in rats, oral doses of lutein of up to 200 mg/kg bw, the highest dose tested, caused no treatment-related effects. In a 52-week study designed primarily to investigate possible adverse

effects on the eye in monkeys, lutein was administered by gavage at a dose of 0.2 or 20 mg/kg bw per day. This study was performed because adverse ocular effects had been seen with canthaxanthin (Annex 1, references *78*, *89*, *117*). There were no treatment-related effects on a wide range of toxicological end-points. Furthermore, comprehensive ophthalmic examinations, including electroretinography, showed no evidence of treatment-related adverse changes.

No long-term studies of toxicity or carcinogenicity had been undertaken.

Lutein gave negative results in several studies of genotoxicity in vitro and in vivo. Although the Committee noted that the doses used in these tests were low, it recognized that maximum feasible doses were used. There was no evidence of tumour promoting activity in animal models.

In a study of developmental toxicity with lutein in rats, there was no evidence for toxicity at doses of up to 1000 mg/kg bw per day, the highest dose tested.

In a 20-week multicentre intervention trial with lutein in healthy human subjects, there were no changes in haematological or biochemical parameters after continuous daily doses of lutein of 15 mg (0.25 mg/kg bw, assuming a body weight of 60 kg). There has been a relatively large number of human studies that have examined correlations between macular degeneration and dietary intake of lutein or zeaxanthin, intakes via dietary supplements, or serum concentrations. Although these studies were designed to look for ocular effects, where clinical or biochemical parameters were also examined, no adverse effects of these xanthophylls were reported.

Intake

Dietary intake data from a number of studies in North America and the United Kingdom indicate that intake of lutein from natural sources is in the range of 1–2 mg/day (approximately 0.01–0.03 mg/kg bw per day). Simulations considering proposed levels of use as a food ingredient resulted in an estimated mean and 90th percentile of intake of lutein plus zeaxanthin of approximately 7 and approximately 13 mg/day, respectively. Formulations containing lutein and zeaxanthin are also available as dietary supplements, but there were no reliable estimates of intakes from these sources.

Evaluation

In several studies of toxicity, including developmental toxicity, no adverse effects were documented in animals, including monkeys,

or humans. Taking into account data showing that lutein was not genotoxic, had no structural alert, did not exhibit tumour promoting activity, and is a natural component of the body (the eye), the Committee concluded that there was no need for a study of carcinogenicity.

Lutein has some structural similarities to β-carotene, which has been reported to enhance the development of lung cancer when given in supplement form to heavy smokers. The available data indicated that lutein in food would not be expected to have this effect. The Committee was unable to assess whether lutein in the form of supplements would have the reported effect in heavy smokers.

The 52-week study in monkeys was designed to evaluate ocular effects, and although there were no adverse toxicological effects at the highest dose tested (20 mg/kg bw per day), this study was considered to be inappropriate for the establishment of an ADI, in view of the much higher doses used in several other studies and found to be that without effect. The available comparative toxicokinetic data for humans and rats indicated that the studies of toxicity in rats could be used to derive an ADI. The Committee concluded that the best study for this purpose was the 90-day study in rats. An ADI of 0–2 mg/kg bw was allocated based on the NOEL of 200 mg/kg bw per day (the highest dose tested in this study) and a safety factor of 100.

Although the ADI was based on the results of a short-term study, the supporting data and lack of effects at much higher doses in some studies (e.g. a study of developmental toxicity), indicated that the safety factor of 100 was appropriate.

In view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin, the Committee decided to include zeaxanthin in the ADI (0–2 mg/kg bw) for lutein, which had a stronger toxicological database, and to make this a group ADI for these two substances. This group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications.

A toxicological monograph, a Chemical and Technical Assessment, and specifications were prepared for lutein from *Tagetes erecta*.

3.1.5 *Peroxyacid antimicrobial solutions containing 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP)*

Explanation

The Committee considered the safety of antimicrobial solutions that are prepared from acetic acid and octanoic acid (singly or in

combination), together with hydrogen peroxide, and using 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) as a sequestrant or stabilizer. Preparations that are ready for use also contain as active compounds the peroxy forms of both acids. Before use, concentrated solutions are diluted to achieve target concentrations of total peroxyacid ranging from 80 to 200 mg/kg. These antimicrobial solutions are intended for use as components of wash solutions on fresh poultry and meat and in wash water for fresh and processed fruits and vegetables. After being applied in process water, they are largely eliminated by drainage, further washing and trimming of products, and evaporation. The safety of the antimicrobial solutions was therefore assessed on a component-by-component basis, considering the potential residue of each component or its breakdown products in food as consumed.

At its seventeenth meeting (Annex 1, reference *32*), the Committee allocated an ADI "not limited"[1] to acetic acid and its potassium and sodium salts. This ADI was retained at the forty-ninth meeting (Annex 1, reference *131*) when the Committee evaluated a group of flavouring agents (saturated aliphatic acyclic linear primary alcohols, aldehydes, and acids) that included acetic acid.

At its forty-ninth meeting, the Committee evaluated octanoic acid for use as a flavouring agent as part of the group of saturated aliphatic acyclic linear primary alcohols, aldehydes, and acids, and concluded that octanoic acid posed no safety concerns at intakes of up to 3800 µg/person per day (or 63 µg/kg bw per day, assuming a body weight of 60 kg).

At its twenty-fourth meeting (Annex 1, reference *53*), the Committee evaluated hydrogen peroxide as a preservative and sterilizing agent for use in milk. While an ADI was not allocated, the Committee noted that hydrogen peroxide should be used only when better methods of milk preservation were not available.

Peroxyacetic acid and peroxyoctanoic acid, and HEDP have not been previously evaluated by the Committee.

At its present meeting, the Committee considered a number of studies on the antimicrobial efficacy of peroxyacid solutions, the toxicity of HEDP, and the effects of peroxyacid solutions on food quality and nutritional value. The Committee also evaluated estimates of the intake of the individual components in these solutions for consideration in the safety evaluation.

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[1] A term no longer used by the Committee, which has the same meaning as ADI "not specified".

Chemical and technical considerations

Antimicrobial washing solutions are manufactured by mixing hydrogen peroxide (4–12%), acetic acid (40–50%), and HEDP (<1%), with or without octanoic acid (3–10%). The concentrations of peroxyoctanoic acid and peroxyacetic acid at equilibrium are in the range of 1–4% and 12–15%, respectively. Peroxyacetic acid was identified as the main antimicrobial substance in the washing solutions. Information submitted indicated that reductions of naturally-occurring microbial flora on treated food were generally less than one order of magnitude. Peroxy compounds in washing solutions used on food were reported to break down rapidly to water, oxygen, acetic acid and octanoic acid. Estimated residues of HEDP and octanoic acid in treated food were ≤0.2 mg/kg and ≤4 mg/kg, respectively. Because the peroxy compounds are highly reactive, these compounds will not leave residues on food and consumers will not be exposed to these substances.

It was noted that there were limited studies available on the effects of the washing solutions on nutrients and there were no studies that identified residues of reaction products that might be formed by the reaction of peroxy compounds with the food components.

The existing specifications for acetic acid, glacial, prepared by the Committee at its nineteenth meeting (Annex 1, reference *38*) were revised to include details of the materials and methods of its manufacture. The maximum limit for lead was reduced to 0.5 mg/kg to be consistent with other monographs for acetic acid, considering the broad use in the food industry. The existing specifications for hydrogen peroxide were initially elaborated in conjunction with the evaluation of the lacto-peroxidase milk sterilization system, carried out by the Committee at its twenty-ninth meeting (Annex 1, reference *70*). They were revised to include details of the materials and methods of manufacture. The functional use descriptor was revised to "antimicrobial agent" to reflect function. The Committee decided to prepare new specifications for octanoic acid as a food additive because of a lower minimum assay value requirement than that listed in the specifications for this compound as a flavouring agent. Specifications for octanoic acid as a flavouring agent were prepared by the Committee at its forty-ninth meeting (Annex 1, reference *131*). New specifications for HEDP, as sequestrant and stabilizer for peroxy-based antimicrobial washing solutions, were also prepared.

Intake

The Committee evaluated estimates of intake of each component used in the peroxyacid solutions on the basis of residual amounts

anticipated to be present on treated food at the time of consumption. Consistent with what was known about the chemistry of peroxy compounds, no residues of hydrogen peroxide, peroxyacetic acid, or peroxyoctanoic acid were anticipated to be present on foods that have been washed in, sprayed with, or otherwise treated using these solutions.

Acetic and octanoic acid present in the solutions and as by-products from the corresponding peroxyacids would be expected to remain on any treated foods that are not washed or further processed after treatment. The Committee noted that the estimate of exposure to octanoic acid resulting from the use of the antimicrobial solutions, 1.9 mg/day, was highly conservative. The mean intake of octanoic acid from foods consumed as part of the diet in the USA was estimated to be approximately 200 mg/day. Intake of acetic acid was not explicitly analysed, but its use in and on foods (vinegar) would result in a greater exposure than that from the use of peroxyacid antimicrobial solutions. The Committee did not further consider exposure to these common food acids.

HEDP is expected to remain on foods that are treated with antimicrobial solutions and that are not further washed, processed, or cooked. The highest estimate of intake of HEDP prepared using GEMS/Food diets was that for the European diet: 3.6 µg/kg bw per day for the upper-bound estimate using a model for vegetables with a high surface area. The Committee also considered national estimates of intake from the Czech Republic, the USA, and the United Kingdom. The upper-bound estimate of exposure was 2.2 µg/kg bw per day for the Czech Republic. The mean and 90th percentile upper-bound estimates of intake for the USA were 2.2 and 4.7 µg/kg bw per day, respectively. The mean and 90th percentile upper-bound estimates of intake for the United Kingdom were 1.8 µg/kg bw per day and 3.3 µg/kg bw per day, respectively.

The Committee was aware of the non-food uses of HEDP. It is used as an anti-scalant for water treatment and in boilers worldwide (the regulatory limit for this use is 25 µg/l in the USA). HEDP is also used as a drug to treat Paget disease, and in some over-the-counter cosmetic and pharmaceutical formulations. The United States Environmental Protection Agency (EPA) estimated that exposure to HEDP from all these uses was not more than 6 µg/kg bw per day, including 0.04 µg/kg bw per day from its use on food (*3*). The Committee noted that this estimate of exposure resulting from food uses of HEDP was much less conservative than that used in the present evaluation.

Antimicrobial efficacy

Information available to the Committee indicated that peroxyacetic acid solutions enhance the action of water sprayed on food surfaces to reduce numbers of bacteria. While reductions in numbers of microbes were demonstrated, some of the data provided suggest that the results of replicate tests were rather inconsistent, with standard deviations close to or greater than the value of the reductions themselves. Testing of food surfaces showed modest reductions in numbers of microbes, when either endogenous microorganisms (represented by total aerobic plate counts) or inoculated ("spiked") pathogens (commonly *Listeria monocytogenes*, *Escherichia coli* O157:H7, and some *Salmonella* serotypes) were monitored. Data from laboratory and in-plant tests indicated that the use of these solutions would minimize the possibility of cross-contamination, although they are unable to remove all adherent viable bacteria from food surfaces.

The Committee did not further consider the antimicrobial efficacy of peroxyacid antimicrobial solutions containing HEDP.

Toxicological data

Antimicrobial solutions are equilibrium solutions that are diluted in water before use in food processing. Hydrogen peroxide in these solutions will dissociate into water and oxygen. Both peroxyacetic acid and peroxyoctanoic acid are also inherently unstable and will breakdown into acetic acid and octanoic acid, respectively, although their stability is enhanced by HEDP. Low residual amounts of these simple organic acids present on food at the time of consumption would pose no safety concern. It is not expected that residues of peroxyacetic acid or peroxyoctanoic acid from these solutions will be present on treated foods at the time of consumption. The peroxide components of the peroxyacid antimicrobial solutions thus pose no toxicological concerns with regard to the uses considered by the Committee. The Committee concluded that HEDP, which sequesters metal ions thereby stabilizing the peroxy compounds in peroxyacid antimicrobial solutions, is the only component of potential toxicological concern.

Data reviewed by the Committee indicated that absorption of HEDP from the gastrointestinal tract is very limited and that its metabolism is negligible. The limited amount of data available to the Committee suggested that absorption may be related to age and species. The skeleton is the target site for the disposition of HEDP in all species.

HEDP did not show evidence of mutagenic activity in assays in five strains of *Salmonella* or in an assay for mutation in L51718 $Tk^{+/-}$

mouse lymphoma cells, with and without metabolic activation from mammalian microsomes.

In two 90-day studies of toxicity, rats were fed diets containing HEDP at doses ranging from 100 to 2500 mg/kg bw per day. The highest dose tested in each study (i.e. 1500 or 2500 mg/kg bw per day) caused mortality and signs of toxicity, but no effects were reported at lower doses in either study. The NOEL was 500 mg/kg bw per day in both studies.

In a 90-day study of toxicity in dogs, HEDP was administered orally at a dose equivalent to 0, 25, 75, or 250 mg/kg bw per day. No adverse effects attributable to treatment were reported. The NOEL for HEDP was 250 mg/kg bw per day. The Committee also evaluated the results of a long-term study to determine the skeletal effects of daily subcutaneous injections of HEDP administered to adult female dogs for varying periods ranging from 1 to 2 years. Some effects on bone parameters were observed at all doses. Profound skeletal effects were associated with the administration of daily subcutaneous doses of HEDP of 2–10 mg/kg bw for 1 year. Spontaneous bone fractures were slightly increased in dogs given daily subcutaneous doses of 0.5 mg/kg bw for 2 years, but no permanent skeletal changes were observed at this dose and healing was normal. No fractures were observed at a daily subcutaneous dose of 0.1 mg/kg bw after 2 years. Assuming that 10–20% of the administered dose were absorbed from the gut in dogs, a subcutaneous dose of 0.1 mg/kg bw per day would correspond to an oral dose of 0.5–1 mg/kg per day. In considering these studies, the Committee noted that 90 days may not be long enough to observe skeletal effects in dogs and that there may be differences in the disposition of HEDP in bone that are related to the route of administration.

In a combined two-generation study of reproductive toxicity and teratogenicity, rats were given HEDP (disodium salt) in the diet at concentrations equivalent to 0, 50 or 250 mg/kg bw per day either during their lifetime or only on days 6–15 of gestation, for two generations. No fetal abnormalities indicative of a teratogenic effect were reported at either dose tested. HEDP was embryotoxic when administered at a dose of 250 mg/kg bw per day during organogenesis. The NOEL for HEDP was 50 mg/kg bw per day.

The effects of HEDP (disodium salt) were determined in a combined study of reproductive toxicity and teratogenicity in rabbits. Two experiments were performed because of the observation of toxicity at the lowest and highest doses, administered by gavage, in the first experiment. In the second experiment, rabbits received HEDP at a

dose of 0, 25, 50, or 100 mg/kg bw per day in the diet, or 100 mg/kg bw per day by gavage. Fetuses from dams receiving HEDP at a dose of 100 mg/kg bw per day by gavage were significantly smaller than those from untreated controls. No fetal abnormalities indicative of a teratogenic effect in rabbits were observed in either experiment. The NOEL was 50 mg/kg bw per day.

Use of HEDP to treat Paget disease

The disodium salt of HEDP, known clinically as sodium etidronate, is administered orally at a starting dose of 5 mg/kg bw per day, for not longer than 6 months, to treat patients with Paget disease. Paget disease is an idiopathic disease characterized by accelerated bone metabolism; fractures and other abnormalities of the bone are common in patients with Paget disease. Owing to its high affinity for solid-phase calcium phosphate, HEDP prevents the growth and dissolution of hydroxyapatite crystals on crystal surfaces of bone. The mechanism of action, however, is not fully understood.

Assessment of the effects on food quality and nutritional value

Limited data on the quality and nutritional value of foods treated with peroxyacid antimicrobial solutions were provided to the Committee. Studies were conducted to determine whether treatment of foods with peroxyacid antimicrobial solutions resulted in significant differences in concentrations of thiobarbituric acid (a measure of rancidity), or in fatty-acid profile testing of raw or cooked poultry products and fresh beef samples, when compared with treatment with water only. No differences were found.

The Committee was aware that studies in the literature indicated potential reactions of hydrogen peroxide with components of food. The Committee noted that such studies are typically conducted using high concentrations and long periods of exposure and that, under the conditions of their intended use, the potential reactivity of peroxyacid antimicrobial solutions is expected to be limited. Studies available to the Committee confirmed the low potential reactivity of two peroxyacid antimicrobial solutions in dilute ready-to-use solutions that are in brief contact with fruits and vegetables.

A study was conducted to determine the effects of peroxyacetic acid and hydrogen peroxide on the content of β-carotene and vitamin C in tomatoes, potatoes and broccoli. These foods were prepared for consumption using "worst-case" exposure conditions, i.e. peroxyacetic acid at 80 mg/kg and hydrogen peroxide at 59 mg/kg for 5 min, and then rinsed. When treated samples were compared with controls, there were no effects on the β-carotene content of tomatoes or

broccoli, on the vitamin C content of potatoes or broccoli, or on the active vitamin C content of tomatoes.

On the basis of the available data, the Committee concluded that peroxyacid antimicrobial solutions are unlikely to have an adverse effect on food quality or nutritional value, with regard to the uses considered by the Committee.

Evaluation

The Committee considered the safety, on a component-by-component basis, of antimicrobial solutions containing HEDP and three or more of the following components: peroxacetic acid, acetic acid, hydrogen peroxide, octanoic acid and peroxyoctanoic acid. These solutions are intended to be diluted before use to achieve peroxyacid concentrations in the range of 80 to 220 mg/kg. The Committee concluded that the peroxy compounds in these solutions (hydrogen peroxide, peroxyacetic acid and peroxyoctanoic acid) would break down into acetic acid and octanoic acid, and that small residual quantities of these acids on foods at the time of consumption would not pose a safety concern. Therefore, the Committee focused its evaluation on the residues of HEDP that are expected to remain on foods treated, in accordance with manufacturers instructions, with peroxyacid antimicrobial solutions that contain HEDP at up to 1%.

The Committee compared the highest estimate of intake of HEDP from the uses of peroxyacid antimicrobial solutions considered by the Committee (i.e. 0.004 mg/kg bw per day) with the starting oral dose used to treat Paget disease (i.e. 5 mg/kg bw per day) and noted that the margin of exposure is >1000. Based on this margin of exposure, the conservative nature of the estimates of intake of HEDP, and the available toxicity data, the Committee concluded that HEDP does not pose a safety concern at the concentrations of residue that are expected to remain on foods.

The Committee noted that the use of peroxyacid antimicrobial solutions does not replace the need for good hygienic practices in handling and processing of food.

A toxicological monograph and new specifications for HEDP and octanoic acid were prepared. Existing specifications for acetic acid and hydrogen peroxide were revised. Chemical and Technical Assessments were prepared for the peroxy-based antimicrobial washing solutions and HEDP.

3.1.6 *Steviol glycosides*

Explanation

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni, a member of the *Compositae* family. The leaves of *S. rebaudiana* Bertoni contain at least ten different glycosides, the major constituents being stevioside and rebaudioside A. The material evaluated at the present meeting contains not less than 95% glycosylated derivatives of steviol, primarily stevioside, rebaudiosides A and C and dulcoside A, with minor amounts of rubusoside, steviolbioside, and rebaudiosides B, D, E and F.

At its fifty-first meeting (Annex 1, reference *149*), the Committee evaluated toxicological data on stevioside and the aglycone steviol. The Committee noted several shortcomings in the available information and requested that specifications should be developed to ensure that the material tested is representative of the material of commerce. Further information was required on the nature of the substance tested, on the metabolism of stevioside in humans and on the activity of steviol in suitable studies of genotoxicity in vivo.

There is no single common or trivial name in common usage for the evaluated mixture of glycosylated derivatives of steviol. At its thirty-third meeting (Annex 1, reference *83*), the Committee developed guidelines for designating titles for specification monographs. According to these guidelines, the title of a monograph should, in such circumstances, be selected from the available scientific, common and trivial names. The name chosen must be nonproprietary and should be a scientifically accurate description of the substance. In addition, the name should communicate to the consumer an accurate description of the substance, within the scope of existing names for food additives. At its present meeting, the Committee established that the evaluated material of commerce for which specifications were developed should be known as "steviol glycosides". The Committee reviewed additional biochemical and toxicological data on the major glycosylated derivatives of steviol and on the aglycone, steviol.

Chemical and technical considerations

Steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water, followed by solvent purification of the water-soluble extract. Ion-exchange resins may also be used during the purification process. *Stevia* extracts generally contain a high percentage of stevioside and rebaudioside A, and smaller amounts of other steviol glycosides. The composition of the extracts depends on the composition of the leaves, influenced by soil and climate conditions, and on the manufacturing process. The data on analytical

chemistry available to the Committee indicated that commercial products contain at least 95% steviol glycosides. However, the remainder of the material was not identified.

The impurities occurring in steviol glycosides consist primarily of compounds extracted from the *Stevia* leaves. Results of analysis of *Stevia* preparations support the setting of maximum limits of 1 mg/kg for both arsenic and lead.

Different methods, mainly involving liquid chromatography, are currently available for the identification and determination of the principal steviol glycosides.

Stevioside and rebaudioside A are reasonably stable at the elevated temperatures used in food processing, and do not undergo browning or caramelization when heated. No information on the hydrolytic stability of steviol glycosides in acidic foods was available to the Committee.

Toxicological data

After oral administration, steviol glycosides are poorly absorbed in experimental animals and in humans.

Intestinal microflora metabolize steviol glycosides to the aglycone, steviol, by successive hydrolytic removal of glucose units. Data reviewed by the Committee at its current and fifty-first meetings (Annex 1, reference *149*) indicated that this process is similar in rats and humans. The hydrolysis of rebaudioside A to steviol was slower than that of stevioside. In humans treated orally with stevioside, small amounts of steviol were detected in the plasma, with considerable interindividual variability. The major route by which steviol is metabolized in humans in vivo appears to be via conjugation with glucuronide and/or sulfate. Studies with liver microsomal preparations indicated that steviol is also metabolized to a number of hydroxy and dihydroxy derivatives via cytochrome P450 (CYP)-dependent pathways.

Stevioside and/or steviol affected a variety of biochemical parameters in models in vitro, indicating possible mechanisms of antihypertensive and antiglycaemic effects that involve modulation of ion channels. High concentrations (e.g. 1 mmol/l) of stevioside were required to produce a maximal increase in insulin secretion, while steviol was effective at a concentration that was about three orders of magnitude lower. Stevioside also affected a variety of biochemical parameters in different animal species in vivo, mostly with parenteral administration; these studies were considered by the Committee to be of limited relevance to dietary exposure.

No new long-term studies of toxicity or carcinogenicity were available at the present meeting. At its fifth-first meeting, the Committee noted that oral administration of stevioside (purity, 95.6%) at a dietary concentration of 2.5%, equal to 970 and 1100 mg/kg bw per day in male and female rats, respectively, for 2 years was not associated with toxicity. Reduced body-weight gain and survival rate were observed with stevioside at a dietary concentration of 5%. In a new study, stevioside was found to inhibit the promotion of skin tumours by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in a model of skin carcinogenesis in mice.

The Committee reviewed new data on genotoxicity that considered together with data reviewed by the Committee at its fifth-first meeting, allowed a number of conclusions to be drawn. Stevioside and rebaudioside A have not shown evidence of genotoxicity in vitro or in vivo. Steviol and some of its oxidative derivatives show clear evidence of genotoxicity in vitro, particularly in the presence of a metabolic activation system. However, studies of DNA damage and micronucleus formation in rats, mice and hamsters in vivo indicate that the genotoxicity of steviol is not expressed at doses of up to 8000 mg/kg bw.

One new study of developmental toxicity was available at the present meeting. Adverse effects on the reproductive apparatus, which could be associated with impaired fertility, were observed in male rats given a crude extract of *S. rebaudiana*, at a dose corresponding to 1.34 g of dried leaves. However, at its fifth-first meeting, the Committee reviewed a number of studies of reproductive and developmental toxicity with stevioside (purity, 90% or 96.5%). Doses of up to 2500 mg/kg bw per day in hamsters and 3000 mg/kg bw per day in rats had no effect in studies of reproductive toxicity. No teratogenic or embryotoxic effects were observed in rats given stevioside at a dose of up to 1000 mg/kg bw per day by gavage. The Committee considered that the adverse reproductive effects associated with administration of aqueous extracts of *S. rebaudiana*, noted at the present and fifty-first meeting, were unlikely to be caused by steviol glycosides.

Stevioside is being investigated as a potential treatment for hypertension and diabetes. Administration of stevioside at a dose of 750 or 1500 mg per day for 3–24 months resulted in decreased blood pressure in hypertensive patients, and no adverse effects. These studies, in a limited number of subjects, provided some reassurance that stevioside at a dose of up to 25 mg/kg bw per day (equivalent to 10 mg/kg bw per day, expressed as steviol) for up to 2 years shows no evidence of significant adverse effects in these individuals. There is no informa-

tion on the effects of repeated administration of stevioside on blood pressure in normotensive individuals. A small study in 12 patients with type-2 diabetes showed that a single dose of 1 g of stevioside reduced postprandial glucose concentrations and had no effect on blood pressure.

Intake

The Committee evaluated information on exposure to steviol glycosides, submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that was prepared for the European Commission by the Scientific Committee on Food (*4*). All the intake results are presented in terms of equivalents of steviol, based on a conversion of 40% from the steviol glycoside, stevioside (relative molecular mass: steviol, 318, steviosid, 805).

The Committee used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). It was assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, 200:1. The intakes ranged from 1.3 mg/kg bw per day (African diet) to 3.5 mg/kg bw per day (European diet).

The Committee evaluated estimates of exposure per capita derived from disappearance (poundage) data supplied by Japan and China. The Committee also evaluated estimates of exposure to steviol glycosides based on the replacement of all dietary sugars in the diets for Japan and the USA. Table 1 summarizes the exposures to steviol glycosides (as steviol) evaluated or derived by the Committee.

The Committee concluded that the replacement estimates were highly conservative and that intake of steviol glycosides (as steviol) would be likely to be 20–30% of these values.

Table 1
Summary of estimates of exposure to steviol glycosides (as steviol)

Estimate	Exposure (mg/kg bw per day)
GEMS/Food (International)[a]	1.3–3.5 (for a 60 kg person)
Japan, per capita	0.04
Japan, replacement estimate[b]	3
USA, replacement estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme

[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the USA would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200 : 1

Evaluation

The Committee noted that most of the data requested at its fifty-first meeting, e.g. data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available.

The Committee concluded that stevioside and rebaudioside A are not genotoxic in vitro or in vivo and that the genotoxicity of steviol and some of its oxidative derivatives in vitro is not expressed in vivo. The NOEL for stevioside was 970 mg/kg bw per day in a long-term study evaluated by the Committee at its fifty-first meeting.

The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw per day (equivalent to 5–10 mg/kg bw per day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g. those with hypotension or diabetes). The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans.

A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw per day (or 383 mg/kg bw per day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.

The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

A toxicological monograph was prepared, incorporating summaries of the key toxicological data on the evaluation of stevioside conducted by the Committee at its fifty-first meeting.

New tentative specifications were prepared, accompanied by a Chemical and Technical Assessment.

In order to be able to remove the tentative designation from the specifications, the following further information for commercially available products was required by 2007:

— analytical data on distribution and concentrations of all component steviol glycosides, including those that were not identified in the tentative specifications;

— method of analysis for the determination of all component steviol glycosides, including those that were not identified in the tentative specifications;

— the nature and concentration of the fractions that do not contain steviol glycosides;

— the quantities of residual solvents from isolation and purification steps of the manufacturing process;

— the hydrolytic stability of the steviol glycosides in acidic foods and beverages.

3.1.7 *D-Tagatose*

Explanation

D-Tagatose is a ketohexose, an epimer of D-fructose isomerized at C4. 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, fifty-seventh and sixty-first meetings (Annex 1, references *149*, *154* and *166*). 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 concentration of uric acid in serum after intake of D-tagatose and other substances. The Committee decided to base its evaluation on the human data reviewed in the course of these two meetings. A NOEL of 0.75 g/kg bw per day was identified in 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 bw for D-tagatose was established on the basis of this NOEL and a safety factor of 10.

At its sixty-first meeting, the Committee reviewed the results of two new studies of toxicity in rats, and of two new studies of plasma concentrations of uric acid in human volunteers; these studies were submitted by the sponsor with a request for a re-evaluation of D-tagatose.

The Committee concluded that the 2-year study in rats demonstrated that the previously-reported liver glycogen deposition and hypertrophy did not result in histopathological changes after long-term administration of D-tagatose, and thus addressed the concerns expressed at the fifty-fifth meeting. However, this study also identified new findings, namely increased adrenal, kidney and testes weights. The Committee considered that these changes might have been caused by 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 results of histopathological examination, the Committee confirmed that the human data provided the most relevant basis for assessing the acceptable intake of D-tagatose.

Results of a study in hyperuricaemic individuals indicated that the NOEL identified for normal individuals was 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 removed, and the Committee allocated a temporary ADI for D-tagatose of 0–125 mg/kg bw on the basis of the NOEL of 0.75 g/kg bw per day and a safety factor of 6.

The Committee considered that the temporary ADI did not apply to individuals with hereditary fructose intolerance resulting from deficiency of 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. This information was provided to the Committee for evaluation at its present meeting, together with additional data on the risk to individuals with hereditary fructose intolerance.

The specifications for D-tagatose established at the sixty-first meeting were not considered by the present Committee.

Toxicological data

Additional histopathological examinations were conducted on the adrenals, kidneys and testes of Wistar rats fed diets containing 2.5, 5 or 10% D-tagatose, or 10% D-tagatose plus 10% fructose for 2 years. The observed changes were similar to those reported in studies with other carbohydrates of low digestibility. The Committee has

previously noted that gross dietary imbalance caused by high doses of polyols may result in metabolic and physiological disturbances in rats, and are associated with changes in calcium uptake and excretion accompanied by nephrocalcinosis and adrenal medullary hyperplasia (Annex 1, reference *62*). These changes were not considered to be of relevance to this safety evaluation. Carbohydrates of low digestibility do not increase the intestinal absorption of calcium in humans to the same extent as in rats. Rats, especially females, are particularly prone to the development of nephrocalcinosis. The Committee has previously noted the unique features of the rat adrenal medulla and concluded that the occurrence of proliferative lesions of the adrenal medulla in rats fed with polyols and lactose is a species-specific phenomenon (Annex 1, reference *122*). An increased incidence of Leydig cell tumours has been reported in male Wistar rats fed diets containing 10% lactitol or 20% D-tagatose. This study demonstrated that there were no toxicologically significant findings in rats fed with D-tagatose at dietary levels of up to 10% for 2 years (equal to approximately 4 and 5 g/kg bw per day for males and females, respectively).

The Committee further considered the risk to individuals with hereditary fructose intolerance, which if untreated leads to metabolic disturbances, liver damage, renal tubular disease and defective blood coagulation. Treatment requires almost complete exclusion of sucrose, fructose and sorbitol. There is no direct evidence establishing that individuals with hereditary fructose intolerance are also intolerant to D-tagatose, but in view of their common biochemical pathways it is probable that D-tagatose could produce the same adverse effects as fructose. At its fifty-fifth meeting (Annex 1, reference *149*), the Committee noted that the absorption of D-tagatose by humans is not expected to exceed 20% of the administered dose. However, the rate of gluconeogenesis from D-tagatose is slower than that from fructose. Thus the Committee could not discount the possibility that, in individuals with hereditary fructose intolerance, tissue concentrations of D-tagatose could be elevated or prolonged compared with those of fructose, leading to adverse reactions.

The Committee has previously noted that gastrointestinal effects (nausea, flatulence, diarrhoea) have been reported in some individuals after the consumption of 30 g of D-tagatose in a single dose.

Intake

The Committee at its fifty-seventh meeting estimated that the mean intake of D-tagatose would range from 3 to 9 g/day and the 95th percentile of consumption would be up to 18 g/day. These estimates,

based on data on food consumption from Australia, Member States of the European Union and the USA, were considered to be still valid.

Evaluation

At its sixty-first meeting, the Committee concluded that, pending provision of the results of histopathological examination from a 2-year study in rats, the human data provided the most relevant basis for assessing the acceptable intake of D-tagatose. The histopathological data had now been provided and demonstrated that there were no toxicologically significant findings in rats given D-tagatose at levels of up to 10% in the diet for 2 years (equal to approximately 4 and 5 g/kg bw per day for males and females, respectively). On the basis of the data reviewed by the Committee at its sixty-first meeting and at its present meeting, and taking into account the fact that D-tagatose has physiological and toxicological properties similar to those of other carbohydrates of low digestibility, the Committee removed the temporary ADI and allocated an ADI "not specified" for D-tagatose.

The fact that ingestion of 30 g or more of D-tagatose on a single occasion may cause gastrointestinal effects in humans should be taken into account when considering appropriate levels of use.

The ADI "not specified" does not apply to individuals with hereditary fructose intolerance arising from 1-phosphofructoaldolase (aldolase B) deficiency or fructose 1,6-diphosphatase deficiency.

An addendum to the toxicological monograph was prepared. The specifications for D-tagatose established at the sixty-first meeting were not considered by the present Committee.

3.1.8 ***Xylanases from* Bacillus subtilis *expressed in* Bacillus subtilis**

Explanation

Xylanases from *Bacillus subtilis* expressed in *B. subtilis* have not been evaluated previously by the Committee. Xylanase is an enzyme that catalyses the hydrolysis of xylans and arabinoxylans to mono- and oligosaccharides. The Committee received information on three xylanases, designated BS1, BS2, and BS3. These xylanases are derived from nonpathogenic and nontoxigenic genetically modified strains of *B. subtilis*. *B. subtilis* has been a source of enzymes used in food for many years. Xylanases BS1 and BS2 are identical to the native xylanase of *B. subtilis*. Xylanase BS3 differs from the native enzyme by two amino acids and is resistant to the xylanase inhibitor present in flour. Xylanases BS2 and BS3 are used as processing aids in baking applications to increase tolerance towards variations in

process parameters, improve the dough, and increase the volume of baked goods. Use levels range from 500 to 13 300 total xylanase units (TXU)/kg of flour for xylanase BS3, and from 3000 to 40 000 TXU/kg of flour for xylanase BS2. The xylanase BS2 preparation contains 0.3 mg of total organic solids per 1000 TXU, and the xylanase BS3 preparation contains 1.5 mg of total organic solids per 1000 TXU.

Genetic modification

Three production strains for xylanases BS1, BS2 and BS3 were developed by transformation of the *B. subtilis* host strain with an appropriate transformation vector. The host strain is derived from the well-characterized nonpathogenic and nontoxigenic *B. subtilis* wild-type strain 168. Three transformation vectors were constructed based on the commonly used plasmid pUB110. The vectors contain the xylanase gene derived from *B. subtilis* strain 168. Two vectors encode xylanases BS1 and BS2, both of which are identical to the native xylanase A from strain 168. The vector encoding xylanase BS1 also contains genes encoding proteins that inactivate the antibiotics kanamycin/neomycin and phleomycin. These proteins are intracellular and are not carried over into the xylanase preparation. The vector encoding xylanase BS2 was genetically modified to remove the genes conferring resistance to the antibiotics. The third transformation vector encodes xylanase BS3, which was genetically modified by two amino acid substitutions to be resistant to the xylanase inhibitor present in flour. This vector does not contain genes conferring resistance to the antibiotics. Each vector was introduced into the host strain to obtain the corresponding xylanase production strain. All the introduced DNA is well-characterized and would not result in the production of any toxic or undesirable substances. The production strain is stable with respect to the introduced DNA.

Product characterization

Each xylanase is produced by pure culture fermentation of the respective production strain. Xylanase is secreted into the fermentation medium from which it is subsequently recovered, concentrated, and formulated using substances suitable for use in food, such as starch and salt. Two xylanase preparations, one containing the native xylanase BS2 and the other containing the modified xylanase BS3, which is resistant to the xylanase inhibitor, have been marketed. These xylanases would be denatured at temperatures >50 °C and would not be enzymatically active in food as consumed. The xylanase preparation containing xylanase BS1 is not intended for commercialization. Therefore, two specification monographs were prepared for xylanase preparations containing xylanases BS2 and BS3. The

respective titles of the monographs are *Xylanase from* Bacillus subtilis *expressed in* Bacillus subtilis, and *Xylanase (resistant to xylanase inhibitor) from* Bacillus subtilis *containing a modified xylanase gene from* Bacillus subtilis. Both xylanase preparations conform to the *General specifications for enzyme preparations used in food processing* (Annex 1, reference *156*).

Toxicological data

Xylanases naturally present in food and xylanases used as enzymes in food processing have not been reported to cause allergic reactions. By analogy, it is not likely that the *B. subtilis* xylanases under evaluation will cause allergic reactions after ingestion of food containing the residues of these enzymes.

Toxicological studies were performed with test batches of the water-soluble liquid enzyme concentrates. These bacterial enzyme preparations were not acutely toxic when tested in rats, nor were they mutagenic in assays in bacteria in vitro or clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro. No significant treatment-related effects were seen in a 4-week study in rats treated by gavage with xylanase BS3 at doses up to and including 200 000 TXU/kg bw per day (equivalent to 304 mg of total organic solids/kg bw per day), the highest dose tested, or in a 13-week study in rats treated by gavage with xylanase BS1 at doses up to and including 80 000 TXU/kg bw per day (equivalent to 63 mg of total organic solids/kg bw per day), the highest dose tested. These highest doses were therefore considered to be the NOELs in these studies.

Intake

Conservative estimates of daily intakes resulting from the use of xylanase in baking applications were 0.2 mg of total organic solids (or 660 TXU)/kg bw per day for xylanase BS2, and 0.3 mg of total organic solids (or 219 TXU)/kg bw per day for xylanase BS3. When these intakes were compared with the NOEL of 200 000 TXU/kg bw per day (equivalent to 304 mg of total organic solids/kg bw per day), the highest dose tested in the 4-week study of oral toxicity, the margins of safety were >1000 for both enzyme preparations. When these intakes were compared with the NOEL of 80 000 TXU/kg bw per day (equivalent to 63 mg of total organic solids/kg bw per day), the highest dose tested in the 13-week study of oral toxicity, the margins of safety were >200 for both enzyme preparations.

Evaluation

The Committee allocated an ADI "not specified" for xylanase from this recombinant strain of *Bacillus subtilis*, used in the

applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared. New specifications were prepared for the native *B. subtilis* xylanase BS2 and for the modified xylanase BS3 that is resistant to the xylanase inhibitor. A Chemical and Technical Assessment was prepared that included both enzymes.

3.1.9 *Zeaxanthin*

Explanation

Zeaxanthin (3*R*,3′*R*-dihydroxy-β-carotene), a naturally occurring xanthophyll pigment, is an oxygenated carotenoid that has no provitamin A activity. It occurs together with the isomeric xanthophyll pigment, lutein, in many foods, particularly vegetables and fruits. It is intended to be used as a food colour and as a nutritional supplement in a wide range of applications at concentrations ranging from 0.5–70 mg/kg. An extract from *Tagetes erecta* L. containing primarily lutein with variable amounts of antheraxanthin and other xanthophylls was considered by the Committee at its thirty-first meeting (Annex 1, reference *77*). At that time, no toxicological data were available and no evaluation was made. For the present meeting, information was received for two different products: synthetic zeaxanthin and zeaxanthin-rich extract from *Tagetes erecta* L. However, the Committee has not received toxicological data supporting the safety evaluation of the extract. A number of toxicological studies have been carried out with respect to the safety of synthetic zeaxanthin for addition to food and these were evaluated at the present meeting.

Zeaxanthin (synthetic)

Chemical and technical considerations

Zeaxanthin (synthetic) is the synthetic all-*trans* isomer of zeaxanthin produced by the Wittig reaction from raw materials that are commonly used in the production of other carotenoids with application in foods. Minor quantities of *cis*-zeaxanthins and by-products 12-apozeaxanthinal, parasiloxanthin, diatoxanthin and triphenyl phosphine oxide, may be present in the final product. The content of *trans*-zeaxanthin is not less than 96%.

Toxicological data

In rats given zeaxanthin in the diet for 5 weeks, the highest tissue concentrations were present in the small intestine, spleen, liver and adipose tissue. Seven days after cessation of administration, the

concentrations in plasma and tissues had decreased by between 2- and 4-fold, indicating that the elimination half-life was about 4–5 days.

In humans, daily administration of zeaxanthin at a dose of 1 or 10 mg for 42 days showed that the time to steady-state plasma concentrations was about 30 days. This is consistent with an elimination half-life of about 5 days. The plasma concentrations indicated that uptake and availability were not proportional to dose.

The food matrix, including its fibre and lipid contents, and the concentrations of other carotenoids in the diet may influence the extent of absorption of carotenoid compounds. Studies have shown that zeaxanthin/lutein does not influence the absorption of β-carotene.

Zeaxanthin has oral LD_{50} values of >4000 mg/kg bw in rats and >8000 mg/kg bw in mice. Ninety-day studies of toxicity with zeaxanthin in rats given doses of up to 1000 mg/kg bw per day, and in dogs given doses of up to 442 mg/kg bw per day, produced no treatment-related effects even at the highest doses. In a 52-week study in monkeys designed primarily to investigate possible adverse effects on the eye, zeaxanthin was administered by gavage at 0.2 or 20 mg/kg bw per day. This study was performed because adverse ocular effects had been seen with canthaxanthin (Annex 1 references *78*, *89*, *117*). There were no treatment-related effects on a wide range of toxicological end-points. Furthermore, comprehensive ophthalmic examinations, including electroretinography, showed no evidence of treatment-related adverse changes.

No long-term studies of toxicity or carcinogenicity were available.

Zeaxanthin gave negative results in several studies of genotoxicity in vitro and in vivo. Although the Committee noted that the doses in these tests were low, it recognized that maximum feasible doses were used.

In a study of developmental toxicity with zeaxanthin in rats, there was no evidence for toxicity at doses of up to 1000 mg/kg bw per day, the highest dose tested.

In the pharmacokinetic study in humans described above, a variety of clinical chemistry measurements as well as any adverse events were recorded during the study. In the groups of five men and five women receiving zeaxanthin at a dose of 1 or 10 mg per day for 42 days, there were no reported treatment-related adverse effects. There has been a relatively large number of human studies that have examined correlations between macular degeneration and exposure to lutein/zeaxan-

thin via intake from traditional food or from dietary supplements, or via measurements of serum concentrations. Although these studies were designed to look for ocular effects, where clinical or biochemical parameters were also examined, no adverse effects of the xanthophylls were reported.

Intake

Dietary intake data from a number of studies in North America and the United Kingdom indicate that intake of zeaxanthin from natural sources is in the range of 1–2 mg/day (about 0.01–0.03 mg/kg bw per day). Simulations considering proposed use levels as a food ingredient resulted in an estimated mean and 90th percentile of lutein plus zeaxanthin intake as approximately 7 and approximately 13 mg/day, respectively. Formulations containing lutein and zeaxanthin are also available as dietary supplements, but there were no reliable estimates of intakes from these sources.

Evaluation

In several studies of toxicity, including developmental toxicity, no adverse effects were documented in animals, including monkeys, or humans. Taking into account data showing that zeaxanthin was not genotoxic, had no structural alert, that the isomeric xanthophyll lutein did not exhibit tumour promoting activity, and that zeaxanthin is a natural component of the body (the eye), the Committee concluded that there was no need for a study of carcinogenicity.

Zeaxanthin has some structural similarities to β-carotene, which has been reported to enhance the development of lung cancer when given in supplement form to heavy smokers. The available data indicated that zeaxanthin in food would not be expected to have this effect. The Committee was unable to assess whether zeaxanthin in the form of supplements would have the reported effect in heavy smokers.

In view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin, the Committee decided to include zeaxanthin in the ADI (0–2 mg/kg bw), for lutein, which had a stonger toxicological database, and to make this a group ADI for these two substances. This group ADI does not apply to other zeaxanthin preparations that do not comply with established specifications.

A toxicological monograph, a Chemical and Technical Assessment and specifications were prepared for synthetic zeaxanthin.

Zeaxanthin-rich extract from Tagetes erecta *L.*

Chemical and technical considerations

Zeaxanthin-rich extract from *Tagetes erecta* L. is obtained by hexane extraction of the red flowers and subsequent purification of the oleoresin by saponification and crystallization. The total content of carotenoids is not less than 30% and the content of zeaxanthin in the total carotenoids is not less than 65%. The remaining 70% consists mainly of uncharacterized fats, oils and waxes originating from the plant material.

New tentative specifications were prepared and information on the non-zeaxanthin components in total carotenoids and on the composition of the non-carotenoid components was requested.

In view of the absence of toxicological information on this material, a toxicological monograph was not prepared. A chemical and technical assessment for zeaxanthin-rich extract was included in a single chemical and technical assessment that also covered synthetic zeaxanthin.

3.2 Revision of specifications

3.2.1 *Aluminium powder, iron oxides and titanium dioxide*

At its fifty-ninth meeting (Annex 1, reference *160*), the Committee concluded that a reconsideration of the full specifications for these inorganic colours was required because of the high heavy metal limits in the existing specifications. Therefore, the Committee maintained the existing limits and decided to call for data on raw materials, manufacturing methods and analytical data on impurities. At the present meeting, the Committee revised the specifications after considering the available data.

Aluminium powder

Aluminium powder is produced by grinding aluminium. This may be carried out in the presence of edible vegetable oils and/or food-grade fatty acids. The functional use of aluminium powder is as a colour for surface applications only.

The existing limit of 20 mg/kg for lead was maintained.

Iron oxides

Iron oxides (yellow, red and black) are produced by heat-soaking ferrous sulfate, removing water and decomposing the product; this is followed by washing, filtration, drying and grinding.

The maximum limit for cadmium was reduced from 10 mg/kg to 1 mg/kg, and limits for barium, chromium, copper, nickel and zinc were

deleted from the specifications, while limits for arsenic, lead and mercury were retained.

Titanium dioxide

Titanium dioxide is manufactured by digesting ilmenite ($FeTiO_3$), or a mixture of ilmenite and titanium slag, with sulfuric acid. The resulting liquor, after dilution with water or dilute acid, is clarified to remove insoluble residues such as silica. Iron is removed by crystallization, followed by filtration. Alkaline hydrolysis produces a precipitate of titanium dioxide that is filtered, washed, calcined, and micronized. Titanium dioxide may be coated with small amounts of aluminium and/or silica to improve its technological properties.

A maximum limit of 1 mg/kg for cadmium was introduced. The limit for antimony was reduced to 2 mg/kg and the limit for zinc was deleted from the specifications, while the limits for arsenic, lead and mercury were retained. The limits for heavy metals are based on the metals that are soluble in 0.5 N hydrochloric acid and do not apply to metals that are not extractable under these conditions.

3.2.2 *Aluminium lakes of colouring matters — general specifications*

General specifications for aluminium lakes of colouring matters were prepared by the Committee at its twenty-eighth meeting (Annex 1, reference *66*). At its present meeting, the Committee made revisions to these specifications, following a suggestion by the Joint Secretariat to consider the limits for heavy metals and any additional relevant data related to the revision of the specifications for aluminium powder.

Aluminium lakes of colouring matters are prepared under aqueous conditions by reacting aluminium oxide with colouring matter complying with purity criteria set out in the appropriate specification monograph. Undried aluminium oxide is usually freshly prepared by reacting aluminium sulfate or aluminium chloride with sodium carbonate, sodium bicarbonate or aqueous ammonia. After lake formation, the product is filtered, washed with water and dried. Unreacted aluminium oxide may also be present in the final product.

The existing specifications for aluminium lakes were revised. The limit for lead was reduced from 10 to 5 mg/kg and the existing limit of 3 mg/kg for arsenic was maintained in the specifications. The title of the specifications monograph was changed to *Aluminium lakes of colouring matters — general specifications*.

3.2.3 *Hydroxypropyl cellulose*

The Committee considered the existing specifications for hydroxypropyl cellulose on the basis of information received following a request for data on the procedure for the analysis of residual propylene chlorohydrin. The specifications were revised to include a method for the determination of residual propylene chlorohydrin.

3.2.4 *Hydroxypropylmethyl cellulose*

The Committee considered the existing specifications for hydroxypropylmethyl cellulose on the basis of information received following a request for data on the procedure for the analysis of residual propylene chlorohydrin. The specifications were revised to include a method for the determination of residual propylene chlorohydrin.

3.2.5 *Magnesium sulfate*

Magnesium sulfate has not been previously evaluated by the Committee. It was added to the agenda at the request of the Codex Committee on Food Additives and Contaminants. At its present meeting, the Committee decided to postpone the safety evaluation of magnesium sulfate because of insufficient information on the intended uses. The preparation of specifications for magnesium sulfate was considered.

Magnesium sulfate occurs naturally in seawater, mineral springs and in minerals such as kieserite and epsomite. It can be recovered from these sources or be prepared by reacting sulfuric acid and magnesium oxide. The commercial product is produced with one or seven molecules of water of hydration or in a dried form containing the equivalent of 2–3 waters of hydration. An anhydrous form is also known to exist.

Magnesium sulfate is used as a nutrient. Although other food-related applications may exist, information on their functional uses and use levels was not received by the Committee. Furthermore, no information on the commercial use of anhydrous magnesium sulfate was available.

The Committee noted the existence of specifications for magnesium sulfate in other internationally recognized compendia and considered these, while preparing new tentative specifications.

Further information is required by the end of 2006 on other functional uses of magnesium sulfate, including their use levels, and on the commercial use of anhydrous magnesium sulfate.

3.2.6 *Polyvinyl alcohol*

Polyvinyl alcohol was placed on the agenda of the present meeting following an industry request for the revision of specifications. After considering the comments and information received, the Committee agreed to remove the formula weight range and include a provision for the measurement of viscosity. Other minor changes were made.

3.3 Revision of metals levels and arsenic specifications

At its fify-fifth meeting (Annex 1, reference *149*), the Committee began implementation of a systematic 5-year programme to replace the outdated test for heavy metals (as lead) in all existing food specifications with appropriate limits for individual metals of concern. At the present meeting, the remaining group of 84 food additives was reviewed (Table 2). All the specifications for food additives previously evaluated by the Committee have now been reviewed for heavy metals and arsenic. As this was a "clearing-up" exercise, the list of food additives considered covered a wide range of functional uses, ranging from acidity regulator to yeast food. Analytical data received was taken into account in setting revised specifications. In general, the procedures adopted at previous meetings were used in setting new limits.

Comments on the Committee"s new proposed limits were invited. If alternative values and supporting data were not received by the deadline for submission of data for the sixty-fifth meeting (30 November 2004), the proposed metal limits would be adopted and supersede the existing limits, replacing those published in FAO Food and Nutrition Paper 52 and its addenda 1 to 11.

4. Flavouring agents

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

Eight groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 3 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*). 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.

Table 2
Limits for heavy metals in 84 food additives

INS		Food additive	Limits, not more than (mg/kg)			
			As	Pb	Cd	Hg
523		Aluminium ammonium sulfate	—	3	—	—
510		Ammonium chloride	—	2	—	—
503	(ii)	Ammonium hydrogen carbonate	—	2	—	—
927	a	Azodicarbonamide	—	2	—	—
901		Bees wax	—	2	—	—
210		Benzoic acid	—	2	—	—
—		Benzyl alcohol	—	2	—	—
—		Butan-1,3-diol	—	2	—	—
—		Butan-1-ol	—	2	—	—
—		Butan-2-ol	—	2	—	—
—		Butyl *p*-hydroxybenzoate	—	2	—	—
263		Calcium acetate	—	2	—	—
213		Calcium benzoate	—	2	—	—
170		Calcium carbonate	3	3	—	—
509		Calcium chloride	—	2	—	—
952		Calcium cyclamate	—	1	—	—
341	(ii)	Calcium hydrogen phosphate	3	4	—	—
516		Calcium sulfate	—	2	—	—
902		Candelilla wax	—	2	—	—
1503		Castor oil	—	2	—	—
925		Chlorine	—	2	—	1
—		Citraxanthin	—	2	—	—
459		Cyclodextrin, β-	—	1	—	—
—		Cyclohexane	—	2	—	—
—		Dammar gum	—	2	—	—
—		Diethyl tartrate	—	2	—	—
—		Diethylene glycol monoethyl ether	—	2	—	—
242		Dimethyl dicarbonate	—	2	—	—
—		Diphenyl	—	2	—	—
—		Edible gelatin	1	1.5	0.5	0.15
—		Ferric ammonium citrate	—	2	—	—
422		Glycerol	—	2	—	—
—		Glycerol diacetate	—	2	—	—
—		Heptanes	—	2	—	—
239		Hexamethylene tetramine	—	2	—	—
—		Isoamyl acetate	—	2	—	—
—		Isobutanol	—	2	—	—
—		Isopropyl acetate	—	2	—	—
270		Lactic acid	—	2	—	—
—		Light petroleum	—	2	—	—
1105		Lysozyme hydrochloride	—	2	—	—

Table 2 *(continued)*

INS		Food additive	Limits, not more than (mg/kg)			
			As	Pb	Cd	Hg
504	(i)	Magnesium carbonate	—	2	—	—
511		Magnesium chloride	—	2	—	—
343	(ii)	Magnesium hydrogen phosphate	3	4	—	—
329		Magnesium lactate	—	2	—	—
—		Methanol	—	2	—	—
905		Mineral oil (high viscosity)	—	1	—	—
—		Monoglyceride citrate	—	2	—	—
234		Nisin	—	1	—	—
—		Norhydroguaiaretic acid	—	2	—	—
451	(ii)	Pentapotassium triphosphate	3	4	—	—
231		Phenyl phenol, *o*-	—	2	—	—
1202		Polyvinylpolypyrrolidone, insoluble	—	2	—	—
1201		Polyvinylpyrrolidone	—	2	—	—
261		Potassium acetate	—	2	—	—
212		Potassium benzoate	—	2	—	—
924	a	Potassium bromate	—	2	—	—
508		Potassium chloride	—	2	—	—
340	(i)	Potassium dihydrogen phosphate	3	4	—	—
917		Potassium iodate	—	2	—	—
252		Potassium nitrate	—	2	—	—
249		Potassium nitrite	—	2	—	—
337		Potassium sodium L(+) tartrate	—	2	—	—
515	(i)	Potassium sulfate	—	2	—	—
—		Propan-1-ol	—	2	—	—
1520		Propylene glycol	—	2	—	—
211		Sodium benzoate	—	2	—	—
466		Sodium carboxy methyl cellulose	—	2	—	—
952		Sodium cyclamate	—	1	—	—
262	(ii)	Sodium diacetate	—	2	—	—
251		Sodium nitrate	—	2	—	—
250		Sodium nitrite	—	2	—	—
232		Sodium *o*-phenyl phenol	—	2	—	—
—		Sodium percarbonate	—	2	—	—
—		Sodium thiocyanate	—	2	—	—
200		Sorbic acid	—	2	—	—
955		Sucralose	—	1	—	—
181		Tannic acid	—	2	—	—
—		Tartaric acid, DL-	—	2	—	—
—		Toluene	—	2	—	—
1518		Triacetin	—	2	—	—
—		Trichlorotrifluoroethane, 1,1,2-	—	2	—	—
927	b	Urea	—	2	—	—

- 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:

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.

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

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 *Pyridine, pyrrole and quinoline derivatives*

The Committee evaluated a group of 22 flavouring agents (Table 3) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). This group included:

— six pyrroles (Nos 1314, 1305–1307, 1310 and 1319);

— two indoles (Nos 1301 and 1304);

— 12 pyridine derivatives (Nos 1308, 1309, 1311–1313, 1315–1318 and 1320–1322); and

— a quinoline derivative and an isoquinoline derivative (Nos 1302 and 1303).

The Committee has not previously evaluated any member of the group.

Nineteen of the 22 substances (Nos 1301–1307, 1309, 1310, 1312–1320 and 1322) have been reported to occur naturally in foods. They have been detected in fresh and cooked vegetables, uncured meats, a variety of whole grains, green and black teas, coffee, alcoholic beverages, whiskeys, shellfish, and a wide variety of fresh fruits (Nijssen et al., 2003).

Estimated daily per capita intake

The total annual volume of production of the 22 flavouring agents in this group is approximately 1000 kg in Europe and 650 kg in the USA. More than 41% of the total annual volume of production in Europe and >79% in the USA is accounted for by a single substance in this

Figure 3
Procedure for the safety evaluation of flavouring agents

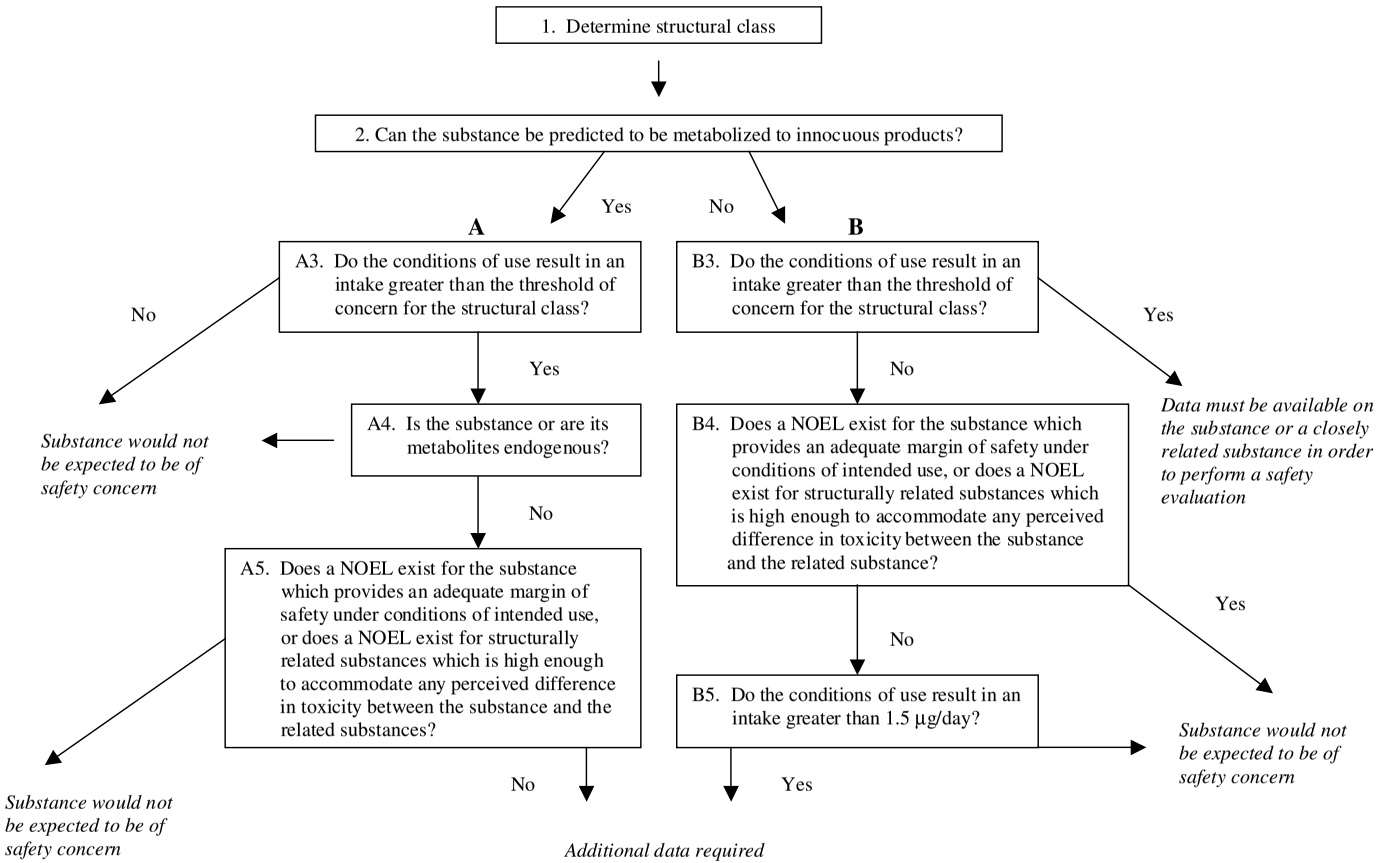


Table 3
Summary of the results of safety evaluations of pyridine, pyrrole and quinoline derivatives used as flavouring agents

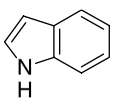
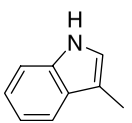
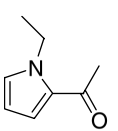
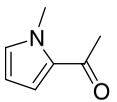
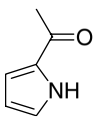
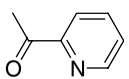
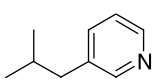
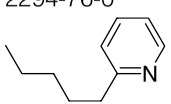
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous metabolites?	Step A3 Does intake exceed the threshold for human intake?[a]	Comments	Conclusion based on current intake
Structural class I						
Indole	1301	120-72-9 	Yes	No Europe: 30 USA: 10	See notes 2, 5	No safety concern
Skatole	1304	83-34-1 	Yes	No Europe: 3 USA: 0.07	See notes 2, 5	No safety concern
Pyrrole	1314	109-97-7 	Yes	No Europe: 0.1 USA: 0.01	See note 1	No safety concern
Structural class II						
1-Ethyl-2-acetylpyrrole	1305	39741-41-8 	Yes	No Europe: ND USA: 0.009	See notes 1, 4	No safety concern

Table 3 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous metabolites?	Step A3 Does intake exceed the threshold for human intake?[a]	Comments	Conclusion based on current intake
1-Methyl-2-acetylpyrrole	1306	932-16-1 	Yes	No Europe: 1 USA: 0.02	See notes 1, 4	No safety concern
Methyl 2-pyrrolyl ketone	1307	1072-83-9 	Yes	No Europe: 4 USA: 0.2	See note 1	No safety concern
2-Acetylpyridine	1309	1122-62-9 	Yes	No Europe: 59 USA: 68	See notes 3, 4	No safety concern
3-(2-Methylpropyl) pyridine	1312	14159-61-6 	Yes	No Europe: ND USA: 0.07	See note 3	No safety concern
2-Pentylpyridine	1313	2294-76-0 	Yes	No Europe: 0.07 USA: 0.07	See note 3	No safety concern
Pyrrole	1314	109-97-7 	Yes	No Europe: 0.1 USA: 0.01	See note 1	No safety concern

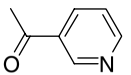
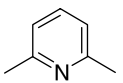
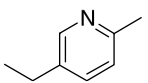
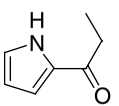
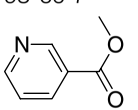
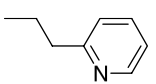
3-Ethylpyridine	1315	536-78-7	Yes	No Europe: 11 USA: 3	See note 3	No safety concern
3-Acetylpyridine	1316	350-03-8 	Yes	No Europe: 27 USA: 0.8	See notes 3, 4	No safety concern
2,6-Dimethylpyridine	1317	108-48-5 	Yes	No Europe: 0.3 USA: 0.007	See note 3	No safety concern
5-Ethyl-2-methylpyridine	1318	104-90-5 	Yes	No Europe: 0.1 USA: 0.04	See note 3	No safety concern
2-Propionylpyrrole	1319	1073-26-3 	Yes	No Europe: 0.01 USA: 2	See notes 1, 4	No safety concern
Methyl nicotinate	1320	93-60-7 	Yes	No Europe: 0.6 USA: 0.2	See note 6	No safety concern
2-Propylpyridine	1322	622-39-9 	Yes	No Europe: ND USA: 0.9	See note 3	No safety concern

Table 3 *(continued)*

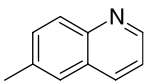
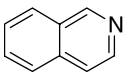
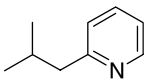
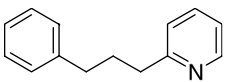
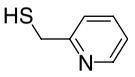
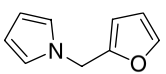
Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous metabolites?	Step A3 Does intake exceed the threshold for human intake?[a]	Comments	Conclusion based on current intake
Structural class III						
6-Methylquinoline	1302	91-62-3 	Yes	No Europe: 4 USA: 0.01	See notes 2, 5	No safety concern
Isoquinoline	1303	119-65-3 	Yes	No Europe: 0.01 USA: 0.07	See note 2	No safety concern
2-(2-Methylpropyl) pyridine	1311	6304-24-1 	Yes	No Europe: ND USA: 0.9	See note 3	No safety concern
2-(3-Phenylpropyl) pyridine	1321	2110-18-1 	Yes	No No Europe: 2 USA: 0.7	See note 3	No safety concern

Table 3 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step 2 Predicted to be metabolized to innocuous metabolites?	Step B3 Does intake exceed the threshold for human intake?[a]	Step B4 Adequate margin of safey for the flavouring agent or related chemical?	Comments	Conclusion based on current intake
Structural class III							
2-Pyridinemethanethiol	1308	2044-73-7 	No	No Europe: 0.001 USA: 0.007	Yes. The NOEL of 3.42 mg/kg bw per day in rats is >20 million times the estimated daily intake of 2-pyridinemethanethiol.	See note 3	No safety concern
N-Furfurylpyrrole	1310	143 8-94-4 	No	No Europe: 0.1 USA: 0.07	Yes. The NOEL of 12.2 mg/kg bw per day in rats is >1 million times the estimated daily intake of *N*-furfurylpyrrole.	See notes 1, 4	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] The thresholds for human intake for structural classes I, II, and III are 1800, 540 and 90μg/day, respectively. All intake values are expressed in μg per day. The combined intake of the flavouring agents in structural class I is 33μg/person per day in Europe and 11μg/person per day in the USA. The combined intake of the flavouring agents in structural class II is 103μg/person per day in Europe and 76μg/person per day in the USA. The combined intake of the flavouring agents in structural class III is 6μg/person per day in Europe and 1μg/person per day in the USA.

Notes to Table 3:

1 The pyrrole ring undergoes hydroxylation at the C-2 position and is excreted in the urine as the corresponding glucuronic acid conjugate.
2 The ring system undergoes hydroxylation at the C-3 position and is excreted in the urine as the corresponding glucuronic acid conjugate.
3 Alkyl side-chain oxidation followed by glucuronic acid conjugation and excretion or oxidation to nicotinic acid.
4 The acetyl group is reduced and conjugated with glucuronic acid.
5 Forms a reactive epoxide metabolite that is detoxified through glutathione conjugation.
6 Ester readily undergoes hydrolysis and resulting nicotinic acid is either used in numerous metabolic processes or excreted as the mercapturic acid conjugate

group, namely 2-acetylpyridine (No. 1309). The estimated daily per capita intakes of 2-acetylpyridine in Europe and the USA are 59 and 68 µg, respectively. The daily per capita intakes of all other flavouring agents in the group ranged from 0.001 to 30 µg, most values being at the lower end of this range. The estimated daily per capita intake of each agent is reported in Table 3.

Absorption, distribution, metabolism, and elimination

Pyridine, pyrrole and quinoline derivatives are expected to be rapidly absorbed from the gastrointestinal tract, oxidized to polar metabolites, and eliminated primarily in the urine and, to a minor extent, in the faeces.

Alkyl-substituted pyrroles and indoles may undergo CYP-mediated side-chain oxidation to yield the corresponding alcohol, which may be excreted as the glucuronic acid or sulfate conjugate. To a lesser extent, the double bond of the indole ring may undergo epoxidation.

Alkyl-substituted pyridines and quinolines are principally subject to side-chain oxidation, primarily at the C-1 position. Minor pathways include ring hydroxylation and epoxidation for substituted quinolines. *N*-Oxide formation has also been reported.

Methyl nicotinate (No. 1320), the only ester in the group, is rapidly hydrolysed by carboxyesterase to yield nicotinic acid and methanol.

Application of the Procedure for the Safety Evaluation of Flavouring Substances

Step 1. In applying the Procedure, the Committee assigned three (Nos 1301, 1304 and 1314) of the 22 agents to structural class I. Thirteen agents (Nos 1305–1307, 1309, 1312, 1313, 1315–1320 and 1322) were assigned to structural class II and the remaining six (Nos. 1302, 1303, 1308, 1310, 1311, and 1321) were assigned to structural class III.

Step 2. Twenty flavouring agents in this group are predicted to be metabolized to innocuous products (Nos 1301–1307, 1309 and 1311–1322). The evaluation of these flavouring agents therefore proceeded via the A-side of the decision-tree. Two flavouring agents (Nos 1308 and 1310) cannot be predicted to be metabolized to innocuous products. The evaluation of these two flavouring agents therefore proceeded via the B-side of the decision-tree.

Step A3. The estimated daily per capita intakes of all three of the flavouring agents in structural class I (Nos 1301, 1304 and 1314), all thirteen of the flavouring agents in structural class II (Nos 1305–1307,

1309, 1312, 1313, 1315–1320 and 1322), and of the four flavouring agents in structural class III (Nos 1302, 1303, 1311 and 1321) are below the respective thresholds of concern (i.e. 1800 µg for class I, 540 µg for class II, and 90 µg for class III). According to the Procedure, the use of these 20 flavouring agents raises no safety concern at estimated current intakes.

Step B3. The estimated daily per capita intakes in Europe and the USA of the remaining two flavouring agents in this group (Nos 1308 and 1310), which cannot be predicted to be metabolized to innocuous products, are also below the threshold of concern for structural class III (i.e. 90 µg). Accordingly, the evaluation of both flavouring agents in the group proceeded to step B4.

Step B4. For *N*-furfurylpyrrole (No. 1310), the NOEL of 12 mg/kg bw per day from a 90-day feeding study in rats is >1 000 000 greater than the estimated current intake of this substance as a flavouring agent. For 2-pyridinemethanethiol (No. 1308), the NOEL of 3.4 mg/kg bw per day from a 90-day feeding study in rats is >20 000 000 times greater than the estimated current intake of this substance as a flavouring agent.

The intake considerations and other information used to evaluate the 22 flavouring agents in this group according to the Procedure are summarized in Table 3.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of <95%.

Consideration of combined intakes from use as flavouring agents

In the event that all three agents in structural class I were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class I (1800 µg/person per day). In the unlikely event that all 13 agents in structural class II were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class II (540 µg/person per day). In the unlikely event that all six agents in structural class III were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class III (90 µg/person per day). Overall evaluation of the data indicated that combined intake would not raise safety concerns at estimated current intakes.

Conclusions

The Committee concluded that none of the flavouring agents in this group of pyridine, pyrrole and quinoline derivatives would present safety concerns at estimated current intakes. Other available data on the toxicity and metabolism of these pyridine, pyrrole and quinoline derivatives 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 and alicyclic hydrocarbons*

The Committee evaluated a group of 20 aliphatic and alicyclic hydrocarbons (Table 4) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). One member of this group, *d*-limonene (No. 1326), was previously evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*) and was assigned an ADI of 0–1.5 mg/kg bw. The Committee at that meeting recommended, however, that intake of this substance as a food additive be restricted to 0.075 mg/kg bw per day, or 5% of the ADI. At its forty-first meeting (Annex 1, reference *107*), the Committee re-evaluated the ADI for *d*-limonene and recommended that it be withdrawn and replaced with an ADI "not specified".

Nineteen of the 20 flavouring agents (Nos 1323, 1324, 1326–1331, 1336–1343 and 1345–1347) have been reported to occur naturally in foods. They have been detected in, for example, coffee, alcoholic beverages, baked and fried potato, heated beans, tea, bread and cheese. The substance with the highest natural occurrence is *d*-limonene (No. 1326).

Estimated daily per capita intake

The total annual volume of production of the 20 flavouring agents in this group is approximately 380 000 kg in Europe and 140 000 kg in the USA. *d*-Limonene (No. 1326) accounts for approximately 73% of the total annual volume of production in Europe and 71% in the USA. The estimated daily per capita intakes of *d*-limonene in Europe and the USA are approximately 40 000 µg and 13 000 µg, respectively. Myrcene (No. 1327), α- and β-pinene (Nos 1329 and 1330, respectively), terpinolene (No. 1331), β-caryophyllene (No. 1324), α-phellandrene (No. 1328), and *p*-mentha-1,4-diene (No. 1340) account for most of the remaining (approximately 26–27%) total annual volume of production. The estimated daily per capita intakes of these flavouring agents are in the range of 92 to 8300 µg in Europe and 70 to 2400 µg in the USA. The reported annual volumes of production of the remainder of the flavouring agents in this group are extremely

Table 4

Summary of the results of safety evaluations of aliphatic and alicyclic hydrocarbons used as flavouring agents[a]

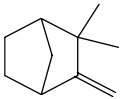
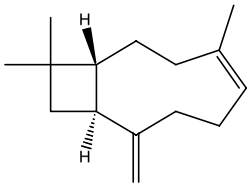
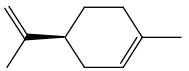
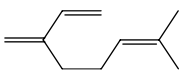
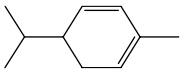
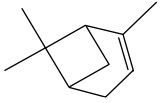
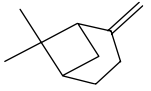
Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Structural class I							
Camphene	1323	79-92-5 	No Europe: 16 USA: 28	NR	NR	See note 1	No safety concern
β-Caryophyllene	1324	87-44-5 	No Europe: 389 USA: 508	NR	NR	See note 1	No safety concern
d-Limonene	1326	5989-27-5 	Yes Europe: 39 307 USA: 12 726	No	Yes. Given that there is an ADI "not specified" for *d*- limonene (see footnote c), the daily intakes of 660 μg/kg bw in Europe and 210 μg/kg bw in the USA were considered not to pose a safety concern.	See note 1	See footnote c

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	Conclusion based on current intake
Myrcene	1327	123-35-3 	Yes Europe: 8287 USA: 156	No	Yes. The LOEL/NOEL of 250 mg/kg bw per day for myrcene is approximately 1800 and 83 000 times the daily intakes of 140 µg/kg bw in Europe and 3 µg/kg bw in the USA, respectively.	See notes 2, 3	No safety concern
α-Phellandrene	1328	99-83-2/4221-98-1 	No Europe: 92 USA: 410	NR	NR	See note 1	No safety concern
α-Pinene	1329	80-56-8 	Yes Europe: 2152 USA: 2444	No	Yes. The daily intakes of 36 µg/kg bw in Europe and 41 µg/kg bw in the USA are approximately 5% and 20%, respectively, of those of the structural analogue *d*-limonene, for which an ADI "not specified" wasestablished (see footnote c).	See note 1	No safety concern
β-Pinene	1330	127-91-3 	No Europe: 1 550 USA: 759	NR	NR	See note 1	No safety concern

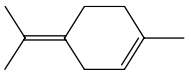
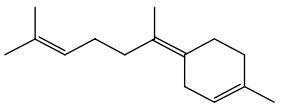
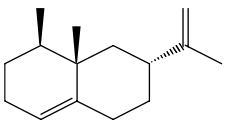
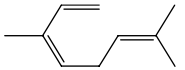
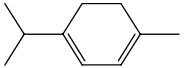
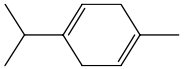
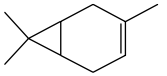
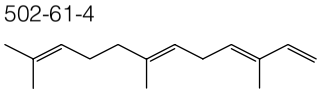
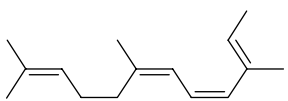
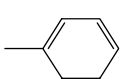
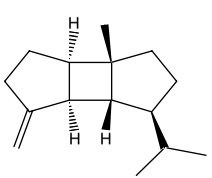
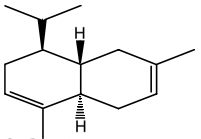
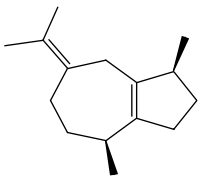
Terpinolene	1331	586-62-9 	No Europe: 772 USA: 70	NR	NR	See note 1	No safety concern
Bisabolene	1336	495-62-5 	No Europe: 15 USA: 10	NR	NR	See note 1	No safety concern
Valencene	1337	4630-07-3 	No Europe: 62 USA: 26	NR	NR	See note 1	No safety concern
3,7-Dimethyl-1,3,6-octatriene	1338	13877-91-3 	No Europe: 65 USA: 11	NR	NR	See notes 2, 3	No safety concern
p-Mentha-1,3-diene	1339	99-86-5 	No Europe: 32 USA: 93	NR	NR	See note 1	No safety concern
p-Mentha-1,4-diene	1340	99-85-4 	No Europe: 1372 USA: 321	NR	NR	See note 1	No safety concern
1,3,5-Undecatriene	1341	16356-11-9/19883-29-5 	No Europe: 0.2 USA: 0.2	NR	NR	See note 3	No safety concern
δ-3-Carene	1342	13466-78-9 	No Europe: ND USA: 40	NR	NR	See note 1	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	Conclusion based on current intake
Farnesene (α and β)	1343	502-61-4   farnesene	No Europe: ND USA: 40	NR	NR	See notes 2, 3	No safety concern
1-Methyl-1, 3-cyclohexadiene	1344		No Europe: ND USA: 313	NR	NR	See note 1	No safety concern
β-Bourbonene	1345	5208-59-3 	No Europe: ND USA: 0.2	NR	NR	See note 1	No safety concern

Cadinene (mixture of isomers)	1346	523-47-7  β-Cadinene, a principal isomer of Cadinene	No Europe: ND USA: 0.05	NR	NR	See note 1	No safety concern
Guaiene	1347	88-84-6 	No Europe: ND USA: 3	NR	NR	See note 1	No safety concern

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

[a] Step 2: All of the 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 per day. The combined intake of flavouring agents in structural class I is 54 111 μg/person per day in Europe and 17 959 μg/person per day in the USA.

[c] An ADI "not specified" was established for *d*-limonene by the Committee at its forty-first meeting (Annex 1, reference *107*), which was maintained at the present meeting.

Notes to Table 4

1. Allylic oxidation, epoxidation and hydrolysis to yield diols or by ring cleavage followed by conjugation with glucuronic acid and excretion in the urine.
2. Side-chain oxidation followed by subsequent conjugation with glycine, glucuronic acid, or glutathione.
3. Epoxidation to yield the corresponding diol that is conjugated with glucuronic acid and excreted in the urine.

low, accounting for <1 and 3% of the total annual volume of production in Europe and the USA, respectively. The estimated daily per capita intakes of these agents range from <0.1 to 93 µg in Europe and the USA, except for 1-methyl-1,3-cyclohexadiene (No. 1344) which has an estimated daily per capita intake of approximately 300 µg in the USA. The estimated daily per capita intake of each agent is reported in Table 4.

Absorption, distribution, metabolism and elimination

Being lipophilic, the aliphatic and alicyclic hydrocarbons in this group are likely to cross biological membranes by passive diffusion. After oral and inhalation exposure, they are rapidly absorbed and distributed to body tissues, elimination from blood being triphasic, with a slow terminal phase.

On the basis of the available data, it is anticipated that all the aliphatic and alicyclic hydrocarbons in this group will participate in similar pathways of metabolic detoxification in mammals, including humans. After absorption, these hydrocarbons are oxidized to polar oxygenated metabolites via cytochrome P450 enzymes and alcohol and aldehyde dehydrogenases. The aliphatic and alicyclic substances are oxidized either by side-chain oxidation or by epoxidation of an exocyclic or endocyclic double bond. Alkyl oxidation initially yields hydroxylated metabolites that may be excreted in conjugated form or undergo further oxidation, yielding more polar metabolites that are also excreted in conjugated form in the urine. If a double bond is present, epoxide metabolites may form and these metabolites are detoxified either by hydrolysis to yield diols, or by conjugation with glutathione.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned all the 20 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 17 of the 20 flavouring agents (Nos 1323, 1324, 1328, 1330, 1331 and 1336–1347) are below the threshold of concern (i.e. 1800 µg/person per day for class I). According to the Procedure, the use of these 17 flavouring agents raises no safety concern at estimated current intakes. The estimated daily per capita intakes of the remaining three agents in this group, *d*-limonene (No. 1326), myrcene (No. 1327) and α-pinene

(No. 1329), exceed the threshold of concern for class I. Accordingly, the evaluation of these three agents proceeded to step A4.

Step A4. *d*-Limonene, myrcene and α-pinene are not endogenous in humans. Therefore, the evaluation of these agents proceeded to step A5.

Step A5. For myrcene (No. 1327) a lowest-observed-effect level (LOEL) of 250 mg/kg bw per day was reported for male mice and male and female rats treated by gavage for 13 weeks, while the same dose was the NOEL in female mice. This dose is approximately 1800 times greater than the estimated intake of myrcene from its use as a flavouring agent in Europe (140 µg/kg bw per day) and 83 000 times greater than the estimated intake of myrcene in the USA (3 µg/kg bw per day). The Committee concluded that myrcene would not pose a safety concern at estimated current intake.

At its forty-first meeting, the Committee established an ADI "not specified" for *d*-limonene (No. 1326) on the basis of short- and long-term studies of toxicity in female rats and male and female mice, and studies of developmental toxicity in mice, rats and rabbits. In these studies, *d*-limonene was tested at doses ranging from 250–2800 mg/kg bw per day. Based on the ADI "not specified", the Committee concluded that *d*-limonene would not pose a safety concern at the estimated current intakes (660 µg/kg bw per day in Europe and 210 µg/kg bw per day in the USA).

No toxicological data on α-pinene (No. 1329) were available. *d*-Limonene shares structural characteristics with α-pinene in that both contain a methyl-substituted cyclohexene ring, which contains a second alkyl substituent. In *d*-limonene, this is an isopropenyl group, whereas in α-pinene the second substituent is a dimethyl-substituted methylene bridge. Based on these chemical structures, it would be predicted that the toxicity of α-pinene would be unlikely to exceed that of *d*-limonene. Both compounds are predicted to be metabolized to innocuous products. Metabolism of both compounds is by hydroxylation of the cyclohexene ring and oxidation of its methyl substituent. *d*-Limonene undergoes epoxidation of the endocyclic and allylic double bonds, leading to dihydroxy products. α-Pinene is converted to several metabolites, including *d*-limonene, by rat liver microsomes in vitro. The Committee concluded that *d*-limonene shared sufficient chemical and metabolic similarities with α-pinene to be used as a structural analogue for α-pinene at this step of the Procedure. The estimated current per capita intakes of α-pinene in Europe (36 µg/kg bw per day) and in the USA (41 µg/kg bw per day) are approximately 5% and 20%, respectively, of those of *d*-limonene, and are

almost four orders of magnitude lower than the lowest doses of *d*-limonene considered in the establishment of its ADI "not specified". On the basis of these considerations, the Committee concluded that α-pinene would not pose a safety concern at estimated current intakes.

The intake considerations and other information used to evaluate the 20 aliphatic and alicyclic hydrocarbons in this group according to the Procedure are summarized in Table 4.

Consideration of secondary components

Nine members (Nos 1323, 1324, 1327, 1337–1339 and 1341–1343) of this group of flavouring agents have assay values of <95%. The Committee evaluated the secondary components in No. 1339 (1,4- and 1,8-cineole) at a previous meeting and considered that they did not present a safety concern. The secondary components in Nos 1323, 1324, 1337 and 1343 ($C_{15}H_{24}$ terpene hydrocarbons) and in No. 1342 (β-pinene, *d*-limonene, myrcene and *p*-cymene) were all evaluated according to the Procedure by the Committee at its present meeting. The Committee did not consider any of these secondary components to present a safety concern. The secondary components in No. 1327 (dihydromyrcene), No. 1338 (*cis*-β-ocimene), No. 1341 (2,4,6-undecatriene), and the remaining secondary components in No. 1343 (other isomers of farnesene) are all structurally related to the primary flavouring agents and are expected to share the same metabolic fate. Therefore none of these secondary components was considered to present a safety concern.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 20 agents in this group were consumed concurrently on a daily basis, the estimated combined intake would exceed the human intake threshold of 1800 μg/person per day for class I. However, these 20 agents are all expected to be efficiently metabolized and would not saturate metabolic pathways. Overall evaluation of the data indicated that combined intake of these agents would not raise a safety concern.

Conclusions

The Committee maintained the previously established ADI "not specified" for *d*-limonene (Annex 1, reference *107*). The Committee concluded that use of the flavouring agents in this group of aliphatic and alicyclic hydrocarbons would not present a safety concern at estimated current intakes. The Committee also noted that the available data on the toxicity and metabolism of these flavouring agents 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 *Aromatic hydrocarbons*

The Committee evaluated a group of five aromatic hydrocarbons (Table 5) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). One member of this group, biphenyl (No. 1332), was previously evaluated by the Committee at its eighth meeting (Annex 1, reference *8*) and was assigned an ADI for its use as a fungistatic agent. The fungistatic use of biphenyl was also evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1966 and 1967 (*5*, *6*), when an ADI of 0–0.125 mg/kg bw was established.

Four (Nos 1325, 1332, 1333 and 1335) of the five flavouring agents in this group have been reported to occur naturally in foods. They have been detected in, for example, coffee, alcoholic beverages, baked and fried potato, heated beans, tea, bread and cheese. The substance with the highest natural occurrence is *p*-cymene (No. 1325).

Estimated daily per capita intake

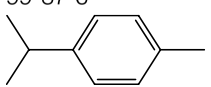
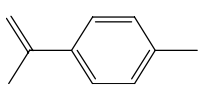
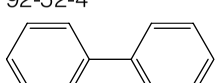
The total annual volume of production of the five flavouring agents in this group is approximately 7800 kg in Europe and 3600 kg in the USA. More than 98% of the total annual volume of production in Europe and the USA is accounted for by the monoaromatic terpene hydrocarbon *p*-cymene. The estimated daily per capita intakes of *p*-cymene in Europe and the USA are approximately 1100 µg and 470 µg, respectively. The reported annual volumes of production of the remainder of the flavouring agents in this group are low to very low. The estimated daily per capita intakes of these agents range from 0.001 to 21 µg in Europe and the USA. The estimated daily per capita intake of each agent is reported in Table 5.

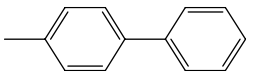
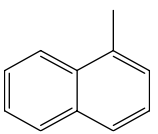
Absorption, distribution, metabolism and elimination

Being lipophilic, the aromatic hydrocarbons in this group are likely to cross biological membranes by passive diffusion. Available data on *p*-cymene and biphenyl indicate that these materials are readily absorbed from the gastro-intestinal tract, widely distributed in the body, metabolized and excreted mainly in the urine.

On the basis of the available data, it is anticipated that the aromatic hydrocarbons in this group will participate in similar pathways of metabolic detoxification in mammals, including humans. After absorption, these hydrocarbons are oxidized to polar oxygenated metabolites via cytochrome P450 enzymes and alcohol and aldehyde

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

Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
p-Cymene	1325	99-87-6 	No Europe: 1 085 USA: 472	See note 1	No safety concern
p-α-Dimethylstyrene	1333	1195-32-0 	No Europe: 21 USA: 0.3	See note 1	No safety concern
Structural class III					
Biphenyl	1332	92-52-4 	No Europe: 0.001 USA: 0.7	See note 2	No safety concern

4-Methylbiphenyl	1334	644-08-6 	No Europe: 0.01 USA: 0.08	See notes 1, 2	No safety concern
1-Methylnaphthalene	1335	90-12-0 	No Europe: 0.9 USA: 0.06	See notes 1, 2	No safety concern

CAS: Chemical Abstracts Service.

[a] Step 2: All the agents in this group are expected to be metabolized to innocuous products.

[b] The thresholds for human intake for structural classes I and III are 1800 μg/day and 90 μg/day, respectively. All intake values are expressed in μg per day. The combined intake of flavouring agents in structural class I is 1106 μg/person per day in Europe and 472 μg/person per day in the USA. The combined intake of flavouring agents in structural class III is 0.9 μg/person per day in Europe and 0.8 μg/person per day in the USA.

Notes to Table 5

1. Side-chain oxidation followed by subsequent conjugation with glycine, glucuronic acid, or glutathione.
2. Ring hydroxylation yielding phenolic derivatives that are subsequently metabolized to glucuronide and sulfate conjugates, which are excreted in the urine.

dehydrogenases. The major metabolic pathway of aromatic terpene hydrocarbons involves hepatic microsomal cytochrome P450-mediated oxidation of ring side-chains, yielding alcohols, aldehydes, and acids. The metabolites are then conjugated with glycine, glucuronic acid, or glutathione, and excreted in the urine and/or bile. The biotransformation of biphenyl proceeds via ring hydroxylation, preferentially at the C-4 position, yielding phenolic derivatives that are subsequently metabolized to glucuronide and sulfate conjugates, which are excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned two (Nos 1325 and 1333) of the five flavouring agents in this group to structural class I. The remaining three flavouring agents (Nos 1332, 1334 and 1335) 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 the two flavouring agents in structural class I and the three flavouring agents in structural class III are all below the thresholds of concern (i.e. 1800 µg for class I and 90 µg for class III). According to the Procedure, the use of these five flavouring agents raises no safety concern at estimated current intakes.

The intake considerations and other information used to evaluate the five aromatic hydrocarbons in this group according to the Procedure are summarized in Table 5.

Consideration of secondary components

All five flavouring agents in this group have minimum assay values of >95%. Hence, it is not necessary to consider secondary components.

Consideration of combined intakes from use as flavouring agents

In the event that the two agents in structural class I were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold of 1800 µg/person per day for class I. In the event that all three agents in structural class III were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold of 90 µg/person per day for class III. Overall evaluation of the data indicated that combined intake would not raise a safety concern.

Conclusions

The Committee concluded that none of the flavouring agents in this group of aromatic hydrocarbons would present safety concerns at estimated current intakes. The Committee noted that all 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, linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters*

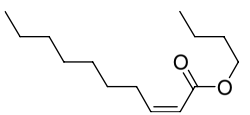
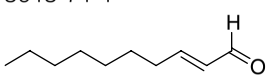
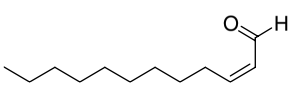
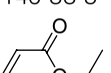
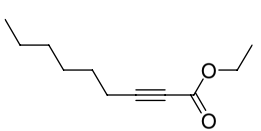
The Committee evaluated a group of 37 aliphatic, linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters flavouring agents (Table 6) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). The Committee has not previously evaluated any member of the group. The group included nine 2-alkenals (Nos 1349, 1350, 1353, 1359, 1360, 1362–1364 and 1366), six 2-alken-1-ols (Nos 1354, 1365, 1369, 1370, 1374 and 1384), five 2-alkenoic acids (Nos 1361, 1371–1373 and 1380), sixteen related alkenoic and alkynoic acid esters (Nos 1348, 1351, 1352, 1355–1358, 1367, 1368, 1375–1379, 1381, 1382), and one acetal (No. 1383).

Twenty-eight of the 37 flavouring agents (Nos 1349–1351, 1353–1355, 1359–1366, 1369–1378, 1380–1382 and 1384) in this group have been reported to occur naturally in foods and have been detected in beef, chicken, fish, fresh fruit, cheese, tea, coffee and beer.

Estimated daily per capita intake

The total annual volume of production of the 37 flavouring agents in this group is approximately 10000 kg in Europe and 7100 kg in the USA. Approximately 95% of the total annual volume of production in Europe and 81% in the USA is accounted for by 2-hexenal (No. 1353), the corresponding alcohol 2-hexen-1-ol (No. 1354), and the corresponding acetate ester *(E)*-2-hexen-yl acetate (No. 1355). Of these, 2-hexenal accounts for approximately 54% of the total annual volume of production in Europe and 44% in the USA. The estimated daily per capita intakes of 2-hexenal in Europe and the USA were 791 and 409 μg, respectively. The daily per capita intakes of all the other flavouring agents in the group were in the range of 0.01 to 395 μg, with most values being at the lower end of this range. The estimated daily per capita intake of each agent is reported in Table 6.

Table 6
Summary of the results of safety evaluations of aliphatic, linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters[a] used as flavouring agents

Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Butyl 2-decenoate	1348	7492-45-7 	No Europe: 0.01 USA: 0.3	See note 2	No safety concern
2-Decenal	1349	3913-71-1 	No Europe: 3 USA: 6	See note 4	No safety concern
2-Dodecenal	1350	4826-62-4 	No Europe: 16 USA: 2	See note 4	No safety concern
Ethyl acrylate	1351	140-88-5 	No Europe: 2 USA: 0.7	See note 2	No safety concern
Ethyl 2-nonynoate	1352	10031-92-2 	No Europe: ND USA: 0.9	See note 2	No safety concern

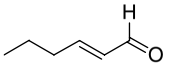
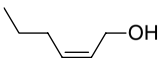
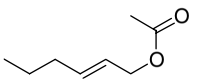
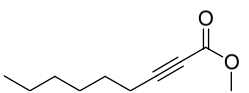
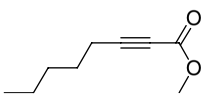
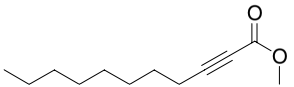
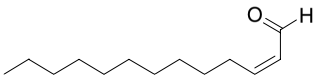
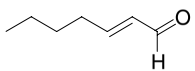
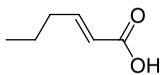
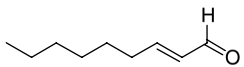
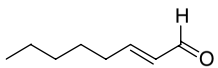
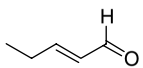
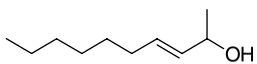
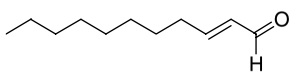
2-Hexenal	1353	6728-26-3 	No Europe: 791 USA: 409	See note 4	No safety concern
2-Hexen-1-ol	1354	2305-21-7 	No Europe: 395 USA: 291	See note 5	No safety concern
(E)-2-Hexen-yl acetate	1355	10094-40-3 	No Europe: 199 USA: 56	See note 2	No safety concern
Methyl 2-nonynoate	1356	111-80-8 	No Europe: 2 USA: 21	See note 2	No safety concern
Methyl 2-octynoate	1357	111-12-6 	No Europe: 21 USA: 38	See note 2	No safety concern
Methyl 2-undecynoate	1358	10522-18-6 	No Europe: ND USA: 0.04	See note 2	No safety concern
2-Tridecenal	1359	7774-82-5 	No Europe: 0.6 USA: 0.7	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?	Comments	Conclusion based on current intake
trans-2-Heptenal	1360	18829-55-5 	No Europe: 6 USA: 30	See note 4	No safety concern
trans-2-Hexenoic acid	1361	13419-69-7 	No Europe: 18 USA: 36	See note 1	No safety concern
2-Nonenal	1362	2463-53-8 	No Europe: 2 USA: 0.4	See note 4	No safety concern
2-Octenal	1363	2363-89-5 	No Europe: 4 USA: 0.9	See note 4	No safety concern
2-Pentenal	1364	764-39-6 	No Europe: 0.9 USA: 0.1	See note 4	No safety concern
trans-2-Nonen-1-ol	1365	31502-14-4 	No Europe: 0.1 USA: 0.03	See note 5	No safety concern
2-Undecenal	1366	2463-77-6 	No Europe: 0.4 USA: 0.4	See note 4	No safety concern

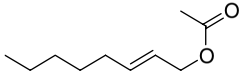
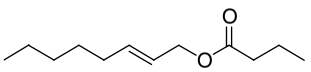
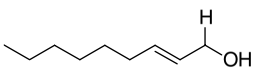
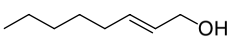
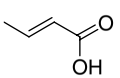
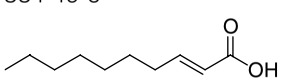
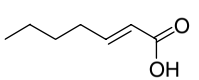
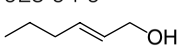
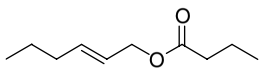
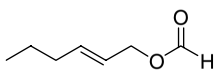
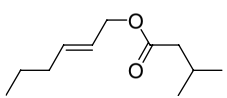
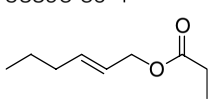
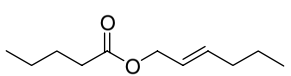
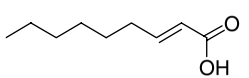
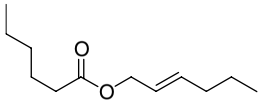
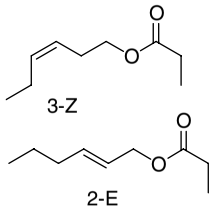
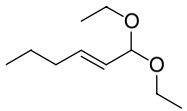
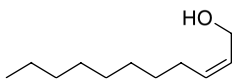
trans-2-Octen-1-yl acetate	1367	913-80-2 	No Europe: 0.3 USA: 0.7	See note 2	No safety concern
trans-2-Octen-1-yl butanoate	1368	84642-60-4 	No Europe: 0.3 USA: 0.7	See note 2	No safety concern
cis-2-Nonen-1-ol	1369	41453-56-9 	No Europe: 0.07 USA: 2	See note 5	No safety concern
(E)-2-Octen-1-ol	1370	18409-17-1 	No Europe: ND USA: 0.2	See note 5	No safety concern
(E)-2-Butenoic acid	1371	107-93-7 	No Europe: ND USA: 7	See note 1	No safety concern
(E)-2-Decenoic acid	1372	334-49-6 	No Europe: ND USA: 4	See note 1	No safety concern
(E)-2-Heptenoic acid	1373	10352-88-2 	No Europe: ND USA: 4	See note 1	No safety concern
(Z)-2-Hexen-1-ol	1374	928-94-9 	No Europe: ND USA: 10	See note 5	No safety concern

Table 6 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
trans-2-Hexenyl butyrate	1375	53398-83-7 	No Europe: ND USA: 4	See note 2	No safety concern
(E)-2-Hexenyl formate	1376	53398-78-0 	No Europe: ND USA: 7	See note 2	No safety concern
trans-2-Hexenyl isovalerate	1377	68698-59-9 	No Europe: ND USA: 4	See note 2	No safety concern
trans-2-Hexenyl propionate	1378	53398-80-4 	No Europe: ND USA: 4	See note 2	No safety concern
trans-2-Hexenyl pentanoate	1379	56922-74-8 	No Europe: ND USA: 4	See note 2	No safety concern
(E)-2-Nonenoic acid	1380	14812-03-4 	No Europe: ND USA: 3	See note 1	No safety concern

(E)-2-Hexenyl hexanoate	1381	53398-86-0 	No Europe: ND USA: 0.09	See note 2	No safety concern
(Z)-3 & *(E)*-2-Hexenyl propionate	1382	33467-74-2 53398-80-4 	No Europe: ND USA: 0.7	See note 2	No safety concern
(E)-2-Hexenal diethyl acetal	1383	67746-30-9 	No Europe: 0.3 USA: 0.09	See notes 3, 4, and 5	No safety concern
2-Undecen-1-ol	1384	37617-03-1 	No Europe: ND USA: 1.0	See note 5	No safety concern

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

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

[b] All 37 flavouring agents (Nos 1348–1384) 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.

[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 intake of the flavouring agents in structural class I is 1461 μg/person per day in Europe and 949 μg/person per day in the USA.

Notes to Table 6:

1. Undergoes β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle.
2. Hydrolysed to corresponding alcohols and acids, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.
3. Hydrolysed to corresponding aldehydes and alcohols.
4. 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.
5. Oxidized to aldehydes and acids, which metabolize completely in the fatty acid β-oxidation pathway.

Absorption, distribution, metabolism and elimination

In general, aliphatic esters formed from 2-alkenols and carboxylic acids are more rapidly hydrolysed than their saturated alcohol counterparts. Ester or acetal hydrolysis has been shown to occur in simulated stomach juice, simulated intestinal fluid, plasma, and liver microsomes. If hydrolysed before absorption, the resulting aliphatic alcohols and carboxylic acids are rapidly absorbed in the gastrointestinal tract. The unsaturated alcohols are successively oxidized to the corresponding aldehydes and carboxylic acids, which participate in fundamental biochemical pathways, including the fatty acid pathway and tricarboxylic acid cycle.

α,β-Unsaturated aldehydes are formed endogenously by lipid peroxidation of polyunsaturated fatty acids (PUFAs) or they can be ingested as naturally occurring constituents of food and, to a minor extent, as added flavouring agents. Under conditions of glutathione depletion and oxidative stress, high intracellular concentrations of α,β-unsaturated aldehydes have been shown to form adducts with proteins and DNA, resulting in cellular toxicity and DNA fragmentation during apoptosis. At low intakes, α,β-unsaturated aldehydes undergo metabolic detoxication by enzymes of the high-capacity β-oxidation pathway or, to a lesser extent, by glutathione conjugation.

It is anticipated that humans will biotransform small quantities of 2-alkenols and 2-alkenals 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 with glutathione, followed by excretion as the mercapturic acid derivative.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned all 37 of the flavouring agents in this group to structural class I.

Step 2. All 37 flavouring agents (Nos 1348–1384) 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.

Step A3. The estimated daily per capita intakes of all 37 flavouring agents in this group in Europe and the USA are below the threshold for concern for class I (i.e. 1800 μg). According to the Procedure, the safety of these 37 flavouring agents raises no concern when they are used at their estimated current intakes.

The intake considerations and other information used to evaluate the 37 aliphatic, linear, α,β-unsaturated aldehydes, acids and related

alcohols, acetals and esters in this group according to the Procedure are summarized in Table 6.

Consideration of secondary components

Nine members of this group of flavouring agents (Nos 1349, 1350, 1353, 1359, 1362, 1363, 1374, 1379 and 1381) have minimum assay values of <95%. Information on the safety of the secondary components of these nine compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). Some of the secondary components of Nos 1363 (ethyl octanoate), 1379 (propanoic acid), and 1381 (hexanoic acid) were evaluated by the Committee at its forty-seventh and forty-ninth meetings (Annex 1, references *125* and *131*). The secondary components of Nos 1349 (2-decenoic acid), 1353 (2-hexenoic acid), 1362 (2-nonenoic acid) and 1374 (*(E)*-2-hexen-1-ol), as well as one of the secondary components of Nos 1379 and 1381 (2-hexenol) were evaluated by the Committee at its present meeting, when it was concluded that these substances were of no safety concern at estimated current intakes. The secondary components of Nos 1350 (2-dodecenoic acid) and 1359 (2-tridecenoic acid), as well as one of the secondary components of No. 1363 (2-octenoic acid), have not been previously evaluated. However, they are structurally related to the primary flavouring agents and are expected to be substrates of the fatty acid cycle, metabolized, and excreted primarily as carbon dioxide and water. On this basis, the secondary components for Nos 1350, 1359, and 1363 were considered not to present a safety concern at estimated current intakes.

Consideration of combined intakes from use as flavouring agents

As many of the flavouring agents in this group are subject to conjugation with reduced glutathione, simultaneous consumption of the α,β-unsaturated aldehydes, at sufficiently high concentrations, could theoretically deplete glutathione, resulting in lipid peroxidation. However, under normal conditions and at the estimated current intakes resulting from use as flavouring agents, replenishable intracellular concentrations of glutathione (approximately 1–10 mmol/l) would be sufficient to detoxify the agents in this group. Additionally, since the α,β-unsaturated aldehydes provide similar flavouring characteristics, it is unlikely that all foods containing these flavouring agents will be consumed concurrently on a daily basis. On the basis of estimated current intakes of α,β-unsaturated aldehydes used as flavouring agents, and the constant replenishment of glutathione by biosynthesis, the Committee therefore concluded that the combined intake of these flavouring agents would not present a safety concern.

Conclusions

The Committee concluded that none of the flavouring agents in this group of aliphatic, linear, α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters would present safety concerns at estimated current intakes. The Committee noted that the available data on the toxicity and metabolism of these aliphatic, linear, α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters were consistent with the results of the safety evaluation conducted according to the Procedure.

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

4.1.5 *Monocyclic and bicyclic secondary alcohols, ketones and related esters*

The Committee evaluated a group of 32 monocyclic and bicyclic secondary alcohols, ketones and related esters (see Table 7) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). The Committee has not previously evaluated any of the members of this group.

Nineteen of the 32 flavouring agents (Nos 1385–1389, 1391, 1394–1400, 1403, 1404, 1407, 1412, 1414 and 1416) have been reported to occur naturally in foods. They have been detected in butter, beef, beer, parmesan and other cheeses, wine, fruit, herbs, spices, mints, and cocoa.

Estimated daily per capita intake

The total annual volume of production of the 32 monocyclic and bicyclic secondary alcohols, ketones and related esters in this group is approximately 11 000 kg in Europe and 14 000 kg in the USA. Approximately two-thirds of the total annual volume of production in Europe is accounted for by one agent in the group, isobornyl acetate (No. 1388), while borneol (No. 1385) and nootkatone (No. 1398) account for an additional 20% of the total volume. Approximately 80% of the total annual volume of production in the USA is accounted for by three agents, isobornyl acetate (No. 1388), *d*-camphor (No. 1395) and 3-*l*-menthoxypropane-1,2-diol (No. 1408). Daily per capita intakes in Europe and the USA were calculated to be 1039 and 236 μg for isobornyl acetate (No. 1388), 155 and 23 μg for borneol (No. 1385), 152 and 20 μg for nootkatone (No. 1398), and 58 and 396 μg for *d*-camphor (No. 1395), respectively. For 3-*l*-menthoxypropane-1,2-diol (No. 1408) and *d*,*l*-menthol-propylene glycol carbonate (No. 1413), the daily per capita intakes in the USA are calculated to be 789 and 140 μg, respectively. The daily per capita intakes of the other

Table 7

Summary of the results of safety evaluations of monocyclic and bicyclic secondary alcohols, ketones and related esters[a] used as flavouring agents

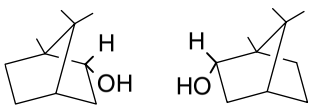
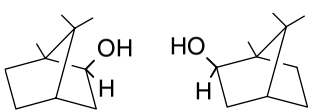
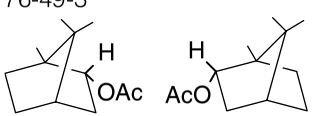
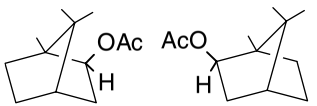
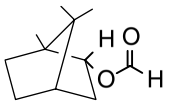
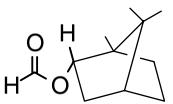
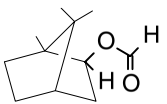
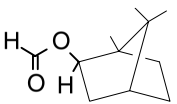
Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Borneol	1385	507-70-0  (–)-Borneol (+)-Borneol	No Europe: 155 USA: 23	See note 1	No safety concern
Isoborneol	1386	124-76-5  (–)-Isoborneol (+)-Isoborneol	No Europe: 24 USA: 0.07	See note 1	No safety concern
Bornyl acetate	1387	76-49-3  (–)-Bornyl acetate (+)-Bornyl acetate	No Europe: 21 USA: 3	See notes 1 and 2	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Isobornyl acetate	1388	125-12-2  (–)-Isobornyl acetate (+)-Isobornyl acetate	No Europe: 1039 USA: 236	See notes 1 and 2.	No safety concern
Bornyl formate	1389	7492-41-3  (–)-Bornyl formate  (+)-Bornyl formate	No Europe: 1 USA: 0.09	See notes 1 and 2	No safety concern
Isobornyl formate	1390	1200-67-5  (–)-Isobornyl formate  (+)-Isobornyl formate	No Europe: 0.7 USA: 0.4	See notes 1 and 2.	No safety concern

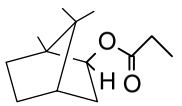
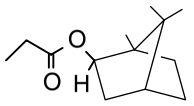
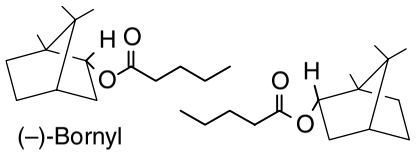
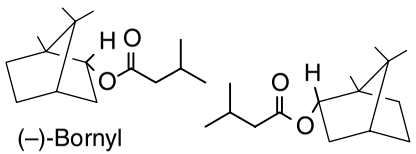
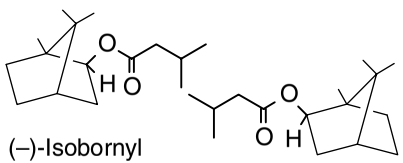
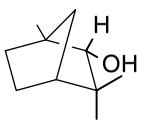
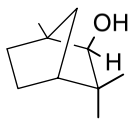
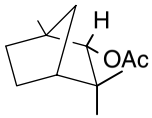
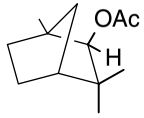
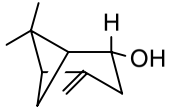
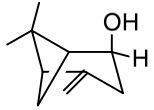
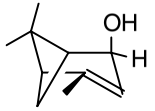
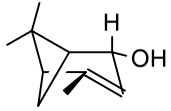
Isobornyl propionate	1391	2756-56-1  (–)-Isobornyl propionate  (+)-Isobornyl propionate	No Europe: 3 USA: 0.007	See notes 1 and 2	No safety concern
Bornyl valerate	1392	7549-41-9  (–)-Bornyl valerate (+)-Bornyl valerate	No Europe: ND USA: 5	See notes 1 and 2	No safety concern
Bornyl isovalerate (endo-)	1393	76-50-6  (–)-Bornyl isovalerate (+)-Bornyl isovalerate	No Europe: 0.1 USA: 0.5	See notes 1 and 2	No safety concern
Isobornyl isovalerate	1394	7779-73-9  (–)-Isobornyl isovalerate (+)-Isobornyl isovalerate	No Europe: 0.01 USA: 0.08	See notes 1 and 2	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Fenchyl alcohol	1397	1632-73-1  *alpha*-Fenchol  *beta*-Fenchol	No Europe: 64 USA: 17	See note 1	No safety concern
1,3,3-Trimethyl-2-norbornanyl acetate	1399	13851-11-1  *alpha-form*  *beta*-form	No Europe: 3 USA: 0.07	See notes 1 and 2	No safety concern
2(10)-Pinen-3-ol	1403	5947-36-4  *trans*-form  *cis*-form	No Europe: 0.01 USA: 0.01	See notes 1 and 3	No safety concern
Verbenol	1404	473-67-6  *cis*-form  *trans*-form	No Europe: 0.3 USA: 0.2	See notes 1 and 3	No safety concern

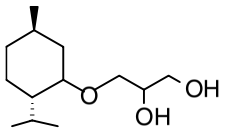
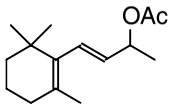
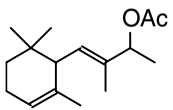
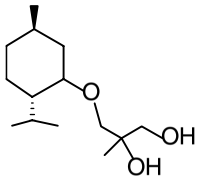
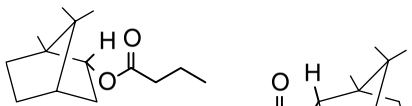
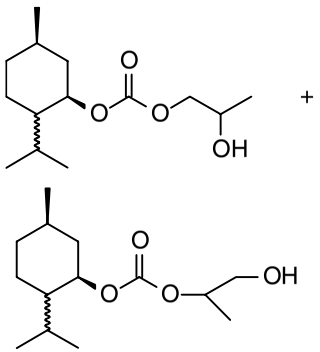
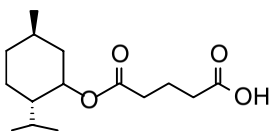
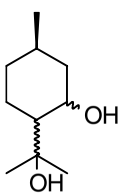
3-*l*-Menthoxypropane-1,2-diol	1408	87061-04-9 	No Europe: ND USA: 789	See notes 1 and 4	No safety concern
β-Ionyl acetate	1409	87061-04-9 	No Europe: ND USA: 9	See notes 1 and 2	No safety concern
α-Isomethylionyl acetate	1410	68555-61-3 	No Europe: ND USA: 9	See notes 1 and 2	No safety concern
3-(*l*-Menthoxy)-2-methyl propane-1,2-diol	1411	195863-84-4 	No Europe: ND USA: 88	See notes 1 and 4	No safety concern
Bornyl butyrate	1412	13109-70-1  (–)-Bornyl butyrate (+)-Bornyl butyrate	No Europe: ND USA: 9	See notes 1 and 2	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
d,l-Menthol(±)-propylene glycol carbonate	1413	156324-82-2 	No Europe: ND USA: 140	See notes 1 and 2	No safety concern
l-Monomenthyl glutarate	1414	220621-22-7 	No Europe: ND USA: 132	See notes 1 and 2	No safety concern
p-Menthane-3, 8-diol	1416	42822-86-6 	No Europe: ND USA: 18	See note 1	No safety concern

Structural class II

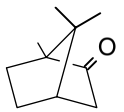
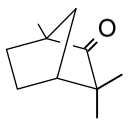
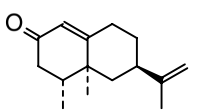
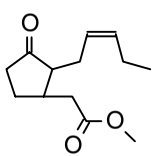
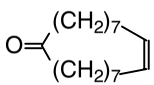
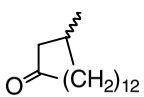
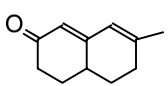
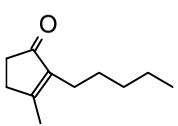
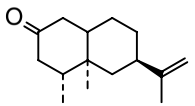
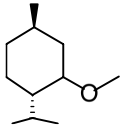
d-Camphor	1395	464-49-3  (*d*)-(+)-Camphor	No Europe: 58 USA: 396	See notes 1, 5, and 6	No safety concern
d-Fenchone	1396	4695-62-9  (*d*)-(+)-Fenchone	No Europe: 7 USA: 5	See notes 1, 5, and 6	No safety concern
Nootkatone	1398	4674-50-4 	No Europe: 152 USA: 20	See notes 1, 5, 6, and 7	No safety concern
Methyl jasmonate	1400	1211-29-6 	No Europe: 31 USA: 0.4	See notes 1 and 2	No safety concern

Table 7 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Cycloheptadeca-9-en-1-one	1401	542-46-1  *trans*-isomer	No Europe: 0.3 USA: 0.05	See notes 1, 5, and 6	No safety concern
3-Methyl-1-cyclopentadecanone	1402	541-91-3 	No Europe: 0.4 USA: 0.009	See notes 1, 5, and 6	No safety concern
7-Methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone	1405	34545-88-5 	No Europe: ND USA: 0.04	See notes 1, 5, and 6	No safety concern
3-Methyl-2-(*n*-pentanyl)-2-cyclopenten-1-one	1406	1128-08-1 	No Europe: 0.4 USA: 0.2	See notes 1 and 5	No safety concern

Dihydronootkatone	1407	20489-53-6 	No Europe: 0.7 USA: 0.9	See notes 1, 5, 6, and 7	No safety concern
Structural class III					
l-Menthyl methyl ether	1415	1565-76-0 	No Europe: ND USA: 53	See notes 1 and 8	No safety concern

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

[a] Step 2: All the agents in this group can be predicted to be metabolized to innocuous products.

[b] The threshold for human intake for structural classes I and II is 1800 and 540 μg per day, respectively. All intake values are expressed in μg per day. The combined intake of flavouring agents in structural class I is 1311 μg/per person per day in Europe and 1479 μg/person per day in the USA. The combined intake of flavouring agents in structural class II is 250 μg/person per day in Europe and 423 μg/person per day in the USA. The intake for the flavouring agent in structural class III is 53 μg/person per day in the USA.

Notes to Table 7:

1. Formation of glucuronic acid conjugates directly or after metabolism, which are subsequently excreted in the urine.
2. Ester hydrolysis to liberate the corresponding alcohol and carboxylic acid.
3. Ring cleavage to polar excretable metabolites.
4. Oxidation of the primary alcohol to the corresponding carboxylic acid.
5. Reduced to yield the corresponding alcohol.
6. Hydroxylation of alkyl ring-substituents and ring positions.
7. Oxidation and hydration of exocyclic and, to a lesser extent, endocyclic double bonds.
8. Oxidized by *O*-demethylation to yield corresponding alcohol.

flavouring agents in the group were in the range of 0 to 132 μg, with most of the values being at the lower end of this range. The estimated daily per capita intake of each agent in Europe and in the USA is reported in Table 7.

Absorption, distribution, metabolism and elimination

Studies in humans, dogs, and rabbits, have shown that the mono- and bicyclic secondary alcohols and ketones in this group are rapidly absorbed, distributed, metabolized, and excreted mainly in the urine. Small amounts may be eliminated in exhaled air. In humans, the esters within this group are expected to be hydrolysed to their component secondary alcohol and carboxylic acid.

The major metabolic pathway for the ketones involves reduction to the corresponding secondary alcohols, which are subsequently excreted, primarily as the glucuronic acid conjugates. Metabolites containing a double bond that are excreted into the bile may be reduced to the corresponding dihydro derivatives by the gut microflora. In addition to reductive pathways, alicyclic ketones and, to a lesser extent, secondary alcohols containing an alkyl side-chain, undergo oxidation of the side-chain to form polar poly-oxygenated metabolites that are excreted mainly in the urine, either unchanged or as the glucuronide or sulfate conjugates.

For more lipophilic ketones (e.g. nootkatone, No. 1398) or those with sterically hindered functional groups (e.g. *d*-camphor, No. 1395), oxidation of a ring position by CYP may compete with reduction of the ketone group or oxidation of the alcohol group. For example, bicyclic ketones tend to show greater lipophilicity and steric hindrance of the carbonyl function than do short-chain aliphatic or monocyclic ketones. As such, bicyclic ketones are expected to be poor substrates for cytosolic reducing enzymes. Consequently, the predominant detoxication route is CYP-mediated ring hydroxylation to yield polar, excretable poly-oxygenated metabolites.

The pathways by which fused ring and macrocyclic ketones are detoxified are similar to those for the bridged bicyclic substances. Activated ring positions (e.g. tertiary and allylic positions) and ring substituents are oxidized primarily by CYP, introducing additional polar groups into the molecule. The resulting metabolites are then excreted, mainly in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned 22 (Nos 1385–1394, 1397, 1399, 1403, 1404, 1408–1414, 1416) of the 32 agents to structural class I. Nine of these agents (Nos 1395, 1396, 1398,

1400–1402, 1405–1407) were assigned to structural class II, and the remaining agent (No. 1415) was assigned to structural class III.

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

Step 3. The estimated daily per capita intakes of all 22 of the flavouring agents in structural class I, all 9 of the agents in structural class II and the agent in structural class III are below the thresholds of concern (i.e. 1800 μg for class I, 540 μg for class II, and 90 μg for class III). According to the Procedure, the safety of these 32 flavouring agents raises no concern when they are used at estimated current intakes.

The intake considerations and other information used to evaluate the 32 monocyclic and bicyclic secondary alcohols, ketones and related esters in this group according to the Procedure are summarized in Table 7.

Consideration of secondary components

Six members (Nos 1386, 1398, 1407, 1409, 1413 and 1414) of this group of flavouring agents have minimum assay values of <95%. Information on the safety of the secondary components of these six compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). The secondary components of No. 1407 (acetic acid and β-ionol) were evaluated by the Committee at its forty-ninth meeting and fifty-first meetings (Annex 1, references *131* and *137*), respectively. The secondary components of No. 1413, *d*,*l*-menthol 2-propylene glycol carbonate, and of No. 1414, dimenthyl glutarate and glutaric acid, have not been previously evaluated. However, *d*,*l*-menthol 2-propylene glycol carbonate and dimenthyl glutarate are structurally related to the primary flavouring agents in this group and are expected to share the same metabolic fate. Glutaric acid is structurally related to valeric acid, which was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*). The secondary components of Nos 1386, 1398 and 1407 (borneol, dihydronootkatone and nootkatone) were evaluated as flavouring agents by the Committee at its current meeting. On the basis of these evaluations, the secondary components for these six flavouring agents were considered not to present a safety concern at current estimated intakes.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 22 agents in structural class I were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class I (1800 μg/person per day). In the unlikely event that all nine agents in structural class II were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class II (540 μg/person per day). Overall evaluation of the data indicated that combined intake of the agents in this group would not present a safety concern.

Conclusions

The Committee concluded that none of the flavouring agents in this group of monocyclic and bicyclic secondary alcohols, ketones and related esters would raise a safety concern at current estimated intakes. Available data on the toxicity and metabolism of these substances were consistent with the results of the safety evaluation.

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

4.1.6 *Amino acids and related substances*

The Committee evaluated a group of 20 flavouring agents comprising amino acids and related substances. The group included 16 α-amino acids (some L-form and some D,L-form) (Nos 1419–1424, 1426, 1428–1432, 1434, 1437–1439) and one α-imino acid (No. 1425, L-proline), which are normally found in protein, and two β-amino acids (β-alanine, No. 1418, and taurine, No. 1435) and the *S*-methyl sulfonium salt of methionine (D,L-(3-amino-3-carboxypropyl) dimethylsulfonium chloride, No. 1427), which are not normally found in protein (see Table 8). L-Glutamic acid (No. 1420) was previously evaluated by the Committee at its thirty-first meeting (Annex 1, reference *77*) and an ADI "not specified" was established for L-glutamic acid and its ammonium, calcium, magnesium, monosodium, and potassium salts.

The Committee was of the opinion that the use of the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*) was inappropriate for 12 members of this group, namely, the eleven L-form α-amino acids (L-cysteine, No. 1419; L-glutamic acid, No. 1420; glycine, No. 1421; L-leucine, No. 1423; L-phenylalanine, No. 1428; L-aspartic acid, No. 1429; L-glutamine No. 1430; L-histamine, No. 1431; L-tyrosine, No. 1434; L-arginine, No. 1438; L-lysine, No. 1439) and the one α-imino acid (L-proline, No. 1425). These substances are macronutrients and normal components of protein and, as such,

human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as flavouring agents.

The Committee also noted that amino acids may react with other food constituents upon heating. The mixtures thus formed are commonly referred to as "process flavours". The safety of process flavours has not been reviewed during this evaluation and may be considered at a future meeting. The present evaluation is therefore on the basis that these flavouring agents are present in an unchanged form at the point of consumption.

For the remaining eight members of the group, namely, the D,L-amino acids (D,L-isoleucine, No. 1422; D,L-methionine, No. 1424; D,L-valine, No. 1426; D,L-phenylalanine, No. 1432; D,L-alanine, No. 1437), the two β-amino acids (β-alanine, No. 1418, and taurine, No. 1435) and the *S*-methyl sulfonium salt of methionine (D,L-(3-amino-3-carboxypropyl)dimethylsulfonium chloride, No. 1427) (see Table 8), the evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents. Although the D-form of the α-amino acids and the other three compounds are not found in protein, they are natural components of food. For these eight members of the group, the evaluation has been conducted only in relation to their use as flavouring agents leading to the current estimated intakes.

Estimated daily per capita intake

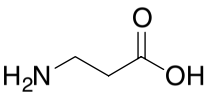
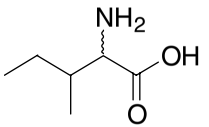
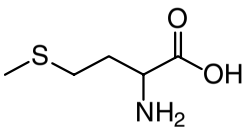
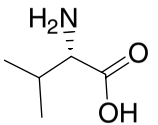
The total annual volume of production for use as flavouring agents only of the 20 substances in this group is approximately 11 200 kg in Europe and 21 100 kg in the USA. The annual volumes of production are equivalent to a total daily per capita intake of 1600 µg in Europe and 2800 µg in the USA.

Approximately 74% of the total annual volume of production in Europe is accounted for by four flavouring agents: L-cysteine (No. 1419), 40%; L-glutamic acid (No. 1420), 20%; glycine (No. 1421), 10%; and D,L-alanine (No. 1437), 8%. Approximately 83% of the total annual volume of production in the USA is accounted for by five flavouring agents in the group: L-cysteine (No. 1419) 11%; L-glutamic acid (No. 1420), 10%; L-aspartic acid (No. 1429), 45%; L-histidine (No. 1431), 9%); and taurine (No. 1435), 8%. The estimated daily per capita intake of each flavouring agent is reported in Table 8.

Absorption, distribution, metabolism and elimination

Amino acids are absorbed readily through the intestinal mucosa, distributed through the bloodstream and transported into cells by a variety of carrier systems. Those L-amino acids that are not needed for new protein synthesis and the D-isomers undergo catabolism,

Table 8
Summary of the results of safety evaluations of amino acids and related substances[a]

Flavouring agent	No.	CAS No. and structure	Step A3 Does intake exceed the threshold for human intake?[a,b]	Comments	Conclusion based on current intake
Structural class I					
β-Alanine	1418	107-95-9 	No Europe: ND USA: 13	See note 1	No safety concern
D,L-Isoleucine	1422	443-79-8 	No Europe: 6 USA: 22	See note 2	No safety concern
D,L-Methionine	1424	59-51-8 	No Europe: 97 USA: 35	See note 3	No safety concern
D,L-Valine	1426	516-06-3 	No Europe: 41 USA: 48	See note 4	No safety concern

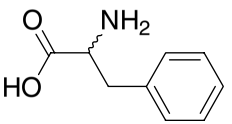
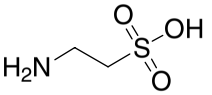
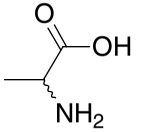
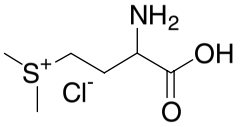
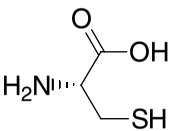
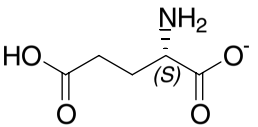
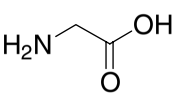
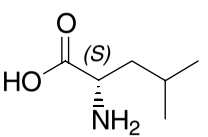
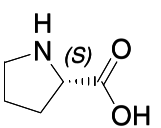
D,L-Phenylalanine	1432	150-30-1 	No Europe: 2 USA: 0.7	See note 5	No safety concern
Taurine	1435	107-35-7 	No Europe: ND USA: 217		No safety concern
D,L-Alanine	1437	302-72-7 	No Europe: 134 USA: 1	See note 6	No safety concern
Structural class III					
D,L-(3-Amino-3-carboxypropyl) dimethylsulfonium chloride	1427	1115-84-0 	No Europe: ND USA: 75	See note 7	No safety concern

Table 8 *(continued)*

Amino acids not evaluated by the Procedure

Flavouring agent	No.	CAS No. and structure	Daily per capita intake[b]	Conclusion based on current intake
L-Cysteine	1419	52-90-4 	Europe: 642 USA: 293	No safety concern
L-Glutamic acid	1420	56-86-0 	Europe: 313 USA: 273	No safety concern
Glycine	1421	56-40-6 	Europe: 158 USA: 5	No safety concern
L-Leucine	1423	61-90-5 	Europe: 14 USA: 24	No safety concern
L-Proline	1425	147-85-3 	Europe: 49 USA: 115	No safety concern

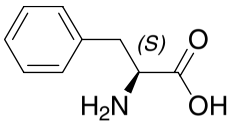
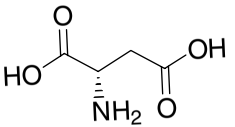
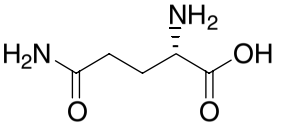
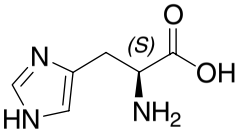
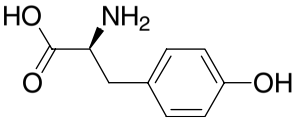
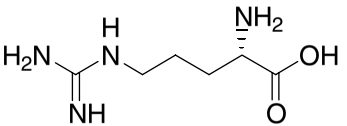
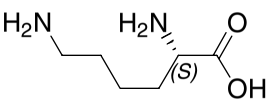
L-Phenylalanine	1428	63-91-2 	Europe: 20 USA: 28	No safety concern
L-Aspartic acid	1429	56-84-8 	Europe: 79 USA: 1240	No safety concern
L-Glutamine	1430	56-85-9 	Europe: 16 USA: 10	No safety concern
L-Histidine	1431	71-00-1 	Europe: 11 USA: 259	No safety concern
L-Tyrosine	1434	60-18-4 	Europe: 12 USA: 4	No safety concern

Table 8 *(continued)*

Flavouring agent	No.	CAS No. and structure	Daily per capita intake[b]	Conclusion based on current intake
L-Arginine	1438	74-79-3 	Europe: ND USA: 57	No safety concern
L-Lysine	1439	56-87-1 	Europe: ND USA: 57	No safety concern

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

[a] Step 2: All eight flavouring agents in this group evaluated using the Procedure are expected to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the decision-tree.

[b] The threshold for human intake is 1800 μg per day for structural class I and 90 μg per day for class III. All intake values are expressed in μg per day. The combined intake of the flavouring agents in structural class I is 1594 μg/person per day in Europe and 2701 μg/person per day in the USA. The intake of the flavouring agent in structural class III is 75 μg/person per day in the USA.

Notes for Table 8:

1 Deaminated to formylacetic acid and metabolized in the citric acid cycle.
2 Deaminated to form acetyl coenzyme A (CoA), propionyl CoA, succinyl CoA, and metabolized in citric acid cycle.
3 Deaminated to form homocysteine, propionyl CoA, succinyl CoA, and metabolized in citric acid cycle.
4 Deaminated to form propionyl CoA, succinyl CoA, and metabolized in citric acid cycle.
5 Converted to tyrosine, then deaminated to form acetoacetyl CoA and metabolized in the citric acid cycle.
6 Deaminated to pyruvate and acetyl CoA and metabolized in the citric acid cycle.
7 Demethylated to methionine or deaminated to homoserine by loss of diemethylsulphide.

primarily in the liver. There is no mechanism for storage of amino acids in humans. Amino acids undergo oxidative deamination, in which amino acids are deaminated to yield α-ketoacids that are either completely oxidized to carbon dioxide and water, or provide three or four carbon units that are converted via gluconeogenesis to yield glucose, or undergo ketogenesis to yield ketone bodies.

The *S*-methyl sulfonium salt of methionine (No. 1427) is demethylated to methionine or converted to homoserine by the loss of dimethylsulfide.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned seven of the eight flavouring agents (D,L-isoleucine, No. 1422; D,L-methionine, No. 1424; D,L-valine, No. 1426; D,L-phenylalanine, No. 1432; D,L-alanine, No. 1437) and the two β-amino acids (β-alanine, No. 1418, and taurine, No. 1435) to structural class I. The remaining flavouring agent (D,L-(3-amino-3-carboxypropyl)dimethylsulfonium chloride, No. 1427) was assigned to structural class III.

Step 2. The eight flavouring agents evaluated using the Procedure were all predicted to be metabolized to innocuous products. Their evaluation therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes of all the flavouring agents in structural class I and that of the one flavouring agent in structural class III are below the thresholds for daily human intake for their respective classes (1800 μg/person per day for class I, and 90 μg/person per day for class III). According to the Procedure, the use of these eight flavouring agents raises no safety concerns at estimated current intakes.

The intake considerations and other information used to evaluate the 20 amino acids and related substances are summarized in Table 8.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of <95%.

Consideration of combined intakes from use as flavouring agents

The eight flavouring agents evaluated using the Procedure are efficiently metabolized and eliminated, and the overall evaluation of the data indicates that combined intake would not raise any safety concerns at estimated current intakes.

Conclusion

In view of the fact that the L-form of the 11 α-amino acids and the one α-imino acid in this group are macronutrients and normal components of protein, the use of these substances as flavouring agents would not raise any safety concerns at estimated current intakes. The Committee also concluded that the use of the other eight substances in the group leading to the estimated current intakes would not raise any safety concerns.

The ADI "not specified" for L-glutamic acid and its ammonium, calcium, magnesium, monosodium and potassium salts was maintained.

A monograph summarizing the safety data on this group of flavouring agents was prepared and specifications were established.

4.1.7 *Tetrahydrofuran and furanone derivatives*

The Committee evaluated a group of 18 tetrahydrofuran and furanone flavouring agents (Table 9) by the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). The Committee has not previously evaluated any member of the group.

Twelve of the 18 flavouring agents in this group (Nos 1443, 1446, 1448–1457) have been reported to occur naturally in various foods. They have been detected in strawberries, pineapple, mango, other fruits, shoyu, cooked beef and pork, fried chicken, roasted hazelnuts and peanuts, cocoa, *maté*, black and green teas, smoked fish, popcorn, and Swiss cheese.

Estimated daily per capita intake

The total annual volume of production of the 18 tetrahydrofuran and furanone derivatives is approximately 40 000 kg in both Europe and in the USA. Approximately 92% of the total annual volume of production in Europe and approximately 98% in the USA is accounted for by 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446). The estimated daily per capita intake of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was 5300 µg in Europe and 5200 µg in the USA. The daily per capita intakes of all the other flavouring agents in this group were in the range of 0.001 to 238 µg, with most values being at the lower end of this range. The estimated daily per capita intake of each agent in Europe and the USA is reported in Table 9.

Absorption, distribution, metabolism and elimination

The four esters in this chemical group (Nos 1442, 1444, 1445 and 1447) are expected to be hydrolysed to tetrahydrofurfuryl alcohol and the

Table 9
Summary of the results of safety evaluations of tetrahydrofuran and furanone derivatives[a] used as flavouring agents

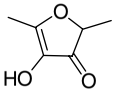
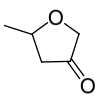
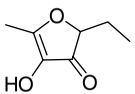
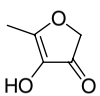
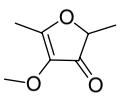
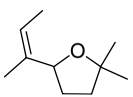
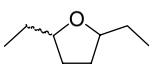
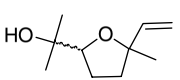
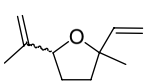
Flavouring agent	No.	CAS No. and structure	Step A3[b] Does intake exceed the threshold for human intake?	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate margin of safety for the flavouring agent or related substance?	Comments	Conclusion based on current intake
Structural class II							
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	1446	3658-77-3 	Yes Europe: 5254 USA: 5203	No	Yes. The NOEL of 200 mg/kg bw per day for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Kelly and Bolte, 2003) is >2300 times the estimated daily intake when used as flavouring agent.	See note 2	No safety concern
2-Methyltetrahydrofuran-3-one	1448	3188-00-9 	No Europe: 24 USA: 9	NR	NR	See note 1	No safety concern
2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone	1449	27538-09-6 	No Europe: 238 USA: 13	NR	NR	See note 2	No safety concern
4-Hydroxy-5-methyl-3(2*H*)-furanone	1450	19322-27-1 	No Europe: 56 USA: 0.07	NR	NR	See note 2	No safety concern

Table 9 *(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	Conclusion based on current intake
2,5-Dimethyl-4-methoxy-3(2*H*)-furanone	1451	4077-47-8 	No Europe: 14 USA: 0.7	NR	NR	See note 4	No safety concern
2,2-Dimethyl-5-(1-methylpropen-1-yl) tetrahydrofuran	1452	7416-35-5 	No Europe: 11 USA: 0.04	NR	NR	See note 5	No safety concern
2,5-Diethyltetrahydrofuran	1453	41239-48-9  *cis* and *trans*	No Europe: 0.01 USA: 0.09	NR	NR	See note 5	No safety concern
Linalool oxide	1454	1365-19-1  *cis* and *trans*	No Europe: 85 USA: 14	NR	NR	See note 2	No safety concern
5-Isopropenyl-2-methyl-2-vinyltetrahydrofuran	1455	13679-86-2  *cis* and *trans*	No Europe: 1 USA: 0.03	NR	NR	See note 5	No safety concern

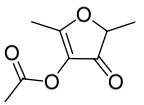
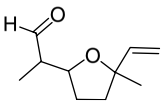
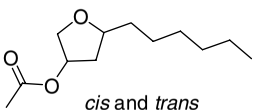
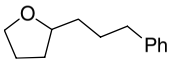
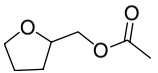
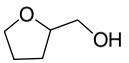
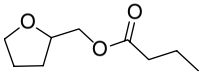
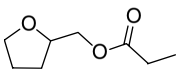
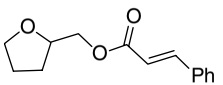
4-Acetoxy-2,5-dimethyl-3(2*H*)-furanone	1456	4166-20-5 	No Europe: ND USA: 8	NR	NR	See note 3	No safety concern
(±)-2-(5-Methyl-5-vinyltetrahydrofuran -2-yl) propionaldehyde	1457	51685-39-3 	No Europe: ND USA: 0.9	NR	NR	See note 5	No safety concern
Structural class III							
2-Hexyl-4-acetoxytetrahydrofuran	1440	10039-39-1  *cis* and *trans*	No Europe: ND USA: 0.7	NR	NR	See note 8	No safety concern
2-(3-Phenylpropyl) tetrahydrofuran	1441	3208-40-0 	No Europe: 0.001 USA: 0.7	NR	NR	See note 5	No safety concern
Tetrahydrofurfuryl acetate	1442	637-64-9 	No Europe: 0.7 USA: 8	NR	NR	See note 7	No safety concern
Tetrahydrofurfuryl alcohol	1443	97-99-4 	No Europe: 39 USA: 22	NR	NR	See note 6	No safety concern
Tetrahydrofurfuryl butyrate	1444	2217-33-6 	No Europe: 0.01 USA: 0.2	NR	NR	See note 7	No safety concern

Table 9 *(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	Conclusion based on current intake
Tetrahydrofurfuryl propionate	1445	637-65-0 	No Europe: 0.06 USA: 5	NR	NR	See note 7	No safety concern
Tetrahydrofurfuryl cinnamate	1447	65505-25-1 	No Europe: ND USA: 0.01	NR	NR	See note 7	No safety concern

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

[a] Step 2: All 18 tetrahydrofuran and furanone derivatives (Nos 1440–1457) 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.

[b] The thresholds for human intake for structural classes II and III are 540 and 90 μg per day, respectively. All intake values are expressed in μg per day. The combined intake of flavouring agents in structural class II is 5683 μg/person per day in Europe and 5249 μg/person per day in the USA. The combined intake of flavouring agents in structural class III is 40 μg/person per day in Europe and 37 μg/person per day in the USA.

Notes to Table 9:

1. Reduced to corresponding alcohol, which is conjugated with glucuronic acid and excreted in the urine.
2. Conjugated with glucuronic acid and excreted in the urine.
3. Ester group is readily hydrolysed and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
4. Ether group is readily oxidized and the resulting furanone is conjugated with glucuronic acid and excreted in the urine.
5. Subjected to side-chain or ring oxidation by human cytochrome P450 to yield ring or side-chain alcohols that may be conjugated with glucuronic acid or further oxidized, conjugated and excreted in the urine.
6. Alcohol is oxidized to the corresponding carboxylic acid, conjugated and excreted in the urine.
7. The ester group is readily hydrolysed and the resulting alcohol is oxidized to the corresponding carboxylic acid, conjugated and excreted in the urine.
8. Ester group is readily hydrolysed or it undergoes side-chain or ring oxidation by human cytochrome P450 to yield ring or side-chain alcohols that may be conjugated with glucuronic acid or further oxidized, conjugated and excreted in the urine.

corresponding carboxylic acids. Tetrahydrofuran derivatives are rapidly absorbed and eliminated primarily in the urine in laboratory animals.

Hydroxyl-substituted tetrahydrofuran and furanone derivatives are predicted to form glucuronic acid conjugates, which are primarily excreted in the urine. In humans fed with fresh strawberries, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) is rapidly absorbed, conjugated in the liver with glucuronic acid and excreted in the urine. The metabolism of the tetrahydrofurfuryl alcohol derivatives is anticipated to be similar to that of the furfuryl alcohol derivatives. These compounds will not form epoxides. After hydrolysis of the tetrahydrofurfuryl esters, the resulting primary alcohol is oxidized to the corresponding carboxylic acid, conjugated and excreted in the urine. The remaining tetrahydrofurfuryl alcohol, linalool oxide (No. 1454) is a tertiary alcohol that is conjugated with glucuronic acid and excreted in the urine.

The alkyl-substituted tetrahydrofuran derivatives are subjected to ring- or side-chain hydroxylation catalysed by human cytochrome P450 to yield ring- or side-chain-substituted alcohols that may be conjugated with glucuronic acid and excreted, or further oxidized, conjugated, and excreted in the urine.

Genotoxicity observed with some members of the group (Nos 1446, 1449 and 1450) was considered to be an effect caused by high dose and related to a mechanism involving reactive oxygen species, rather than the generation of a reactive metabolite, such as an epoxide. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (No 1446) showed no evidence of carcinogenicity in a 2-year study in which rats were given a dose of up to 400 mg/kg bw per day.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned 11 of the 18 agents (Nos 1446, 1448–1457) to structural class II. Seven agents (Nos 1440–1445 and 1447) were assigned to structural class III.

Step 2. All 18 tetrahydrofuran and furanone derivatives (Nos 1440–1457) in this group are expected to be metabolized to innocuous products. The evaluation of these 18 flavouring agents therefore proceeded via the A-side of the decision-tree.

Step A3. The estimated daily per capita intakes in Europe and the USA of 10 of the 11 flavouring agents in structural class II, and of all 7 of the flavouring agents in structural class III are below the threshold of concern (i.e. 540 µg for class II, and 90 µg for class III).

According to the Procedure, the safety of these 17 flavouring agents raises no concern when they are used at their estimated current intakes. One of the flavouring agents in structural class II, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446), exceeds the threshold of concern for that class. The daily per capita intake of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was 5300 µg in Europe and 5200 µg in the USA. According to the Procedure, the evaluation of this flavouring agent proceeded to step A4.

Step A4. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) or its metabolites are not endogenous. Therefore, the evaluation of this flavouring agent proceeded to step A5.

Step A5. For 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446), the NOEL of 200 mg/kg bw per day from a 2-year dietary study in rats is >2300 times the estimated daily per capita intake of this agent from its use as a flavouring agent in Europe or the USA. The Committee therefore concluded that the safety of this agent would not be a concern at the estimated current intake.

The intake considerations and other information used to evaluate the 18 tetrahydrofuran and furanone derivatives in this group according to the Procedure are summarized in Table 9.

Consideration of secondary components

Two members of this group of flavouring agents (Nos 1456 and 1457) have minimum assay values of <95%. Information on the safety of the secondary components of these two compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). The secondary component of No. 1456 (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, No. 1446) was evaluated at the present meeting and was considered not to be a concern at current estimated intakes. The secondary component of No. 1457 (6-hydroxy-2,6-dimethyl-2,7-octadienal) has not been previously evaluated by the Committee. The Committee did evaluate a structurally related compound (hydroxycitronellal, No. 611) at its fifty-third meeting (Annex 1, reference *143*) and concluded that it did not present a safety concern at estimated current intakes. On this basis, the Committee considered that 6-hydroxy-2,6-dimethyl-2,7-octadienal did not pose a safety concern at current estimated intakes.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that all seven agents in structural class III were consumed concurrently on a daily basis, the estimated combined intake would not exceed the intake threshold for class III (90 µg/person

per day). In the unlikely event that all 11 agents in structural class II were consumed concurrently on a daily basis, the estimated combined intake would exceed the human intake threshold for class II (540 µg/person per day). Nevertheless, all these flavouring agents are expected to be efficiently metabolized and would not saturate metabolic pathways. Overall evaluation of the data indicated that combined intake would not raise a safety concern.

Conclusions

The Committee concluded that none of the flavouring agents in this group of tetrahydrofuran and furanone derivatives would present safety concerns at estimated current intakes. The Committee noted that the available data on the toxicity and metabolism of these tetrahydrofuran and furanone derivatives were consistent with the results of the safety evaluation using the Procedure.

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

4.1.8 *Phenyl-substituted aliphatic alcohols and related aldehydes and esters*

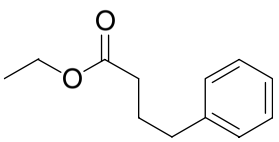
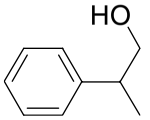
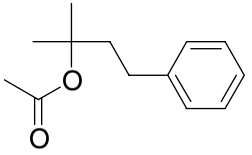
The Committee evaluated a group of 22 phenyl-substituted aliphatic alcohols and related aldehydes and esters (Table 10) using the Procedure for the Safety Evaluation of Flavouring Agents (Figure 3). The Committee has not previously evaluated any member of the group.

Seven of the 22 flavouring agents (Nos 1465, 1467, 1472–1474, 1478 and 1479) have been reported to occur naturally in various foods. They have been detected in roasted nuts, cooked potatoes, cheese, wine, fruit, vegetables, coffee, tea, and cocoa.

Estimated daily per capita intake

The total annual volume of production of the 22 phenyl-substituted aliphatic alcohols and related aldehydes and esters in this group is approximately 1300 kg in Europe and 3000 kg in the USA. Approximately 70% of the total annual volume of production in Europe is accounted for by 2-phenylpropionaldehyde (No. 1467), while approximately 87% of the total annual volume of production in the USA is accounted for by 2-methyl-3-(*p*-isopropylphenyl) propionaldehyde (No. 1465). The daily per capita intake of 2-phenylpropionaldehyde (No. 1467) was calculated to be 125 and 6 µg in Europe and the USA, respectively. The daily per capita intake of 2-methyl-3-(*p*-isopropylphenyl)propionaldehyde (No. 1465) was calculated to be 22 and 343 µg, in Europe and the USA, respectively. The daily per capita intake values for each agent are reported in Table 10.

Table 10
Summary of the results of safety evaluations of phenyl-substituted aliphatic alcohols and related aldehydes and esters[a] used as flavouring agents

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
Structural class I					
Ethyl 4-phenylbutyrate	1458	10031-93-3 	No Europe: ND USA: 0.01	See notes 4 and 1	No safety concern
β-Methylphenethyl alcohol	1459	1123-85-9 	No Europe: 0.1 USA: 0.01	See note 1	No safety concern
2-Methyl-4-phenyl-2-butylacetate	1460	103-07-1 	No Europe: 0.4 USA: 0.04	See notes 4 and 1	No safety concern

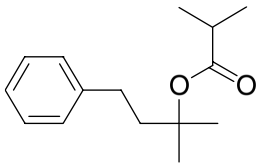
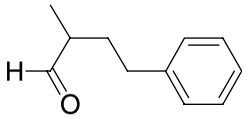
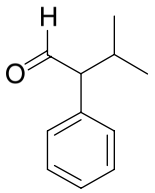
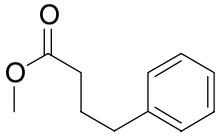
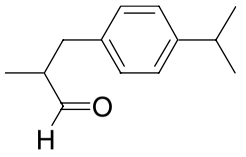
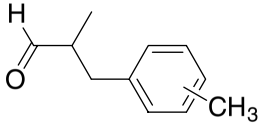
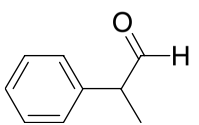
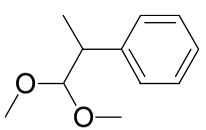
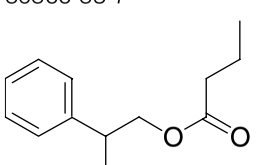
2-Methyl-4-phenyl-2-butylisobutyrate	1461	10031-71-7 	No Europe: 2 USA: 1	See notes 4 and 1	No safety concern
2-Methyl-4-phenylbutyraldehyde	1462	40654-82-8 	No Europe: 0.4 USA: 0.4	See note 2	No safety concern
3-Methyl-2-phenylbutyraldehyde	1463	2439-44-3 	No Europe: ND USA: 0.07	See note 2	No safety concern
Methyl 4-phenylbutyrate	1464	2046-17-5 	No Europe: ND USA: 0.01	See notes 4 and 1	No safety concern
2-Methyl-3-(*p*-isopropylphenyl) propionaldehyde	1465	103-95-7 	No Europe: 22 USA: 343	See note 2	No safety concern

Table 10 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Methyl-3-tolylpropionaldehyde (*o*, *m*, and *p*)	1466	41496-43-9 	No Europe: 0.6 USA: 27	See note 2	No safety concern
2-Phenylpropionaldehyde	1467	93-53-8 	No Europe: 125 USA: 6	See note 2	No safety concern
2-Phenylpropionaldehyde dimethyl acetal	1468	90-87-9 	No Europe: 5 USA: 3	See notes 3 and 2	No safety concern
2-Phenylpropyl butyrate	1469	80866-83-7 	No Europe: 0.004 USA: 0.5	See notes 4 and 1	No safety concern

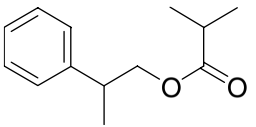
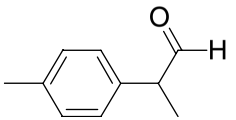
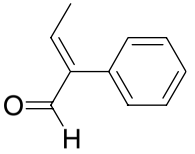
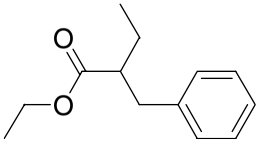
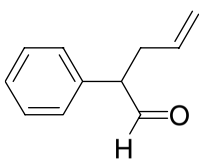
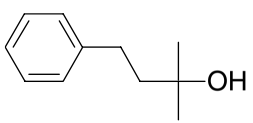
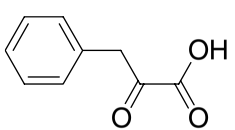
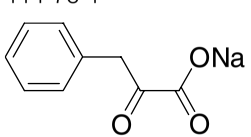
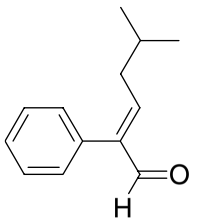
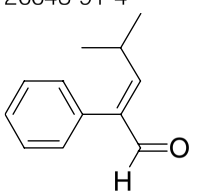
2-Phenylpropyl isobutyrate	1470	65813-53-8 	No Europe: 2 USA: 0.05	See notes 4 and 1	No safety concern
2-(*p*-Tolyl) propionaldehyde	1471	99-72-9 	No Europe: 0.04 USA: 0.01	See note 2	No safety concern
2-Phenyl-2-butenal	1474	4411-89-6 	No Europe: 2 USA: 0.07	See note 7	No safety concern
Ethyl 2-ethyl-3-phenylpropanoate	1475	2983-36-0 	No Europe: ND USA: 0.9	See notes 4 and 1	No safety concern

Table 10 *(continued)*

Flavouring agent	No.	CAS No. and structure	Step A3[a,b] Does intake exceed the threshold for human intake?	Comments	Conclusion based on current intake
2-Phenyl-4-pentenal	1476	24401-36-3 	No Europe: 0.03 USA: 0.04	See note 2	No safety concern
2-Methyl-4-phenyl-2-butanol	1477	103-05-9 	No Europe: 4 USA: 0.01	See note 1	No safety concern
2-Oxo-3-phenylpropionic acid	1478	156-06-9 	No Europe: ND USA: 0.09	See notes 5 and 6	No safety concern
Sodium 2-oxo-3-phenylpropionate	1479	114-76-1 			

Structural class II					
5-Methyl-2-phenyl-2-hexenal	1472	21834-92-4 	No Europe: 18 USA: 6	See note 7	No safety concern
4-Methyl-2-phenyl-2-pentenal	1473	26643-91-4 	No Europe: 0.4 USA: 5	See note 7	No safety concern

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

[a] Step 2: All the agents in this group can be predicted to be metabolized to innocuous products.

[b] The thresholds for human intake for structural classes I and II are 1800 and 540 μg per day, respectively. All intake values are expressed in μg per day. The combined intake of flavouring agents in structural class I is 164 μg/person per day in Europe and 382 μg/person per day in the USA. The combined intake of flavouring agents in structural class II is 18 μg/person per day in Europe and 11 μg/person per day in the USA.

Notes to Table 10:

1. Readily forms glucuronic acid conjugates, which are subsequently excreted in the urine.
2. Oxidized to the corresponding carboxylic acid and conjugated with glucuronic acid and is eliminated in the urine.
3. Rapidly hydrolysed to liberate the corresponding aldehyde and 2 equivalents of methanol.
4. Esters undergo rapid hydrolysis to liberate the corresponding alcohol and carboxylic acid.
5. Primarily decarboxylated to form phenylacetate which is excreted in the urine as such.
6. Readily undergoes transamination to form phenylalanine.
7. Readily forms glutathione conjugates and is rapidly eliminated in the urine.

Absorption, distribution, metabolism and elimination

The esters of phenyl-substituted flavouring agents (Nos 1458, 1460, 1461, 1464, 1469, 1470 and 1475) will be hydrolysed rapidly by carboxyesterases to the corresponding 2-phenyl substituted alcohol or acid. Before absorption these esters, as well as 2-phenylpropionaldehyde dimethyl acetal (No. 1468), are predicted to undergo hydrolysis in the gastrointestinal tract to yield compounds such as 2-phenylpropionaldehyde, β-methylphenethyl alcohol, 2-ethyl-3-phenylpropionic acid, 4-phenylbutyric acid, and 2-methyl-4-phenyl-2-butanol, which would be rapidly absorbed.

Once absorbed, the phenyl-substituted alcohols, aldehydes and acids may follow multiple metabolic pathways. The alcohols and aldehydes can be converted to phenyl-substituted carboxylic acids. These acids can be conjugated with glucuronic acid and excreted in the urine. They can also undergo β-oxidation to benzoic acid or phenylacetic acid derivatives, which are conjugated with glycine or glutamine before being excreted in the urine. Phenyl-substituted alcohols can also be conjugated directly with glucuronic acid before excretion.

2-Oxo-3-phenylpropionic acid (phenylpyruvate and its sodium salt, Nos 1478 and 1479) is a metabolite of phenylalanine. It is primarily decarboxylated to yield phenylacetate and is readily excreted in the urine.

α,β-Unsaturated 2-phenylaldehyde derivatives (Nos 1472–1474) are electrophilic in nature and are predicted to be detoxified by glutathione conjugation. The structurally related substance 2-phenylpropenal (atropaldehyde) readily forms glutathione conjugates when incubated with glutathione in vitro.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure, the Committee assigned 20 of the 22 flavouring agents in this group (Nos 1458–1471, 1474–1479) to structural class I. The other two flavouring agents (Nos 1472 and 1473) were assigned to structural class II.

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

Step A3. The estimated daily per capita intakes of the 20 flavouring agents in structural class I and the two flavouring agents in structural class II, are below the respective thresholds of concern (i.e. 1800 μg for class I and 540 μg for class II). According to the Procedure, the safety of these 22 flavouring agents raises no concern when they are used at estimated current intakes.

The intake considerations and other information used to evaluate the 22 phenyl-substituted aliphatic alcohols and related aldehydes and esters in this group according to the Procedure are summarized in Table 10.

Consideration of secondary components

One member of this group of flavouring agents, 2-methyl-3-(*p*-isopropylphenyl)propionaldehyde (No. 1465), 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 less than 95%). The secondary component, 2-methyl-3-(*p*-isopropylphenyl)propionic acid, is structurally related to the primary flavouring agent and is expected to share the same metabolic fate. On this basis, the Committee considered that 2-methyl-3-(*p*-isopropylphenyl)propionaldehyde does not present a safety concern at estimated current intakes.

Consideration of combined intakes from use as flavouring agents

In the event that all 20 agents in structural class I were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class I (1800 µg/person per day). In the event that the two agents in structural class II were consumed concurrently on a daily basis, the estimated combined intake would not exceed the human intake threshold for class II (540 µg/person per day). Overall evaluation of the data indicated that combined intake of the agents in this group would not present a safety concern.

Conclusions

The Committee concluded that none of the flavouring agents in this group of phenyl-substituted aliphatic alcohols and related aldehydes and esters would raise a safety concern at estimated current intakes. Available data on the toxicity and metabolism of these substances were consistent with the results of the safety evaluation.

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

5. A natural constituent: glycyrrhizinic acid

Explanation

The Committee was asked to comment on the safety of glycyrrhizinic acid and its monoammonium salt as a natural constituent of liquorice

(licorice) and in its use as a flavouring substance in various food products. In the call for data, the term "glycyrrhizic acid" was used rather than the alternative term "glycyrrhizinic acid". The Committee agreed to use the latter term. Glycyrrhizinic acid and its monoammonium salt have not been evaluated previously by the Committee.

Glycyrrhizinic acid is a naturally occurring triterpenoid saponin found in the extracts of roots and rhizomes from *Glycyrrhiza glabra*, the liquorice plant. Dried extracts of the roots of the liquorice plant, which may contain between 4% and 25% glycyrrhizinic acid, are present in liquorice confectionery, liquorice herbal teas and in some health products. Glycyrrhizinic acid and the monoammonium salt are both used as flavouring agents. It should be noted that in the literature, some authors have used the term "glycyrrhizin" interchangeably with "glycyrrhizinic acid"; however, this is not technically correct. Glycyrrhizin is the term historically used to describe the crude acid extract of the liquorice plant.

Toxicological data

The absorption, distribution, biotransformation and excretion of glycyrrhizinic acid and/or its monoammonium salt have been investigated in rats and humans. In both species, glycyrrhizinic acid, whether in the free form or as the monoammonium salt, is poorly absorbed from the gastrointestinal tract. In the gastrointestinal tract, glycyrrhizinic acid is hydrolysed, mainly by the activity of intestinal microflora, to 18β-glycyrrhetic acid (the aglycone of glycyrrhizinic acid), a substance that is readily absorbed. 18β-Glycyrrhetic acid is subject to enterohepatic circulation and can be further metabolized by intestinal bacteria to 3-dehydro-18β-glycyrrhetic acid and 3-epi-18β-glycyrrhetic acid. The time at which maximum plasma concentrations of glycyrrhetic acid are achieved after oral ingestion of glycyrrhizinic acid are reported to be in the range of 12–16 and 8–12 h in rats and humans, respectively. Doses in excess of 25 mg/kg bw may saturate the capacity of intestinal microflora to hydrolyse glycyrrhizinic acid to glycyrrhetic acid. In humans, absorption of glycyrrhetic acid from the gut is virtually complete, regardless of whether it is formed from the hydrolysis of glycyrrhizinic acid or is initially present as either the glycoside or the aglycone in a food matrix (e.g. liquorice). In humans, at a dose of 0.5 g the half-life was approximately 2 h, while at doses of 1.0 and 2.0 g, a second, slower phase of elimination occurred.

The results of studies in rats, and inferences that can be drawn from the results of studies in humans, indicate that both glycyrrhizinic acid

and its hydrolysis product glycyrrhetic acid are largely confined to the plasma. In plasma, glycyrrhizinic acid and glycyrrhetic acid are bound to serum albumin and are not taken up in body tissues to a significant extent.

Absorbed glycyrrhetic acid has been reported to produce effects that are similar to those of the adrenal steroid aldosterone. The mechanism of action of glycyrrhetic acid involves the inhibition of the type-2 11β-hydroxysteroid dehydrogenase, an enzyme that converts cortisol to cortisone. As a result, levels of cortisol, which has mineralocorticoid activity reportedly equivalent to that of aldosterone, increase. The high renal cortisol levels produce sodium retention and potassium excetion. This electrolyte imbalance has been referred to as "apparent mineralocorticoid excess" or "pseudohyperaldosteronism".

The oral LD_{50} values for glycyrrhizinic acid and various salts in mice, guinea-pigs and dogs were reported to be in the range of 308 to 12 700 mg/kg bw. The toxicity of glycyrrhizinic acid and/or its monoammonium salt has been evaluated in a number of short-term studies in rats and mice. At high doses, effects reported included those related to apparent mineralocorticoid excess or pseudohyperaldosteronism. Mild myolysis of the heart papillary muscles was reported in female Sprague-Dawley rats treated with glycyrrhizin (crude extract) at 30 mg/kg bw per day or 18α- or 18β-glycyrrhetic acid at 15 mg/kg bw per day for 30 days (note: glycyrrhizinic acid is not metabolized to 18α-glycyrrhetic acid).

In a study of carcinogenicity, $B6C3F_1$ mice were treated for 96 weeks with the disodium salt of glycyrrhizinic acid at a dose of up to 229 mg/kg bw per day in males and 407 mg/kg bw per day and observed for an additional 14 weeks. There was a dose-related reduction in the amount of water consumed by the treated animals when compared with the control animals (statistical significance not stated); however, no dose-related increase was reported in the incidence of tumours or in the specific distribution of benign and malignant neoplasms in treated mice compared with controls.

Oral administration of glycyrrhizin, like glycyrrhizinic acid, has been reported to inhibit the development of chemical-induced neoplasms in several models in mice and rats.

The available data indicated that glycyrrhizinic acid and its salts do not have carcinogenic activity.

Several glycyrrhizinic acid salts and liquorice extracts and/or various components of liquorice containing glycyrrhizinic acid have been

investigated in a number of tests for mutagenicity and/or genotoxicity. Overall, although some positive findings were reported, the available data indicated that glycyrrhizinic acid and its related salts are not genotoxic in vitro or in vivo.

Ammonium and disodium salts of glycyrrhizinic acid at doses of up to 1.5 g/kg bw per day have been evaluated in several studies of developmental toxicity in mice, rats, hamsters and rabbits. In one of these studies, embryotoxicity was observed, but overall the data indicated that glycyrrhizinic acid and its salts are not teratogenic.

There have been many case reports of effects related to excessive liquorice consumption (i.e. equivalent to an intake of glycyrrhizinic acid of >200 mg per day). These included serum sodium retention, serum potassium depletion, oedema, hypertension, and myopathy. The case reports also documented that consumption of liquorice-containing products that would result in exposures to glycyrrhizinic acid of <100 mg per day could be associated with the development of effects characteristic of pseudohyperaldosteronism, including increased blood pressure. The basis for susceptibility in such cases was not known, although several explanations are possible.

The available clinical studies have been reported to demonstrate mild clinical effects, consisting of hypokalemia, reduced plasma renin activity, and reduced urinary aldosterone concentrations.

In a randomized double-blind study, glycyrrhizinic acid at a dose of 0, 1, 2, or 4 mg/kg bw per day was administered to 39 healthy female volunteers for 8 weeks. No adverse effects were observed in the groups receiving a dose of 1 or 2 mg/kg bw per day. In the group receiving a dose of 4 mg/kg bw per day, decreases in plasma renin activity and serum aldosterone were found. There was an apparent increase in the concentration of atrial natriuretic peptide, which returned to normal after discontinuation of exposure, but there was no increase in blood pressure. However, mean blood pressure was greater at the highest dose than in the controls, owing to a reduction in the blood pressure of the latter over the course of the study.

A physiologically-based pharmacokinetic-pharmacodynamic model has been developed to characterize the probability of humans developing pseudohyperaldosteronism as a result of the consumption of glycyrrhizinic acid. On the basis of modelling, it was calculated that at a glycyrrhizinic acid intake of 100 mg/day (about 2 mg/kg bw per day), approximately 18% of the exposed population would have glycyrrhizinic acid concentrations of >800 µg/l. Also, it was predicted that disturbances of the ratio of cortisol to corticosterone would occur

in 26% of the exposed population, and that clinical manifestations of "pseudohyperaldosteronism" would appear in 0.04% of exposed persons (95% confidence interval (CI), 0.00046–3.0%).

Intake

Exposure to glycyrrhizinic acid through consumption of liquorice confectionery was assessed on the basis of a number of food surveys lasting 1–14 days. Assuming a mean content of 2000 mg of glycyrrhizinic acid per kg of liquorice confectionery, the exposures for consumers only in these surveys were calculated to be in the range of 5 to 50 mg per day at the mean and reached 100 to 300 mg per day at the 95th percentile.

On the basis of a mean content of 126 mg glycyrrhizinic acid per litre of herbal tea containing liquorice, high levels of exposure may be expected in regular consumers of these beverages.

Evaluation

The most significant effect of glycyrrhizinic acid, after hydrolysis in the gut to glycyrrhetic acid and subsequent absorption, is inhibition of the type-2 11β-hydroxysteroid dehydrogenase, with a consequent increase in cortisol concentrations, which leads to increased mineralocorticoid activity with sodium and water retention and symptoms of "apparent mineralocorticoid excess". This physiological action of glycyrrhizinic acid (glycyrrhetic acid) is reversible, but when sustained can lead to elevated blood pressure.

The Committee concluded that the safety evaluation of glycyrrizinic acid should be based on the human data. It was observed that there is a sensitive subset of the population who appear to show signs of pseudohyperaldosteronism at lower exposures than those which produce effects in the general population, but the available data did not allow the Committee to adequately characterize this subgroup, and hence the human data could not be used to assign an ADI. The available data suggest that an intake of 100 mg per day would be unlikely to cause adverse effects in the majority of adults. The Committee recognized that, in certain highly susceptible individuals, physiological effects could occur at exposure levels somewhat below this figure. The intake data indicate that consumers with a high intake of liquorice confectionery or herbal tea containing liquorice may be exposed to glycyrrhizinic acid with an intake of >100 mg/day.

A toxicological monograph was prepared.

6. Future work

- The Committee considered it advantageous to consolidate and minimize the number of methods used for the analysis of members of the carotenoid family, which are currently described in the numerous existing specifications for these substances. The methods should be published in FAO Food and Nutrition Paper, No. 5.
- The Committee recommended that the existing specifications for diphenyl (No. 1332) should be withdrawn, unless information on food additive uses (other than as a flavouring agent) is provided by the end of 2005.

7. Recommendations

1. The Committee noted that the term ADI "not specified" is used for food additives that are of low toxicity and that have defined technological purposes. The Committee considered that it is not appropriate to apply the same term for a material used, often at higher levels, as a food ingredient. The Committee recommended that procedure for the evaluation of food ingredients of low toxicity for which it is not appropriate to establish a numerical ADI be further clarified. The implication of this for substances that have an ADI "not specified" for use as food additives and may have additional uses as ingredients needs further consideration.
2. In view of the detailed data requirements identified in this report for the evaluation of flavour complexes derived from natural sources, the Committee recommended that the Secretariat should develop procedures in collaboration with industry to ensure that the necessary data are submitted in good time for thorough assessment by the Committee at future meetings.

Acknowledgements

The Committee was saddened to learn of the passing of Professor Kohei Kojima, who served on the Committee from its ninth meeting in 1965 until its forty-seventh meeting in 1996. Professor Kojima will be remembered for his dedicated service to the Committee and for the wise leadership and direction he provided during his many years as Chairman and Vice-Chairman. He was a mentor and inspiration to his colleagues and will be missed.

The Committee expressed its recognition to Dr. Manfred Luetzow, FAO Joint Secretary, at the end of his three year assignment in FAO. Dr Luetzow's dedication, expertise, communication skills and commitment to the work of the Committee

improved its working procedures, transparency and reporting system as well as the relationship of the Committee with stakeholders and Codex.

The Committee wishes to thank Dr Heidi Mattock, St Jean d"Ardières, France, for her assistance in the preparation of the report.

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Annex 1

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81. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41, 1988.
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87. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
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90. *Specifications for identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 49, 1990.

91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
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98. *Toxicological evaluation of certain veterinary residues in food.* WHO Food Additives Series, No. 29, 1991.
99. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/4, 1991.
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103. *Compendium of food additive specifications: addendum 1.* FAO Food and Nutrition Paper, No. 52, 1992.
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105. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and food.* FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 32, 1993.
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112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
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117. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 35, 1996.
118. *Compendium of food additive specifications: addendum 3.* FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
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124. *Compendium of food additive specifications, addendum 4.* FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
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127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
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131. *Evaluation of certain food additives and contaminants* (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 40, 1998.
133. *Compendium of food additive specifications: addendum 5.* FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
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142. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/12, 2000.
143. *Evaluation of certain food additives and contaminants* (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000
144. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 44, 2000.
145. *Compendium of food additive specifications, addendum 7.* FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. *Evaluation of certain veterinary drug residues in food* (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001
147. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 45, 2000.
148. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/13, 2000.
149. *Evaluation of certain food additives and contaminants* (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 901, 2001.
150. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 46, 2001.
151. *Compendium of food additive specifications: addendum 8.* FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
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153. *Safety evaluation of certain mycotoxins in food.* WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74, 2001.
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155. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 48, 2002.
156. *Compendium of food additive specifications: addendum 9.* FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. *Evaluation of certain veterinary drug residues in food* (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 49, 2002.
159. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/14, 2002.
160. *Evaluation of certain food additives and contaminants* (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 50, 2003.

162. *Compendium of food additive specifications: addendum 10.* FAO Food and Nutrition Paper No. 52, Add. 10, 2002.
163. *Evaluation of certain veterinary drug residues in food* (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 51, 2003.
165. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/15, 2003.
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168. *Compendium of food additive specifications: addendum 11.* FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
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170. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/16, 2004.
171. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 53, 2005 (in press).
172. *Compendium of food additive specifications: addendum 12.* FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.

Annex 2
Acceptable daily intakes, other toxicological information and information on specifications

Food additives and ingredients evaluated toxicologically

Food additive	Specifications[a]	Acceptable daily intake (ADI) in mg/kg bw and other toxicological recommendations
Benzoyl peroxide	R	Treatment of whey with benzoyl peroxide at a maximum concentration of 100 mg/kg does not pose a safety concern
a-Cyclodextrin	—	α-Cyclodextrin does not pose a safety concern at the proposed use levels and resulting predicted consumption as food ingredient and food additive The previously established ADI "not specified" for use as a carrier and stabilizer for flavours, colours, and sweeteners, as a water-solubilizer for fatty acids and certain vitamins, as a flavour modifier in soya milk, and as an absorbent in confectionery was maintained
Hexose oxidase from *Chondrus crispus* expressed in *Hansenula polymorpha*	N	Not specified[b]
Lutein from *Tagetes erecta* L.	N	0–2 (group ADI for lutein and zeaxanthin)[c]
Peroxyacid antimicrobial solutions containing 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP) *Containing HEDP and three or more of the following components: peroxacetic acid, acetic octanoic acid and acid, hydrogen peroxide, peroxyoctanoic acid*		The peroxy compounds in these solutions (hydrogen peroxide, peroxyacetic acid and peroxyoctanoic acid quantities of acid) would break down into acetic acid and octanoic acid, and small residual these acids on foods at the time of consumption would not pose a safety concern. HEDP does not pose a safety concern at the levels of residue that are expected to remain on foods at the time consumption.
Acetic acid	R	
1-Hydroxyethylidene-1,1-diphosphonic acid (HEDP)	N	

Food additive	Specifications[a]	Acceptable daily intake (ADI) in mg/kg bw and other toxicological recommendations
Hydrogen peroxide Octanoic acid (as food additive)	R N	
Steviol glycosides	N, T	0–2 (temporary)
D-Tagatose	—	Not specified[b]
Xylanase from Bacillus *subtilis expressed* in *Bacillus subtilis*	N	Not specified[b]
Xylanase (resistant to xylanase inhibitor) from *Bacillus subtilis* containing a modified xylanase gene from *Bacillus subtilis*	N	Not specified[b]
Zeaxanthin	N	0–2 (group ADI for lutein and zeaxanthin)[c]

[a] N: new specifications prepared; R: existing specifications revised; T: tentative specifications.
[b] ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired 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.
[c] This group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications.

Food additives considered for specifications only

Food additive	Specifications[a]
Aluminium lakes of colouring matters — general specifications	R
Aluminium powder	R
Hydroxypropyl cellulose	R
Hydroxypropylmethyl cellulose	R
Iron oxides	R
Magnesium sulfate[b]	N, T
Polyvinyl alcohol	R
Titanium dioxide	R
Zeaxanthin-rich extract from Tagetes erecta L.	N, T

[a] R, existing specifications revised; R: existing specifications revised; T: tentative specifications.
[b] Magnesium sulfate was not evaluated at the present meeting because the intended use and use levels were not identified.

Revision of heavy metals limits for food additives

Limits for heavy metals in 84 food additives were established. For a complete list please see Table 2 on page 52.

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

A. Pyridine, pyrrole and quinoline derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Indole	1301	N	No safety concern
6-Methylquinoline	1302	N	No safety concern
Isoquinoline	1303	N	No safety concern
Skatole	1304	N	No safety concern
1-Ethyl-2-acetylpyrrole	1305	N	No safety concern
1-Methyl-2-acetylpyrrole	1306	N	No safety concern
Methyl 2-pyrrolyl ketone	1307	N	No safety concern
2-Pyridinemethanethiol	1308	N	No safety concern
2-Acetylpyridine	1309	N	No safety concern
N-Furfurylpyrrole	1310	N	No safety concern
2-(2-Methylpropyl)pyridine	1311	N	No safety concern
3-(2-Methylpropyl)pyridine	1312	N	No safety concern
2-Pentylpyridine	1313	N	No safety concern
Pyrrole	1314	N	No safety concern
3-Ethylpyridine	1315	N	No safety concern
3-Acetylpyridine	1316	N	No safety concern
2,6-Dimethylpyridine	1317	N	No safety concern
5-Ethyl-2-methylpyridine	1318	N	No safety concern
2-Propionylpyrrole	1319	N	No safety concern
Methyl nicotinate	1320	N	No safety concern
2-(3-Phenylpropyl)pyridine	1321	N	No safety concern
2-Propylpyridine	1322	N	No safety concern

[a] N: new specifications prepared.

B. Aliphatic and alicyclic hydrocarbons

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
Camphene	1323	N	No safety concern
β-Caryophyllene	1324	N	No safety concern
d-Limonene	1326	N, T	ADI not specified[b]
Myrcene	1327	N	No safety concern
α-Phellandrene	1328	N	No safety concern
α-Pinene	1329	N	No safety concern
β-Pinene	1330	N	No safety concern
Terpinolene	1331	N	No safety concern
Bisabolene	1336	N	No safety concern
Valencene	1337	N	No safety concern
3,7-Dimethyl-1,3,6-octatriene	1338	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
p-Mentha-1,3-diene	1339	N	No safety concern
p-Mentha-1,4-diene	1340	N	No safety concern
1,3,5-Undecatriene	1341	N	No safety concern
d-3-Carene	1342	N	No safety concern
Farnesene (α and β)	1343	N	No safety concern
1-Methyl-1,3-cyclohexadiene	1344	N	No safety concern
b-Bourbonene	1345	N	No safety concern
Cadinene (mixture of isomers)	1346	N	No safety concern
Guaiene	1347	N	No safety concern

[a] N: New specifications prepared.
[b] An ADI "not specified" was established for d-limonene by the Committee at its forty-first meeting (Annex 1, reference 107), which was maintained at the present meeting.

C. Aromatic hydrocarbons

Flavouring agent	No.	Specifications[a]	Conclusion based on current intake
p-Cymene	1325	N	No safety concern
Biphenyl	1332	N	No safety concern
p,α-Dimethylstyrene	1333	N	No safety concern
4-Methylbiphenyl	1334	N	No safety concern
1-Methylnaphthalene	1335	N	No safety concern

[a] N: new specifications prepared.

D. Aliphatic, linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Butyl 2-decenoate	1348	N	No safety concern
2-Decenal	1349	N	No safety concern
2-Dodecenal	1350	N	No safety concern
Ethyl acrylate	1351	N	No safety concern
Ethyl 2-nonynoate	1352	N	No safety concern
2-Hexenal	1353	N	No safety concern
2-Hexen-1-ol	1354	N	No safety concern
2-(*E*)Hexen-1-yl acetate	1355	N	No safety concern
Methyl 2-nonynoate	1356	N	No safety concern
Methyl 2-octynoate	1357	N	No safety concern
Methyl 2-undecynoate	1358	N	No safety concern
2-Tridecenal	1359	N	No safety concern
trans-2-Heptenal	1360	N	No safety concern
trans-2-Hexenoic acid	1361	N	No safety concern
2-Nonenal	1362	N	No safety concern
2-Octenal	1363	N	No safety concern
2-Pentenal	1364	N	No safety concern
trans-2-Nonen-1-ol	1365	N	No safety concern
2-Undecenal	1366	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
trans-2-Octen-1-yl acetate	1367	N	No safety concern
trans-2-Octen-1-yl butanoate	1368	N	No safety concern
cis-2-Nonen-1-ol	1369	N	No safety concern
(*E*)-2-Octen-1-ol	1370	N	No safety concern
(*E*)-2-Butenoic acid	1371	N	No safety concern
(*E*)-2-Decenoic acid	1372	N	No safety concern
(*E*)-2-Heptenoic acid	1373	N	No safety concern
(*Z*)-2-Hexen-1-ol	1374	N	No safety concern
trans-2-Hexenyl butyrate	1375	N	No safety concern
(*E*)-2-Hexenyl formate	1376	N	No safety concern
trans-2-Hexenyl isovalerate	1377	N	No safety concern
trans-2-Hexenyl propionate	1378	N	No safety concern
trans-2-Hexenyl pentanoate	1379	N	No safety concern
(*E*)-2-Nonenoic acid	1380	N	No safety concern
(*E*)-2-Hexenyl hexanoate	1381	N	No safety concern
(*Z*)-3- & (E)-2-Hexenyl propionate	1382	N	No safety concern
(*E*)-2-Hexenal diethyl acetal	1383	N	No safety concern
2-Undecen-1-ol	1384	N	No safety concern

[a] N: new specifications prepared.

E. Monocyclic and bicyclic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Borneol	1385	N	No safety concern
Isoborneol	1386	N	No safety concern
Bornyl acetate	1387	N	No safety concern
Isobornyl acetate	1388	N	No safety concern
Bornyl formate	1389	N	No safety concern
Isobornyl formate	1390	N	No safety concern
Isobornyl propionate	1391	N	No safety concern
Bornyl valerate	1392	N	No safety concern
Bornyl isovalerate (endo-)	1393	N	No safety concern
Isobornyl isovalerate	1394	N	No safety concern
d-Camphor	1395	N	No safety concern
d-Fenchone	1396	N	No safety concern
Fenchyl alcohol	1397	N	No safety concern
Nootkatone	1398	N	No safety concern
1,3,3-Trimethyl-2-norbornanyl acetate	1399	N	No safety concern
Methyl jasmonate	1400	N	No safety concern
Cycloheptadeca-9-en-1-one	1401	N	No safety concern
3-Methyl-1-cyclopentadecanone	1402	N	No safety concern
2(10)-Pinen-3-ol	1403	N	No safety concern
Verbenol	1404	N	No safety concern
7-Methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone	1405	N	No safety concern
3-Methyl-2-(*n*-pentanyl)-2-cyclopenten-1-one	1406	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Dihydronootkatone	1407	N	No safety concern
3-l-Menthoxypropane-1,2-diol	1408	N	No safety concern
β-Ionyl acetate	1409	N	No safety concern
α-Isomethylionyl acetate	1410	N	No safety concern
3-(*l*-Menthoxy)-2-methylpropane-1,2-diol	1411	N	No safety concern
Bornyl butyrate	1412	N	No safety concern
DL-Menthol(+/–)-propylene glycol carbonate	1413	N	No safety concern
L-Monomenthyl glutarate	1414	N	No safety concern
L-Menthyl methyl ether	1415	N	No safety concern
p-Menthane-3,8-diol	1416	N	No safety concern

[a] N: new specifications prepared.

F. Amino acids and related substances

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
β-Alanine	1418	N	No safety concern
L-Cysteine	1419	N	No safety concern[b]
L-Glutamic acid	1420	N	No safety concern[b,c]
Glycine	1421	N	No safety concern[b]
DL-Isoleucine	1422	N	No safety concern
L-Leucine	1423	N	No safety concern[b]
DL-Methionine	1424	N	No safety concern
L-Proline	1425	N	No safety concern[b]
DL-Valine	1426	N	No safety concern
DL-(3-Amino-3-carboxypropyl) dimethylsufonium chloride	1427	N	No safety concern
L-Phenylalanine	1428	N	No safety concern[b]
L-Aspartic acid	1429	N	No safety concern[b]
L-Glutamine	1430	N	No safety concern[b,c]
L-Histidine	1431	N	No safety concern[b]
DL-Phenylalanine	1432	N	No safety concern
L-Tyrosine	1434	N	No safety concern[b]
Taurine	1435	N	No safety concern
DL-Alanine	1437	N	No safety concern
L-Arginine	1438	N	No safety concern[b]
L-Lysine	1439	N	No safety concern[b]

[a] N: new specifications prepared.

[b] Not evaluated using the Procedure for the Safety Evaluation of Flavouring Agents. The substance is a macronutrient and normal component of protein and, as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as flavouring agent.

[c] The group ADI "not specified" established at the thirty-first meeting for L-glutamic acid and its ammonium, calcium, magnesium, monosodium and potassium salts was maintained.

G. Tetrahydrofuran and furanone derivatives

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
2-Hexyl-4-acetoxytetrahydrofuran	1440	N	No safety concern
2-(3-Phenylpropyl)tetrahydrofuran	1441	N	No safety concern
Tetrahydrofurfuryl acetate	1442	N	No safety concern
Tetrahydrofurfuryl alcohol	1443	N	No safety concern
Tetrahydrofurfuryl butyrate	1444	N	No safety concern
Tetrahydrofurfuryl propionate	1445	N	No safety concern
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	1446	N	No safety concern
Tetrahydrofurfuryl cinnamate	1447	N	No safety concern
2-Methyltetrahydrofuran-3-one	1448	N	No safety concern
2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone	1449	N	No safety concern
4-Hydroxy-5-methyl-3(2*H*)-furanone	1450	N	No safety concern
2,5-Dimethyl-4-methoxy-3(2*H*)-furanone	1451	N	No safety concern
2,2-Dimethyl-5-(1-methylpropen-1-yl) tetrahydrofuran	1452	N	No safety concern
2,5-Diethyltetrahydrofuran	1453	N	No safety concern
cis,*trans*-2-Methyl-2-vinyl-5-(2-hydroxy-2- propyl)tetrahydrofuran (linalool oxide)	1454	N	No safety concern
5-Isopropenyl-2-methyl-2-*trans* mixture) vinyltetrahydrofuran(*cis* and	1455	N	No safety concern
4-Acetoxy-2,5-dimethyl-3(2*H*)furanone	1456	N	No safety concern
(+/–)-2-(5-Methyl-5-vinyl-tetrahydrofuran-2-yl)propionaldehyde	1457	N	No safety concern

[a] N: new specifications prepared.

H. Phenyl-substituted aliphatic alcohols and related aldehydes and esters

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Ethyl 4-phenylbutyrate	1458	N	No safety concern
β-Methylphenethyl alcohol	1459	N	No safety concern
2-Methyl-4-phenyl-2-butyl acetate	1460	N	No safety concern
2-Methyl-4-phenyl-2-butyl isobutyrate	1461	N	No safety concern
2-Methyl-4-phenylbutyraldehyde	1462	N	No safety concern
3-Methyl-2-phenylbutyraldehyde	1463	N	No safety concern
Methyl 4-Phenylbutyrate	1464	N	No safety concern
2-Methyl-3-(*p*-isopropylphenyl) propionaldehyde	1465	N	No safety concern
2-Methyl-3-tolylpropionaldehyde (mixed *o*-,*m*-, *p*-)	1466	N	No safety concern
2-Phenylpropionaldehyde	1467	N	No safety concern
2-Phenylpropionaldehyde dimethyl acetal	1468	N	No safety concern
2-Phenylpropyl butyrate	1469	N	No safety concern
2-Phenylpropyl isobutyrate	1470	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
2-(*p*-Tolyl)propionaldehyde	1471	N	No safety concern
5-Methyl-2-phenyl-2-hexenal	1472	N	No safety concern
4-Methyl-2-phenyl-2-pentenal	1473	N	No safety concern
2-Phenyl-2-butenal	1474	N	No safety concern
Ethyl 2-ethyl-3-phenylpropanoate	1475	N	No safety concern
2-Phenyl-4-pentenal	1476	N	No safety concern
2-Methyl-4-phenyl-2-butanol	1477	N	No safety concern
2-Oxo-3-phenylpropionic acid	1478	N	No safety concern
Sodium 2-oxo-3-phenylpropionate	1479	N,T	

[a] N: new specifications prepared; T: tentative specifications.

Flavouring agents considered for specifications only

No.	Flavouring agent	Specifications[a]
53	Citronellyl formate	R
55	Neryl formate	R
68	Rhodinyl butyrate	R
399	Methyl-β-ionone	R
471	2,8-Dithianon-4-ene-4-carboxaldehyde	R
504	*S*-Methyl benzothioate	R
557	1-Mercapto-2-propanone	R
570	Propenyl propyl disulfide	R
605	1,3-Nonanediol acetate (mixed esters)	R
615	Butyl ethyl malonate	R
628	Ethyl aconitate (mixed esters)	R
631.2	Sodium salt of 3-methyl-2-oxobutanoic acid	S[b]
632.2	Sodium salt of 3-methyl-2-oxopentanoic acid	S[b]
633.2	Sodium salt of 4-methyl-2-oxopentanoic acid	S[b]
919	Glyceryl monooleate	R
1203	Ammonium isovalerate	R
1218	4-Ethyloctanoic acid	R
1263	Isoeugenyl phenylacetate	R
1273	Ethyl 5-hexenoate	R
1291	3-Mercapto-2-methylpentan-1-ol (racemic)	R
1296	spiro[2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane]	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.
[b] Specifications will be withdrawn at the next meeting at which flavouring agents are discussed if no information becomes available by that time.

Evaluation of a natural constituent of food

Constituent	Toxicological recommendations
Glycyrrhizinic acid	Available data suggest that an intake of 100 mg/day would be unlikely to cause adverse effects in the majority of adults. In certain highly susceptible individuals, physiological effects could occur at exposure levels somewhat below this figure. The intake data indicate that consumers with a high intake of liquorice confectionery or herbal tea containing liquorice may be exposed to glycyrrhizinic acid at more than 100 mg/day.

Annex 3

Further information required or desired

Magnesium sulfate

The Committee required further information by the end of 2006 on functional uses of magnesium sulfate, including use levels, and on the commercial use of anhydrous magnesium sulfate.

Steviol glycosides

The Committee required additional information by 2007 on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics. In order to be able to remove the tentative designation from the specifications, further information for commercially available products is required on:

— Analytical data on distribution and concentrations of all component steviol glycosides, including those that are not identified in the tentative specifications.

— Method of analysis for the determination of all component steviol glycosides, including those that are not identified in the tentative specifications;

— The nature and concentration of the fractions that do not contain steviol glycosides.

— The quantities of residual solvents from isolation and purification steps of the manufacturing process.

— The hydrolytic stability of the steviol glycosides in acidic foods and beverages.

Zeaxanthin-rich extract from *Tagetes erecta* L.

Information is required on the non-zeaxanthin components in total carotenoids and on the composition of the non-carotenoid components.

Annex 4

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

References for Annex 4

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

No.	Name	Minimum assay value (%)	Secondary components	Comments on secondary components
B. Aliphatic and alicyclic hydrocarbons				
1323	Camphene	80% of $C_{10}H_{16}$	15–19% $C_{15}H_{24}$ terpene hydrocarbons (e.g. valencene)	The $C_{15}H_{24}$ terpene hydrocarbons most likely to be found as secondary components in camphene include+ valencene (No. 1337), β-caryophyllene (No. 1324), bisabolene (No. 1336), and farnesene (No. 1343). The Committee evaluated all these agents at its present meeting and concluded that they were of no safety concern at estimated current intakes.
1324	β-Caryophyllene	80%	15–19% $C_{15}H_{24}$ terpene hydrocarbons (e.g. valencene)	The $C_{15}H_{24}$ terpene hydrocarbons most likely to be found as secondary components in β-caryophyllene include valencene (No. 1337), bisabolene (No. 1336), and farnesene (No. 1343). The Committe evaluated all these agents at its present meeting and concluded that they were of no safety concern at estimated current intakes.
1327	Myrcene	90% of $C_{10}H_{16}$	5–6% dihydromyrcene	Dihydromyrcene has not been evaluated previously by the Committee. It is expected to share the same metabolic fate as the structurally related substance myrcene (No. 1327), which was evaluated by the Committee at its present meeting. A LOEL/NOEL of 250 mg/kg bw per day was reported in 13-week studies in mice and rats treated by gavage (*1*, *2*). The Committee concluded that this substance was of no safety concern at estimated current intakes.
1337	Valencene	94%	2–4% other sesquiterpenes	The $C_{15}H_{24}$ terpene hydrocarbons most likely to be found as secondary components in valencene include β-caryophyllene (No. 1324), bisabolene (No. 1336), and farnesene (No. 1343). The Committe evaluated all these agents at its present meeting and concluded that they were of no safety concern ats estimated current intakes.

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95% (*continued*)

No.	Name	Minimum assay value (%)	Secondary components	Comments on secondary components
1338	3,7-Dimethyl-1,3,6-octatriene	80%	15–19% *cis*-β-Ocimene	*cis*-β-Ocimene has not been previously evaluated by the Committee. It is the *cis*-isomer of the primary compound 3,7-dimethyl-1,3,6-octatriene. *cis*-β-Octimene is expected to share the same metabolic fate as the primary compound and the structurally related acyclic hydrocarbons in this group of flavouring agents, which include myrcene (No. 1327). Myrcene was evaluated by the Committee at its present meeting, when a LOEL/NOEL of 250 mg/kg bw per day was identified in a 13-week study in rats and mice treated by gavage (*1*, *2*). The Committee concluded that myrcene was of no safety concern at estimated current intakes.
1339	*p*-Mentha-1,3-diene	89% of $C_{10}H_{16}$	6–7% 1,4- and 1,8-Cineole	The Committee evaluated 1,4-cineole (No. 1233) at its sixty-first meeting and concluded that it was not a safety concern at estimated current intakes. The Committee also evaluated 1,8-cineole (eucalyptol, No. 1234) at its sixty-first meeting and concluded that it was not a safety concern at estimated current intakes. In an 80-week study in mice, a NOEL of >32 mg/kg bw per day was reported (*3*).
1341	1,3,5-Undecatriene	94% (sum of isomers)	1–3% 2,4, 6-Undecatriene (*Z*,*Z*,*E*)	2,4,6-Undecatriene has not been evaluated previously by the Committee. It is expected to share the same metabolic fate as the primary compound 1,3,5-undecatriene and the other acyclic hydrocarbons in this group of flavouring agents, which are oxidized to oxygenated metabolites and excreted in the urine. The Committee concluded that this substance was of no safety concern at estimated current intakes.
1342	δ-3-Carene	92%	2–3% β-Pinene; 1–2% limonene; 1–2% myrcene; 0–1% *p*-cymene	β-Pinene (No. 1330) was evaluated by the Committee at its present meeting, when it was concluded that this substance was of no safety concern at estimated current intakes.

				d-Limonene (No. 1326) was evaluated by the Committee at its present meeting. Based on the ADI "not specified" that was established for *d*-limonene at the forty-first meeting of the Committee, the Committee concluded that this substance was of no safety concern at estimated current intakes. Myrcene (No. 1327) was evaluated by the Committee at its present meeting. The LOEL/NOEL for myrcene was 250 mg/kg bw per day in 13-week studies in rats and mice treated by gavage (*1*, *2*). The Committee concluded that this substance was of no safety concern at estimated current intakes. *p*-Cymene (No. 1325) was evaluated by the Committee at its present meeting, when it was concluded that this substance was of no safety concern at estimated current intakes.
1343	Farnesene (α and β)	67% (sum of isomers)	21% bisabolene (sum of isomers); 10% other isomers of farnesene and other $C_{15}H_{24}$ terpene hydrocarbons (e.g. valencene, bourbonene, cadinene, guaiene)	Bisabolene (No. 1336) was evaluated by the Committee at its present meeting, when it was concluded that this substance was of no safety concern at estimated current intakes Other isomers of farnesene are expected to share the same metabolic fate as the primary compounds α- and β-farnesene and the structurally related acylic hydrocarbons in this group of flavouring agents, which include myrcene (No. 1327). Myrcene was evaluated by the Committee at its present meeting. The LOEL/NOEL for myrcene was 250 mg/kg bw per day in 13-week studies in rats and mice treated by gavage (*1*, *2*). The Committee concluded that this substance was of no safety concern at estimated current intakes. The $C_{15}H_{24}$ terpene hydrocarbons found as secondary components of farnesene include valencene (No. 1337), bourbonene (No. 1345), cadinene (No. 1346), and guaiene (No. 1347). The Committe evaluated all these agents at its present meeting and concluded that they were of no safety concern at estimated current intakes.

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95% (*continued*)

No.	Name	Minimum assay value (%)	Secondary components	Comments on secondary components
D. Aliphatic, linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters				
1349	2-Decenal	92% (sum of *E* & *Z* isomers)	3–4% 2-decenoic acid	(*E*)-2-Decenoic (No. 1372) acid is a substrate for the fatty acid cycle and is metabolized and excreted primarily as carbon dioxide and water (*4*). The related material, 2,4-decadienal, which is oxidized to 2,4-decadienoic acid, exhibited NOELs of 100 and 200 mg/kg bw per day for male and female mice, respectively, in a 90-day feeding study (*5*). NOELs of 100 and 33.9 mg/kg bw per day were reported for rats in two separate 90-day studies (*5*, *6*). The Committee concluded that this substance was of no safety concern at estimated current intakes.
1350	2-Dodecenal	93% (sum of *E* & *Z* isomers)	3–4% 2-dodecenoic acid	2-Dodecenoic acid is structurally related to the primary material and is expected to be metabolized in the same way. It is a substrate for the fatty acid cycle, and is metabolized and excreted primarily as carbon dioxide and water (*4*), and thus does not present a safety concern at estimated current intakes.
1353	2-Hexenal	92% (sum of *E* & *Z* isomers)	3–4% 2-hexenoic acid	(*E*)-2-Hexenoic acid (No. 1361) is a substrate for the fatty acid cycle and is metabolized and excreted primarily as carbon dioxide and water (*4*). A 98-day study with the structurally related material 2,4-hexadienal, which oxidizes to 2,4-hexadienoic acid, exhibited NOELs of 15 and 60 mg/kg bw for male and female rats, respectively (*7*). The Committee concluded that this substance was of no safety concern at estimated current intakes.
1359	2-Tridecenal	92% (sum of *E* & *Z* isomers)	3–4% 2-tridecenoic acid	2-Tridecenoic acid is structurally related to the primary material and is expected to be metabolized in the same way. It is a substrate for the fatty acid cycle, and is metabolized and excreted primarily as carbon dioxide and water (*4*), and thus does not present a safety concern at estimated current intakes.

1362	2-Nonenal	92% (sum of *E* & *Z* isomers)	3–4% 2-Nonenoic acid	(*E*)-2-Nonenoic acid (No. 1380) is a substrate for the fatty acid cycle, and is metabolized and excreted primarily as carbon dioxide and water (*4*), and thus does not present a safety concern at estimated current intakes.
1363	2-Octenal	92% (sum of *E* & *Z* isomers)	3–4% 2-octenoic acid and ethyl octanoate	2-Octenoic acid is structurally related to other α,β-unsaturated acids and is expected to be metabolized in the same way. It is a substrate for the fatty acid cycle, and is metabolized and excreted primarily as carbon dioxide and water (*4*), and thus does not present a safety concern at estimated current intakes. Ethyl octanoate (No. 33) has been evaluated by the Committee, which concluded that it was of no safety sconcern at estimated current intakes.
1374	(*Z*)-2-Hexen-1-ol	92%	3–5% (*E*)-2-hexen-1-ol	Both the *E* and *Z* isomers are oxidized in vivo, first to the corresponding aldehyde and then to the acid (*8–10*). They then enter the fatty acid cycle where they are completely metabolized and excreted (*4*). A NOEL of 120 mg/kg bw per day was identified in a 98-day study in rats given drinking-water containing the structurally related material *cis*-3-hexen-1-ol (*11*). The Committee concluded that these substances were of no safety concern at estimated current intakes.
1379	*trans*-2-Hexenyl pentanoate	93%	1–3% propanoic acid; 1–3% 2-hexenol	Propanoic acid (No. 84) has been evaluated by the Committee, which concluded that it was of no safety concern at estimated current intakes. For 2-hexenol see No. 1374 above.
1381	(E)-2-Hexenyl hexanoate	93%	2–3% hexanoic acid; 2–3% 2-hexenol	Hexanoic acid (No. 93) has been evaluated by the Committee, which concluded that it was of no safety concern at estimated current intakes. For 2-hexenol see No. 1374 above.

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95% (*continued*)

No.	Name	Minimum assay value (%)	Secondary components	Comments on secondary components
E. Monocyclic and bicyclic secondary alcohols and ketones				
1386	Isoborneol	92%	3–5% borneol	Borneol (No. 1385) was evaluated by the Committe at its present meeting. NOELs of 526 and >1300 mg/kg bw per day were reported in 31- and 90-day studies in dogs, respectively (*12*). In addition, NOELs of 15 and 90 mg/kg bw per day were identified for males and females, respectively, for the related material isobornyl acetate (No.1388) in a 90-day study in rats (*13*). The Committee thus concluded that borneol does not present a safety concern at estimated current intakes.
1398	Nootkatone	93%	2–3% dihydronootkatone	Dihydronootkatone (No. 1407) is metabolized primarily throught the epoxidation and hydration of the isoprenyl side-chain to form the corresponding 13,14-diol (*14*). To a minor extent, reduction of the ketone to the secondary alcohol followed by conjugation with glucuronic acid may occur as with the aliphatic and monocyclic secondary ketones (*15–17*) (Williams, 1959; Lington & Bevan, 1994; Topping et al., 1994). The Committee thus concluded that dihydronootkatone does not present a safety concern at estimated current intakes.
1407	Dihydronootkatone	90%	5–6% nootkatone	Nootkatone (No. 1398) is metabolized primarily throught the epoxidation and hydration of the isoprenyl side-chain to form the corresponding 13,14-diol (*14*) (Asakawa et al., 1986). To a minor extent, reduction of the ketone to the secondary alcohol followed by conjugation with glucuronic acid may occur as with the aliphatic and monocyclic secondary ketones (*15–17*). The Committee thus concluded that nootkatone does not present a safety concern at estimated current intakes.

1409	β-Ionyl acetate	92%	2–3% acetic acid; 1–2% β-ionol	Acetic acid (No. 81) has been evaluated by the Committee, which concluded that it was of no safety concern at estimated current intakes. β-Ionol (No. 392) has been evaluated by the Committee, which concluded that it was of no safety concern at estimated current intakes.
1413	D,L-Menthol(+/–)-propylene glycol carbonate	87%	12% *d,l*-Menthol 2-propylene glycol carbonate	The secondary component is structurally related to the primary material and is expected to be metabolized in the same way. Both are hydrolysed in the carbonate liver, producing menthol and propylene glycol. Menthol (No. 427) and propylene glycol (No. 925) have both been previously evaluated by the Committee and considered not to be a safety concern at estimated current intakes.
1414	L-Monomenthyl glutarate	72%	22–24% dimenthyl glutarate; 1–2% glutaric acid	The metabolism of dimenthyl glutarate is expected to follow the same pathway as that for monomenthyl glutarate. The ester functions are hydrolysed in vivo yielding menthol and glutaric acid (*18*, *19*), and thus does not present a safety concern at estimated current intakes. Menthol (No. 427) has been evaluated by the Committee, which concluded that it was of no safety concern at estimated current intakes. Glutaric acid has not been evaluated previously by the Committee, but is endogenous in humans and is structurally related to valeric acid (No. 90), which has been evaluated previously by the Committee, and thus does not present a safety concern at estimated current intakes.
G. Tetrahydrofuran and furanone derivatives				
1456	4-Acetoxy-2,5-dimethyl-3(2*H*)furanone	85%	9–8% 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (No. 1446) is expected to share the same metabolic fate as the primary material, i.e. conjugation with glucuronic acid and excretion in the urine (*20*). A 2-year study with this material reported a NOEL of 200 mg/kg bw per day in both male and female rats (*21*). The Committee concluded that 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone does not present a safety concern at estimated current intakes.

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95% (*continued*)

No.	Name	Minimum assay value (%)	Secondary components	Comments on secondary components
1457	(+/–)-2-(5-Methyl-5-vinyl-tetrahydrofuran-2-yl) propionaldehyde	90% (sum of 4 isomers)	5–6% 6-hydroxy-2, 6-dimethyl-2, 7-octadienal	A related substance, hydroxycitronellal (No. 611) was evaluated by the Committee at its 1999 meeting and was concluded to be of no safety concern at estimated current intakes. A NOEL of 250 mg/kg bw per day was reported for hydroxycitronellal in a 2-year study in rats (*22*). The Committee concluded that 6-hydroxy-2,6-dimethyl-2, 7-octadienal does not present a safety concern at estimated current intakes.
H. 2-Phenylpropanol derivatives				
1465	2-Methyl-3-(*p*-isopropylphenyl) propionaldehyde	90%	3–5% 2-methyl-3-(p-isopropylphenyl) propionic acid	The initial step in the metabolic pathway for the primary material is oxidation to its corresponding acid. As the secondary material is the corresponding acid of the primary material, it is expected to share the same metabolic fate of conjugation with glucuronic acid and excretion in the urine (*23*). The Committee concluded that 2-methyl-3-(*p*-isopropylphenyl)propionic acid does not present a safety concern at estimated current intakes.

Corrigenda

WHO Technical Report Series 922: Evaluation of certain food additives and contaminants, 2004

p28, line 9:

Replace “2 mg” with “21 mg”.

The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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