WHO Technical Report Series
934

EVALUATION OF CERTAIN FOOD ADDITIVES

Sixty-fifth report of the joint
FAO/WHO Expert Committee on food additives

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

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents), assessments of intake, and the establishment and revision of specifications for food additives.

A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (Beeswax, Candelilla wax, Calcium L-5-Methyltetrahydrofolic acid (L-5-MTHF), Phospholipase A1 from *Fusarium venenatum* expressed in *Aspergillus oryzae*, Pullulan, Quillaia extract Type 1, Quillaia extract Type 2) and seven groups of flavouring agents. Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, changes in the status of specifications, and further information requested or desired.

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EVALUATION OF CERTAIN FOOD ADDITIVES

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EVALUATION OF CERTAIN FOOD ADDITIVES

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

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Geneva, 7–16 June 2005

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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. **WHO Food Additive Series, No. 56, 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.**

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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

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

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 6 to 17 June 2005. The meeting was opened by Dr Kerstin Leitner, Assistant Director-General for Sustainable Development and Healthy Environment, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr Leitner noted that the work of the Committee plays an important role in improving global food safety, by providing a scientific foundati(on for international food standards. Equally important is its work on formulating principles for assessing the safety of food chemicals. In order that the Committee continue to respond to the increased need for international, independent scientific advice, despite limited financial resources, priorities must be set. Strengthening the system for the provision of scientific advice is a central part of FAO's and WHO's normative work, and there is a clear commitment to continue support for this activity.

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-four 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); and

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

2.1 Modification of the agenda

Three substances, Arpink red, sucralose and the flavouring agent guanilic acid, were withdrawn from the agenda as no data had been received. Arpink red and guanilic acid had been referred to the Committee by the Codex Committee on Food Additives and Contaminants for safety evaluation and sucralose for revision of specifications. Evaluation of Arpink red had been scheduled as a priority for the second time, but no data were submitted on

either occasion. The JECFA Secretariat re-emphasized that, when the Codex Committee requests evaluations by JECFA, it must apply the established criteria for priorities and ensure that data are available.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in 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.

2.2.1 *Safety evaluation of flavouring agents*

Estimating dietary exposure to flavouring agents

At its forty-sixth meeting (Annex 1, reference *122*), the Committee adopted a procedure for the safety evaluation of flavouring agents. In formulating this procedure, the Committee recognized the need for an approach that could be used efficiently for a large class of substances. In view of the availability from industry of data on production volumes for several thousand flavouring ingredients, the Committee decided that a method for calculating per capita exposure, the maximum survey-derived daily intake (MSDI), could be readily used for assessing exposure as part of the procedure for safety evaluation. The Committee re-endorsed the MSDI approach at its forty-ninth (Annex 1, reference *131*), fifty-fifth (Annex 1, reference *149*), sixty-first (Annex 1, reference *166*) and sixty-third (Annex 1, reference *173*) meetings.

The Committee has also discussed limitations to use of the MSDI for estimating dietary exposure. At its fifty-fifth meeting (Annex 1, reference *149*), the Committee noted that use of the MSDI could result in underestimates of the dietary exposure of persons with high levels of consumption of certain foods. At its sixty-first meeting (Annex 1, reference *166*), the Committee decided that it would not evaluate flavouring agents for which poundage data had not been reported; at its sixty-third meeting

(Annex 1, reference *173*), the Committee recognized that the estimates of current dietary exposure are difficult to reconcile with reported maximum use levels of some flavouring agents in foods.

At its current meeting, the Committee considered how better to identify and deal with flavouring agents for which the MSDI estimates, as used in the procedure, are substantially lower than the dietary exposures estimated from the levels of use. The Committee anticipated that, in most cases, the existing data would provide assurance about safety at levels of exposure higher than the MSDI (particularly for flavouring agents that are not used in a wide range of food products). Nevertheless, this assumption would need to be confirmed case by case.

Implications for toxicological data requirements of using model diets for estimating exposure to flavouring agents

Estimates of exposure based on levels of use (model diets) are higher than MSDI estimates. Thus, with this method, the exposure thresholds of more flavouring agents would be exceeded at steps A3 and B3 of the decision tree, which are central to the procedure for evaluating flavouring agents.

The Committee at its present meeting explored alternative approaches for estimating dietary exposure , on the basis of use levels[1] for about 90% of the flavouring agents submitted for evaluation. These data made it possible to prepare conservative estimates of dietary exposure by several methods, including a model diet. Dietary exposure to most of the flavouring agents was above the threshold for the respective structural class when estimated by methods based on use levels, while this was true for only a few compounds when exposure was estimated as the MSDI. A preliminary comparison of the dietary exposure estimates with the no-observed effect level (NOEL) values for selected agents indicated, however, that additional, more conservative estimates of dietary exposure would suggest a safety concern in only a few cases. Comprehensive risk characterizations based on dietary exposure estimates from use levels could not be performed for all agents at the present meeting because appropriate toxicological data were not required for assessment of safety with the procedure and had therefore not been submitted.

The Committee will have to consider carefully the most appropriate approach for evaluating the safety of flavouring agents on the basis of conservative methods for estimating dietary exposure .

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[1] Use levels were taken from the 'GRAS [generally recognized as safe] lists' published by the Expert Panel of the Flavor and Extract Manufacturers Association. of the United States

Recommendation to the JECFA Secretariat to form a working group

To address concerns raised by the Committee at its fifty-fifth meeting (Annex 1, reference *149*), at a recent FAO/WHO workshop on dietary exposure assessment (see section 2.4) and in several recent publications, the Committee recommended that the Secretariat form a small working group, shortly after the conclusion of the present meeting, to consider further all relevant aspects of use of an additional screening method based on use levels, to complement the MSDI, the method used by JECFA for estimating dietary exposure to flavouring agents. The Committee also recommended that experts on exposure should work with the temporary advisers during the preparation of monographs.

The terms of reference for this working group will be determined by the JECFA Secretariat but might include:

(i) a detailed analysis of the effects of various methods for estimating dietary exposure on the safety assessment of flavouring agents according to the procedure;

(ii) formulation of an approach based on dietary exposure estimates derived from the MSDI and use levels to be used before a meeting to identify flavouring agents that require special consideration at the meeting;

(iii) revision of the dietary exposure sections of the safety evaluations of flavouring agents, for discussion by JECFA at its next meeting; and

(iv) consideration of an approach for estimating combined dietary exposure to a group of substances using use level-based and model diets.

Interactions with industry and requests for data

The Committee emphasized that flavouring agents should be evaluated on the basis of complete, up-to-date information. It therefore welcomed a proposal from industry to update and extend the existing surveys of flavour usage.

The Committee recommended that data on poundage be collected regularly for all flavouring agents, so that rolling averages of poundage can be calculated. This information should be collected with attention to adequate quality control.

The apparent discrepancy in dietary exposure to some flavouring agents between that estimated from reported poundage and that estimated from

published use levels requires further investigation to ensure that the safety evaluations are based on exposure estimates that reflect current (and future) practice in the food and flavouring industries. The Committee recommended that studies be undertaken in this area, giving priority to substances of potential toxicological concern, for which there is only a low margin of safety between the potential exposure level and the NOEL observed in studies in experimental animals with the same compound or a structural analogue.

In a recent analysis made available to JECFA, the margins of safety for 808 flavouring agents previously evaluated by JECFA were compared by use of estimates derived from either the MSDI or a model diet. The margin of safety was less than 100 for 16 of 808 substances evaluated by the model diet method and for one (methyl salicylate) identified by MSDI analysis. On the basis of estimates of dietary exposure and data on toxicity, a subset of these flavouring substances were identified as priorities for further investigation: *para*-ethylphenol, 2,5-xylenol, 2,6-xylenol, 3,4-xylenol, *para*-vinylphenol, 2,3,6-trimethylphenol, 4-phenyl-2-butyl acetate, heptanal dimethyl acetal, thiamine hydrochloride, 4-[(2-furanmethyl)thio]-2-pentanone, 4,8-dimethyl-3,7-nonadien-2-one, 2-(methylthio)ethanol, 2,3,5-trithiahexane, 3-L-menthoxypropane-1,2-diol and 3-(l-menthoxy)-2-methylpropane-1,2-diol.

The Committee recommended that the JECFA Secretariat ensure that data on use levels are included in submissions from sponsors for safety evaluation of flavouring agents, as requested in the call for data. The Committee noted that such data were not submitted by the sponsors at the current meeting. Subsequent submissions that do not contain this information will not be evaluated by the Committee.

Anticipated poundage data

The Committee noted that no poundage was recorded in either the European Union or the United States of America (USA) for an increasing number of flavouring agents submitted for evaluation, and MSDI values could be calculated only on the basis of the annual poundage anticipated by the manufacturer. This was the situation for 60 of 135 flavouring agents on the agenda of the present meeting and for a number of those evaluated at the fifty-ninth, sixty-first and sixty-third meetings. As these MSDI estimates contain additional uncertainty, the Committee decided that dietary exposure to such substances, in the future, should either be assessed by an alternative approach or the assessment should be deferred until actual poundage data become available.

The Committee requested actual use levels or poundage data for the flavouring agents that it had previously assessed on the basis of an MSDI calculated from anticipated poundage. These include substances for which the MSDI based on anticipated poundage for one region (the European Union or the USA) was higher than that based on recorded poundage in the other region. A list of these flavouring agents will be published by the Secretariat, and the existing assessments will be revoked if the necessary data are not forthcoming by December 2007.

The Committee decided that the procedure would be applied to evaluate the safety of the flavouring agents for which data were submitted to the present meeting, including those for which anticipated poundage data for the European Union or the USA were submitted. For this group, the evaluations were made conditional if based on MSDI values derived from anticipated poundage estimates. The results of the conditional assessments will be revoked if use levels or poundage data are not provided before December 2007. This decision was not unanimous, and two members registered a minority opinion (see Annex 6).

2.2.2 *Safety evaluation of enzymes produced by genetically modified microorganisms*

In 1987, the Committee outlined criteria for evaluating the safety of enzymes (Annex 1, reference *76*) and proposed to categorize enzyme preparations into five main groups on the basis of their origin: animal tissues, portions of edible plants, microorganisms traditionally accepted as constituents of food or normally used in the preparation of foods, non-pathogenic microorganisms commonly found as contaminants of foods or micro-organisms that are less well known. At the same time, the Committee envisaged three cases for assessing the safety of enzymes—those added directly to food and not removed, those added to food but removed and immobilized enzyme preparations—and indicated guidelines appropriate for evaluations of safety in each case.

In 1987, enzymes produced by genetically modified microorganisms were not considered. Subsequently, the Committee evaluated several enzymes in this category, including laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae* and xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*. The Committee evaluated the safety of these enzyme preparations on the basis of toxicological data files, both of which included a 90-day study in rats, a test for reverse mutation in vitro and a test for chromosomal aberrations. The Committee allocated an ADI ‘not specified’ to these enzyme preparations.

The present Committee evaluated an enzyme preparation of phospholipase A1 produced by the same host strain of *A. oryzae* that has been modified to produce other enzymes. It could not, however, assess the safety of this preparation by comparison with the information available on one of the other enzymes. Therefore, the Committee concluded that guidelines should be drawn up for the safety assessment of enzymes produced by genetically modified microorganisms. These guidelines should include the essential information for various situations and details of molecular characterization of the producing microbial strain necessary to allow adequate assessment of the safety of the preparation. These guidelines should be used by the Committee to adopt a coherent approach to evaluations and should be useful for sponsors in preparing their applications. The Committee reiterated that the existing *General specifications and considerations for enzyme preparations used in food processing* (Annex 1, reference *156*) should be revised at the same time as guidelines for the safety evaluation of enzyme preparations.

The report from the Joint FAO/WHO Expert Consultation on Safety Assessment of Foods Derived from Genetically Modified Microorganisms (*2*) should constitute a starting point for this task.

2.3 Food additive specifications

2.3.1 *Compendium of Food Additive Specifications*

The current Compendium of food additive specifications was published in 1992 (Annex 1, reference *96*), consolidating all the specifications for food additives that had been elaborated by the Committee up to that time. Specifications that were updated and developed at subsequent meetings have been published in a series of addenda (Annex 1, references *103*, *109*, *118*, *124*, *133*, *139*, *145*, *151*, *156*, *162*, *168*, *172*). FAO now plans to consolidate all the specifications in a second edition of the Compendium.

At its current meeting, the Committee considered a report on a number of issues that had arisen during drafting of an introduction to the second edition. The new introduction is intended to update the current one and also to serve as the basis for revising those sections of the *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*) dealing with specifications. The Committee noted that the new introduction emphasizes the importance of setting specifications as an inherent part of the risk assessment of food additives, and that the safety evaluation of an additive should therefore always be read in conjunction with the specifications of identity and purity for that additive.

The Committee also discussed the conditions under which a 'tentative' designation is given to additive specifications and the possible link with the 'temporary ADI' designation. It agreed that, although a clear link between the specifications and the safety assessment is essential, the situations in which the 'tentative' specifications and the 'temporary ADI' designations are used should continue to be judged case by case. The Committee also reaffirmed that these designations should be time-limited.

2.3.2 *Residual solvents*

At its sixty-first meeting (Annex 1, reference *166*), the Committee recognized that the general method for determining residual solvents in food additives, published in FAO Food and Nutrition Paper, No. 5 (*3*), should be revised. A tentative general method based on head-space capillary gas chromatography with flame ionization detection for determination of residual solvents was published in FAO Food and Nutrition Paper, No. 52, Add. 11 (Annex 1, reference *168*).

At its present meeting, the Committee recognized that several issues had to be addressed before a single method could be decided upon. For example, the method should be widely applicable, and its sensitivity should meet the requirements of the specifications. In addition, preference would be given to methods studied collaboratively; in the absence of such studies, methods with other validation would be considered.

In reviewing the responses to the call for data and comments on the tentative general method, the Committee noted that the critical aspects of the determination are liberation of solvent residues from the food additive and their capture by head-space sampling before the gas chromatographic step. Therefore, the Committee decided that the critical steps should be included in future specifications rather than in the general method. The Committee also considered that the tentative general method for determination of residual solvents by gas chromatography should be revised to include more solvents.

The Committee further recommended that methods for the analysis of many common solvents used in the preparation of food additives should be reviewed during further revision of *Guide to specifications—general notices, general analytical techniques, identification tests, test solutions and other reference materials*(*3*).

2.3.3 *Standard curves in analytical methods*

The Committee noted that many specifications mention analytical methods in which standard or calibration curves are used. These curves are typically

constructed by plotting instrument readings against known concentrations of a series of standard solutions. It was noted that metrologists reserve the term 'calibration' for the metrological relationship between a reading and a traceable standard. An example of such a relationship is calibration of an analytical balance with weights that have been calibrated during an unbroken chain of comparisons with a national or international measurement standard for mass. The Committee agreed that the term 'standard curve' should be used in the analytical methods in preference to 'calibration curve'.

2.3.4 *Use of the terms 'anhydrous' and 'dried basis' in specifications*

The Committee agreed that use of the terms 'anhydrous', 'dried basis' and 'dry basis' in food additive specifications has been a source of misunderstanding. To clarify its position, the Committee agreed to discontinue use of the term 'dry basis' and recommended that provisions in future food additive specifications should refer to either 'anhydrous' or 'dried basis'. It was agreed to interpret these terms as described below.

'Anhydrous' refers to:

– the calculated amount of substance, adjusted for the known stoichiometric number of molecules of water of hydration

– the amount of substance adjusted for the measured amount of water, as determined by a method such as the Karl Fischer method described in *Guide to specifications—general notices, general analytical techniques, identification tests, test solutions and other reference materials*(*3*).

'Dried basis' refers to:

– the amount of substance remaining after a sample has been subjected to the stated conditions for loss on drying (e.g. duration, temperature, pressure, presence of a desiccant).

2.3.5 *Withdrawal of certain food additive specifications*

Eugenol, methyl anthranilate, methyl *N*-methylanthranilate, ethyl 3-phenylglycidate and ethyl methylphenylglycidate were on the agenda for evaluation as flavouring agents and were found to have specifications in the standard food additive format. As these substances are used solely as flavouring agents, the Committee prepared specifications in the format for flavouring agents and withdrew the specifications in the standard food additive format.

2.3.6 *Revision of specifications for ethyl maltol and maltol*

Ethyl maltol and maltol were on the agenda for evaluation as flavouring agents and were found to have specifications in the standard food additive format. Specifications in the format for flavouring agents were prepared. As the substances have uses other than as flavouring agents, the Committee decided to revise the specifications in the standard food additive format but to designate them as 'tentative', pending information on functional uses and methods of assay.

2.4 Project to update the principles and methods for the risk assessment of chemicals in food

The Committee was informed of progress on the Joint FAO/WHO project to update the principles and methods for the risk assessment of chemicals in foods, including a recent workshop convened to draft guidance on how to perform and interpret dietary exposure assessments at international, regional, national and local levels. The workshop participants reviewed the report of the Joint FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals (*4*), and, on this basis, drafted additional guidance for determining the concentrations of chemicals in food, food consumption and dietary exposure. The final draft of the report of the workshop is in preparation. Once finalized, it will be incorporated into the draft *General principles and methods for the risk assessment of chemicals in food*. The Committee took note of this important activity, the outcome of which will affect the work of JECFA.

The Committee recommended that all the chapters of the *General principles and methods for the risk assessment of chemicals in food* and the comments received during public review undergo external peer review before they are considered and applied by JECFA.

2.5 FAO/WHO workshop on nutrient risk assessment

The Committee was informed of a joint FAO/WHO workshop on nutrient risk assessment, held in Geneva on 2–6 May 2005, which was convened to formulate a model for establishing upper levels of exposure to nutrients and related substances. The goal was to specify a general approach for scientific risk assessment that could be used internationally. It is anticipated that the report of the workshop will be available in autumn 2005.

The Committee took note of this important activity, the outcome of which might affect the work of JECFA.

2.6 Application of approaches to thresholds of toxicological concern for risk characterization

A threshold of toxicological concern is the level of human exposure below which no significant risks to health are anticipated, even in the absence of toxicological data. Risk assessments based on this approach include a variety of scientific data, such as information on the structure of the substance; data on absorption, distribution, metabolism and excretion and on the toxicity of compounds in the same structural class; and, most importantly, data on exposure. Such pragmatic risk assessments can be used when more comprehensive evaluations are not possible, to provide timely advice for risk managers. They are also useful in making priorities for risk management.

The approach should not be used if there are sufficient, chemical-specific toxicological data for hazard characterization. Furthermore, it should be used only for defined chemical entities of low relative molecular mass.

The approach of using thresholds of toxicological concern is based on analyses of the relationships between chemical structures and long-term toxicity, including carcinogenicity (*5–10*). Similar approaches have been proposed for other toxicological end-points (*11–13*).

JECFA has adopted a decision-tree approach which is based on a series of considerations of threshold of toxicological concern for the evaluation of flavouring agents (Annex 1, references *107*, *116*, *131*).

The Committee noted that the following considerations should be taken into account for further application of 'threshold of toxicological concern' approaches:

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

– Additional data on the toxicity of structurally related substances might be required.

The Committee reaffirmed use of this approach for flavouring agents. It recommended that guidance be drawn up on application of the approach with regard to substances present in the diet in small amounts, such as certain residues of processing aids, packaging materials and contaminants, to provide advice on the risk assessment of substances for which full toxicological datasets are not available or are unnecessary. The Committee recommended that such guidance be developed by a special task group appointed by the Joint FAO/WHO secretaries and incorporated into the *Principles and methods for the risk assessment of chemicals in food.*

3. Specific food additives and ingredients (other than flavouring agents)

At its present meeting, the Committee evaluated three food additives for the first time, i.e. calcium L-5-methyltetrahydrofolate, phospholipase A1 from *Fusarium venenatum* expressed in *Aspergillus oryzae* and pullulan. Four others that had been considered at prevous meetings, i.e. beeswax, candelilla wax, quillaia extract type 1 and quillaia extract type 2, were re-evaluated. In additon, five substances that had been evaluated at previous meetings, i.e. aspartame-acesulfame salt, hexanes, laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*, mono-magnesium phosphate, trisodium diphosphate and sucrose esters of fatty acids, were considered for review of specifications only. Details are given in sections 3.1 and 3.2, and the conclusions are summarized in Annex 2.

3.1 Safety evaluations

3.1.1 *Beeswax*

Explanation

Beeswax was first evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*). At that time, the only toxicological data available were an LD_{50} in rats of > 5 g/kg body weight (bw) per day and results showing lack of mutagenic potential in microbial assays in vitro. The Committee concluded at that time that "although an evaluation in the traditional manner could not be carried out, the long history of use of natural yellow beeswax without apparent adverse effects provided a degree of assurance that its present functional uses (release and glazing agent in bakery products, glazing agent on fresh and frozen fruit, glazing agent on candy, carrier for flavours and component of chewing-gum bases) did not raise any toxicological concerns." The processing necessary to obtain bleached white beeswax was considered not to alter this conclusion. The Committee also noted that "beeswax might have allergenic potential and that the consumer should be made aware of its presence in foods" and that "attention should be paid to the possibility that toxic substances present in honey in some parts of the world might also occur in beeswax".

Beeswax was evaluated at the present meeting at the request of the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session, in order to consider the acceptability of its use as a carrier for flavours in 'water-based flavoured drinks, including "sport", "energy" or "electrolyte" drinks and "particulated" drinks, based on adopted provisions in the GSFA [Codex General Standard for Food Additives]' (*14*).

Chemical and technical considerations

Beeswax (INS no. 901) is marketed in two forms: yellow beeswax (CAS no. 8006-40-4) and white beeswax (CAS no. 8012-89-3). Yellow beeswax is a yellow or light-brown solid that is somewhat brittle when cold and has a characteristic odour of honey. White beeswax is a white or yellowish-white solid (thin layers are translucent) with a characteristic, but faint, odour of honey.

Beeswax is obtained from the honeycombs of honeybees (*Apis mellifera* L, family Apidae) after removal of the honey. The combs are melted with hot water, steam or solar heat. After removal of insoluble impurities, the liquid wax is cast into cakes for further purification to obtain food-grade yellow beeswax. Yellow beeswax can be bleached with e.g. hydrogen peroxide, sulfuric acid or sunlight to produce white beeswax.

The composition of beeswax depends to some extent on the subspecies of bee, the age of the wax and the climatic circumstances of its production. The variations in composition are, however, mainly in the quantitative ratios of the components and not in their chemical identity. Beeswax consists primarily of five main groups of components: (1) free fatty acids (typically 12–14%), most of which (about 85%) are saturated and have a chain length of C24–C32; (2) free primary fatty alcohols (about 1%) with a chain length of C28–C35; (3) linear wax monoesters and hydroxymonoesters (35–45%) with chain lengths generally of C40–C48, which are derived almost exclusively from palmitic acid, 15-hydroxypalmitic acid and oleic acid; (4) complex wax esters (15–27%) containing 15-hydroxypalmitic acid or diols, which, through their hydroxyl group, are linked to another fatty acid molecule; and (5) odd-numbered, straight-chain hydrocarbons (12–16%) with a predominant chain length of C27–C33.

The food applications of beeswax include its use as a component in dietary food supplements (soft gelatine capsules and tablets), as a glazing and coating agent, as a texturizer for chewing-gum base and as a carrier for food additives (including flavours and colours). When used as a flavour carrier in water-based flavoured drinks, beeswax imparts a cloudy appearance.

At its sixty-third meeting (Annex 1, reference *173*), the Committee revised the limit for lead established for beeswax at its thirty-ninth meeting. At the present meeting, the Committee incorporated this change to the limit into the full specification monograph and added a purity test for the presence of carnauba wax; it also modified the section on functional uses to include the technical effect of clouding.

Toxicological data

The Committee evaluated additional biochemical and toxicological studies on the main components of beeswax (linear monoesters, complex esters, hydrocarbons, free fatty acids and free fatty alcohols) and considered the use of beeswax in water-based, flavoured drinks. The toxicological studies conducted on the various components of beeswax included short-term studies with oral administration, long-term studies of toxicity and carcinogenicity and studies of reproductive toxicity. The components, which are common in other foods, were not toxic. A search of the literature did not reveal the presence of naturally occurring toxic substances in commercial beeswax. It was noted that beeswax administered topically or by intracutaneous injection did not induce an allergic response in humans.

Assessment of dietary exposure

Information was submitted on the food uses and resulting exposures to beeswax. Dietary exposure would be about 350 mg per person per day for a person with 90th percentile exposure to foods containing beeswax, in addition to consumption as a component of food supplement tablets or capsules. The Committee received information on the poundage of beeswax sold to the food market in the European Union in 2003. If it is assumed that 1–10% of the population consumes products containing beeswax, the average dietary exposure to beeswax per consumer would be 10–100 mg per person per day.

The Committee estimated the dietary exposure to beeswax from reports of its current use and maximum levels of use in foods, including as a flavour carrier in water-based drinks. On the basis of the conservative assumption that a person would consume all foods (and food supplement tablets or capsules) containing beeswax at the 95th percentile in each food category and that all those foods would contain beeswax, exposure to beeswax would be < 650 mg per person per day. Addition of use as a carrier for flavours in water-based drinks would result in an increase in the estimated dietary exposure of 200 mg per person per day, about 50% higher than the estimated exposure from current uses (450–650 mg per person per day).

Evaluation

The Committee concluded that current uses of beeswax, including that as a carrier for flavours and as a clouding agent in water-based drinks, would not result in dietary exposure that raised concern about safety, especially in view of the long history of use of beeswax and the absence of toxicity of the main components. As the available information was very limited, the

Committee was unable to reach a conclusion about the potential allergenicity of beeswax noted by the Committee at its thirty-ninth meeting.

A revised toxicological monograph and a chemical and technical assessment were prepared. The existing specifications were revised.

3.1.2 *Candelilla wax*

Explanation

Candelilla wax was first evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*). At that time, the Committee evaluated a number of studies in mice, rats and dogs. No compound-related toxicity was observed in a 6-month study in dogs or in a 2-year study in rats treated orally, even at the highest dietary levels, equivalent to 600 and 750 mg/kg bw per day, respectively. These studies were considered to be of fundamental importance in the safety assessment. Furthermore, candelilla wax was not mutagenic in microbial assays in vitro with or without metabolic activation. Deficiencies in the studies in experimental animals, in the light of current criteria, were considered by the Committee at that time to be counterbalanced to some extent by the consistent absence of adverse effects.

Candelilla wax was evaluated at the present meeting at the request of the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session, in order to consider the acceptability of its use as a carrier for flavours in 'water-based flavoured drinks, including "sport", "energy" or "electrolyte" drinks and "particulated" drinks, based on adopted provisions in the GSFA [Codex General Standard for Food Additives]' (*14*).

Chemical and technical considerations

Candelilla wax (INS no. 902; CAS no. 8006-44-8) is a yellowish-brown, hard, brittle, lustrous solid with an aromatic odour when heated. It consists primarily of odd-numbered saturated straight-chain hydrocarbons (C29–C33) and esters of acids and alcohols with even-numbered carbon chains (C28–C34). The most abundant n-alkane, C31, comprises more than 80% of total n-alkanes. Free acids, free alcohols, sterols, neutral resins and mineral matter (< 1%) are also present.

Candelilla wax is obtained from the slender, leafless, wax-coated, cylindrical stalks of several species of *Euphorbiacea*; the primary source is *E. antisyphilitica*. The crude wax is obtained by boiling the dried stalks in water acidified with sulfuric acid to release the wax. The molten wax, known as 'cerote', is then skimmed off and allowed to solidify. It is refined by treatment with sulfuric acid and subsequent passage through filter presses.

The principal food applications of candelilla wax previously considered by the Committee were as a glazing and surface-finishing agent, as a texturizer for chewing-gum and as a carrier for food additives (including flavours and colours). When used as a flavour carrier for water-based flavoured drinks, candelilla wax imparts a cloudy appearance.

At its sixty-third meeting (Annex 1, reference *173*), the Committee revised the limit for lead established for candelilla wax at its thirty-ninth meeting. At the present meeting, the Committee incorporated this change into the full specification monograph, expanded the definition to give more information on the production method, and modified the section on 'functional uses' to include the technical effect of clouding agent.

Toxicological data

No new information was available to the Committee on the safety of candelilla wax.

Assessment of dietary exposure

The only information submitted to the Committee was poundage data in the European Union for 2004 and in the USA for 1995. The per capita dietary exposure in the European Union (379 million persons) on the basis of this poundage would be 0.02 mg per person per day, while that for the USA (260 million persons) would be 0.01 mg per person per day. These poundages do not include use of candelilla wax in tablets or capsules for dietary supplements.

Although the poundage data suggest little current use, candelilla wax is currently listed in the Codex General Standard for Food Additives for use within good manufacturing practice in the same food categories as beeswax (also reviewed at the present meeting). As no other information was available, the Committee estimated dietary exposure to candelilla wax on the basis of the data submitted for beeswax, including use as a carrier for flavours in water-based drinks. Using the conservative assumption that an individual would consume all the foods (and tablets or capsules) containing candelilla wax at the highest percentile in each food category and that all those foods contained candelilla wax, the Committee calculated that the dietary exposure would be < 650 mg per person per day. Addition of use as a carrier for flavours in water-based drinks would result in an increase in the estimated dietary exposure to 200 mg per person per day, approximately 50% higher than the estimated exposure from current uses (450–650 mg per person per day).

Evaluation

The Committee concluded that the uses of candelilla wax, including as a carrier for flavours and as a clouding agent in water-based drinks, would not result in dietary exposure that raises concern about safety.

No toxicological monograph was prepared. The existing specifications were revised, and a chemical and technical assessment was prepared.

3.1.3 *Calcium L-5-methyltetrahydrofolate*

Explanation

Calcium L-5-methyltetrahydrofolate is the calcium salt of L-5-methyltetrahydrofolic acid, which is the predominant naturally occurring form of folate. It contains a reduced and methylated pteridine ring system. This compound has not been evaluated previously by the Committee.

Calcium L-5-methyltetrahydrofolate is structurally analogous to the reduced form of folic acid (pteroyl-L-glutamic acid), which is the nutritionally active form. The form of naturally occurring reduced folate found predominantly in food is a polyglutamyl folic acid. L-5-Methyltetrahydrofolic acid is a co-factor for key enzymatic reactions in the transfer and processing of the one-carbon units needed for re-methylation of homocysteine to methionine, to serve as the methyl donor for numerous methyltransferases, which methylate a range of biological substrates (lipids, proteins, myelin, dopamine). It also serves as a carbon donor in the pathway leading to nucleotide synthesis, supporting the biosynthesis of DNA.

The safety of folic acid was evaluated by the European Commission Scientific Committee for Food (*15*), which established a tolerable upper intake level of folate at 1 mg per adult per day in order to avoid masking vitamin B_{12} deficiency. The same tolerable upper intake level for folate was established by the Institute of Medicine in the USA (*16*) and by the FAO/WHO consultation on human vitamin and mineral requirements (*17*). The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority concluded that use of calcium L-5-methyltetrahydrofolate as a source of folate in foods for specific nutritional uses, food supplements and foods intended for the general population, with a tolerable upper intake level of 1 mg per adult per day, was not a concern with regard to safety (*15*).

For calcium, the tolerable upper intake level established by the European Commission Scientific Committee for Food and the Institute of Medicine

was 2.5 g, and that established by the FAO/WHO consultation on human vitamin and mineral requirements was 3 g.

At the request of a WHO Member State, the Committee was asked to evaluate the safety of calcium L-5-methyltetrahydrofolic acid as an alternative to folic acid in food fortification and supplementation.

Chemical and technical considerations

Calcium L-5-methyltetrahydrofolate is a white to light-yellowish, almost odourless crystalline powder. Its full chemical name is *N*-{4-[[((6*S*)-2-amino-3,4,5,6,7,8-hexahydro-5-methyl-4-oxo-6-pteridinyl)methyl]amino]-benzoyl}-L-glutamic acid, calcium salt. It is also referred to as:

— L-methylfolate, calcium;

— L-5-methyltetrahydrofolic acid, calcium salt;

— (6*S*)-5-methyltetrahydrofolic acid, calcium salt;

— (6*S*)-5-methyl-5,6,7,8-tetrahydropteroyl-L-glutamic acid, calcium salt; and

— L-5-methyltetrahydrofolic acid, the cation being unspecified.

Calcium L-5-methyltetrahydrofolate is proposed for use as an alternative to folic acid in dry crystalline or microencapsulated form in dietary supplements, foods for special dietary uses and other foods, in food supplements (providing 400 μg of folate per day), meal replacements (providing 200 μg of folate per meal), starch-based fortified foods (containing folate at 400 μg/kg of prepared food) and milk-type products (containing folate at 300 μg/l).

Calcium L-5-methyltetrahydrofolate is synthesized by reduction of folic acid to tetrahydrofolic acid, followed by methylation and diastereoselective crystallization (in water) of L-5-methyltetrahydrofolic acid as its calcium salt. The product contains variable amounts of water of crystallization.

The purity of the substance evaluated at the present meeting was stated to be 95% or greater. Specifications for the identity and purity of the material intended for addition to food include limits for water content, calcium, lead and organic folate impurities and related substances. The organic impurities, which are determined by liquid chromatography, can be categorized as minor amounts of other folates, breakdown and oxidation products and other products, including the D stereoisomer of 5-methyltetrahydrofolic acid and the dimethylated form of tetrahydrofolic acid. The specifications set by the Committee include a combined limit of 2.5% for the first two classes and a limit of 1.0% for the D diastereoisomer of 5-methyltetrahydrofolic acid.

Calcium L-5-methyltetrahydrofolate in crystalline micronized form is stable during food processing and long-term storage under conditions of high temperature and humidity. It is stable in multivitamin tablets and in micro-encapsulation and food matrix systems. It is less stable in aqueous solution at elevated temperature, forming products with and without folate vitamin activity as a result of oxidative degradation.

Toxicological data

Studies in humans indicate that L-5-methyltetrahydrofolic acid is the only form of folate normally taken up by cells and appearing in plasma, and that cellular uptake is mediated by a reduced folate carrier and folate receptors, which are integral plasma membrane proteins. At exposure to folate of > 200–300 μg/day per person, the metabolic capacity of the human intestinal mucosa for folic acid begins to be exceeded, resulting in small amounts of unaltered folic acid in circulating blood.

In humans given ^{3}H- and ^{14}C-folic acid orally, the bioavailability of synthetic folic acid was estimated to be 90–95%. A study of the absorption of calcium L-5-methyltetrahydrofolate indicated that it dissociates in aqueous media into Ca^{2+} and L-5-methyltetrahydrofolic acid. After absorption, the latter enters the circulation directly, becoming indistinguishable from other absorbed and metabolized natural folates or from L-5-methyltetrahydrofolate formed from synthetic folic acid. The bioavailability of calcium L-5-methyltetrahydrofolate and synthetic folic acid (400 μg/day per person as folate) was compared in a randomized, double-blind, cross-over study of 21 healthy women. The bioavailability of the two compounds was found to be similar. In a 24-week placebo-controlled study in women, the appearance of folate derived from equimolar concentrations of calcium L-5-methyltetrahydrofolate and folic acid was compared in plasma and erythrocytes by a microbiological assay: similar values were found for the two supplements.

A comparison of the bioavailability of naturally occurring folate from food and synthetic folic acid in humans showed significant differences, with a lower level of bioavailability from natural folate than from synthetic folic acid. Another study found that synthetic folic acid appeared in human plasma more slowly than natural folate. The Committee noted that the differences in bioavailability might reflect rate-limiting kinetics for the metabolic conversion of folic acid to L-5-methyltetrahydrofolic acid, as low doses of radiolabelled compounds showed similar short-term distribution, metabolism and kinetics in vivo. Moderately high doses (several hundred micrograms) of folic acid are likely to result in significant hepatic uptake, enterohepatic circulation, tissue distribution and urinary reabsorption.

Calcium L-5-methyltetrahydrofolate was not acutely toxic to rats after a single oral dose (LD_{50} > 2000 mg/kg bw): no gross changes in organs were observed at necropsy, and all animals gained weight and survived until end of the 15-day observation period. In a short-term study of toxicity in male and female Wistar rats given calcium L-5-methyltetrahydrofolate orally for 13 weeks, no adverse effects were seen. The NOEL was 400 mg/kg bw per day, the highest dose tested. A study of embryotoxicity and teratogenicity in Wistar rats given the compound showed no effects up to the highest dose tested (1000 mg/kg bw). The results of a battery of assays for genotoxicity in vitro and in vivo did not indicate any genotoxic potential.

No long-term studies of toxicity or carcinogenicity were submitted; however, the Committee noted that, given the well-characterized metabolism and nutritional function and the known fate of naturally occurring reduced L-5-methyltetrahydrofolic acid as an essential vitamin in humans, such studies were not required.

The Committee took note of a case report in which L-5-methyltetrahydrofolic acid did not mask the clinical features of vitamin B_{12} deficiency. Vitamin B_{12} is essential for the activity of methionine synthase, which converts homocysteine to methionine, with L-5-methyltetrahydrofolic acid as a co-factor. Recycling of homocysteine back to methionine is part of the methylation cycle necessary for methyltransferases, which methylate a wide range of substrates, such as hormones, lipids and proteins, including neural myelin basic protein. In vitamin B_{12} deficiency, the recycling of homocysteine back to methionine diminishes with the level of methionine synthase activity, resulting in neuropathy. Owing to the diminished activity of the enzyme, administration of L-5-methyltetrahydrofolic acid has no effect on the methylation cycle. Likewise, administration of synthetic folic acid has no effect on the methylation cycle because it is not a substrate of the enzyme. The pernicious anaemia arising from vitamin B_{12} deficiency is corrected by synthetic folic acid because it replenishes the supply of tetrahydrofolate and thereby restores the metabolic pathway leading to DNA biosynthesis and red blood cell formation. Folic acid does not restore methylation reactions via methionine synthase, so that neuropathy can progress in the absence of pernicious anaemia (masking). L-5-Methyltetrahydrofolic acid is not expected to correct the pernicious anaemia caused by vitamin B_{12} deficiency because diminished methionine synthase activity leads to failure to convert L-5-methyltetrahydrofolic acid to tetrahydrofolate, the pathway leading to DNA biosynthesis and red blood cell formation. No data were available to determine whether long-term administration of L-5-methyltetrahydrofolic acid would not mask vitamin B_{12} deficiency.

Epidemiological studies provided evidence that high plasma homocysteine levels are a risk factor for cardiovascular disease. A meta-analysis of clinical trials showed that 0.5–5 mg of folic acid could reduce blood homocysteine concentrations by 25–33%. In three intervention studies, lasting up to 24 weeks, groups of healthy persons were given folic acid, vitamin B_{12} or calcium L-5-methyltetrahydrofolate at a dose of ≤ 950 μg per person per day. Significantly elevated plasma folate levels were found in response to folate treatment, accompanied by significantly reduced (by 9–19%) levels of plasma homocysteine. Calcium L-5-methyltetrahydrofolate and synthetic folic acid had similar effects.

Methylenetetrahydrofolate reductase is a key enzyme in folate metabolism, converting methylenetetrahydrofolate to L-5-methyltetrahydrofolic acid. Persons homozygous for a mutation in the gene encoding 5-methyltetrahydrofolic acid reductase have decreased specific enzymatic activity (~ 34% of normal), lower plasma folate levels and higher plasma homocysteine concentrations than persons who express the wild-type gene. The prevalence of this mutant genotype is related to ethnic group, but elevated homocysteine levels are not a specific indicator of inadequate intake of folate.

In a series of studies with healthy persons who had not been not genotyped for 5-methyltetrahydrofolic acid reductase activity, L-5-methyltetrahydrofolic acid was found to be as effective as folic acid in lowering plasma homocysteine, at doses as low as 400 μg/day as folate.

In a case–control study of the risk for colorectal adenoma associated with two polymorphisms in thymidylate synthase, a key enzyme in folate metabolism downstream of L-5-methyltetrahydrofolic acid, an intake of folic acid > 440 μg/day was associated with a 1.5-fold increase in risk for colorectal adenomas in polymorphic individuals with a double-repeat in the enhancer region of the thymidylate synthase gene and an estimated threefold decreased risk in individuals with a more common triple repeat. The Committee noted the existence of several common inherited polymorphisms in folate-metabolizing enzymes; however, the influence and human health significance of such gene–nutrient interactions on overall folate status is unclear.

No controlled studies on human tolerance to calcium L-5-methyltetrahydrofolate were submitted to the Committee. Circumstantial evidence for high tolerance to the compound and to calcium DL-5-methyltetrahydrofolate was provided by studies in which oral doses of 15–17 mg/day for up to 6 months were given to haemodialysis or psychiatric patients. Although no toxic effects were reported, the scope and design of the studies were inadequate to contribute to a safety evaluation.

Assessment of dietary exposure

Both in Europe and the USA, the average folate intake from food sources is about 300 μg/day for men and 250 μg/day for women. Assessments of exposure to folate were available from three countries with a history of supplementation and food fortification with folic acid. It was assumed that calcium L-5-methyltetrahydrofolate would be substituted for synthetic folic acid in the same products and at the same levels. Supplementation leads to increases in the average intake of folate in the adult population of 15–90 μg/day (Ireland and the United Kingdom), and mandatory fortification of foods could increase the average intake of folate by about 200 μg/day (USA). Overall intake from natural foods, fortified foods and supplements could reach 1 mg or more per day for some segments of the adult population.

The calcium provided by 1 mg of calcium L-5-methyltetrahydrofolate amounts to 0.08 mg per adult per day which is insignificant in comparison with the tolerable upper intake levels for calcium.

Evaluation

The Committee concluded that, in humans, the bioavailability of calcium L-5-methyltetrahydrofolate is similar to that of folic acid and that synthetic calcium L-5-methyltetrahydrofolate has the same metabolic fate as other absorbed natural folates. The Committee evaluated the intended use of calcium L-5-methyltetrahydrofolate as a substitute for folic acid but did not evaluate the safety of folate fortification and supplementation. The Committee had no concern about the safety of the proposed use of calcium L-5-methyltetrahydrofolate in dry crystalline or microencapsulated form as an alternative to folic acid in dietary supplements, foods for special dietary uses and other foods.

In view of a number of common inherited polymorphisms in folate metabolism, the Committee recommended that the health effects of folates be evaluated further when there is better understanding of the role of relevant genetic polymorphisms in the population.

A toxicological monograph and a chemical and technical assessment were prepared. New specifications were drawn up.

3.1.4 *Phospholipase A1 from* Fusarium venenatum *expressed in* Aspergillus oryzae

Explanation

At the request of the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*), the Committee evaluated the enzyme

phospholipase A1 (phosphatidylcholine 1-acylhydrolase, E.C. 3.1.1.32), which it had not evaluated previously. Phospholipase A1 is an enzyme that acts specifically on the fatty acid in position 1 in phospholipid substrates, resulting in the formation of lysophospholipids and free fatty acids. It is intended to be used in the dairy industry as a processing aid in the manufacture of cheese.

Genetic modification

The phospholipase A1 enzyme preparation under evaluation is produced by submerged fermentation of *Aspergillus oryzae* carrying a gene encoding phospholipase A1 from *Fusarium venenatum*. The host organism *A. oryzae* is not pathogenic and has a long history of safe use in food. The specific host strain used, *A. oryzae* BECh2, and the production strain derived therefrom, *A. oryzae* PFJo142, were claimed to constitute a safe strain lineage, given the genetic modifications to remove genes encoding amylases and proteases and to remove or strongly reduce the potential for producing secondary metabolites (aflatoxins, cyclopiazonic acid, 3-β-nitropropionic acid and kojic acid). Furthermore, the expression plasmid was fully characterized, as known DNA sequences were used in the construction and the DNA derived from *F. venenatum* was limited to the phospholipase A1 coding sequence. The plasmid does not contain antibiotic resistance genes, nor does it contain any unidentified DNA or DNA sequences that would result in the production of toxic substances. Phospholipase A1 expressed by the production strain has no significant amino acid sequence homology with known allergens or toxins. Two test batches of the phospholipase A1 enzyme preparation were shown not to contain secondary metabolites.

Chemical and technical considerations

Phospholipase A1 is produced by submerged fed-batch pure culture fermentation of the *A. oryzae* PFJo142 production strain. It is secreted into the fermentation medium, from which it is recovered and concentrated. It is subsequently stabilized, formulated and standardized with glycerol, sucrose, water, sodium benzoate and potassium sorbate. The phospholipase A1 enzyme preparation conforms to the *General specifications and considerations for enzyme preparations used in food processing* prepared by the Committee at its fifty-seventh meeting (Annex 1, reference *156*). The enzyme preparation is free from the production organism and recombinant DNA.

The phospholipase A1 preparation is added to milk before the coagulation step in the manufacture of cheese, in order to modify phospholipids in milk. The modified phospholipids have improved emulsification properties and help to retain more solids in the cheese. After coagulation, most of the enzyme

is drained off with the whey stream, which is pasteurized, causing inactivation of phospholipase A1. Any enzyme remaining in the cheese can no longer function, either because there is no substrate left or because the substrate is occluded by the solid cheese matrix and therefore unavailable to the enzyme. Cheese can contain the reaction products, lysophospholipids and free fatty acids, which are considered normal constituents of the diet.

The recommended dosage is up to 10 lecitase units/g milk fat, corresponding to 350 lecitase units (or 3.5 mg of total organic solids) per litre of milk if the milk contains 3.5% milk fat.

Toxicological data

Only two toxicological studies were performed in vitro with the phospholipase A1 enzyme under evaluation, because the *A. oryzae* BECh2 host strain and the production strain *A. oryzae* PFJo142 derived therefrom were considered to constitute a safe strain lineage. Additionally, summaries were provided of toxicological studies performed with four enzymes derived from the same host strain, *A. oryzae* BECh2: a modified lipase, glucose oxidase and two xylanases. The DNA introduced into the production strains of these enzymes is essentially the same as that introduced into the phospholipase A1 production strain, except for the sequence encoding the specific enzyme. At the request of the Committee, full toxicological data were also provided on one of the four enzymes, a xylanase on which studies had recently been conducted. The studies were a 13-week study of oral toxicity in rats, an assay for mutagenicity in bacteria in vitro and two assays of cytogeneticity in human lymphocytes in vitro.

In the two toxicological studies with the phospholipase A1 enzyme, a test batch of the liquid enzyme concentrate was used, without stabilization, formulation or standardization. The liquid enzyme concentrate was not cytotoxic in an assay in mammalian cells in vitro. Considerable cytotoxicity was, however, observed in bacteria in vitro, making it impossible to interpret the result. The experiment was not repeated. Although no explanation was given for the observed cytotoxicity, the Committee considered that it might have been the result of enzymatic activity on the cells. In contrast to the finding for phospholipase A1, no cytotoxicity was observed when the enzyme xylanase, which is also derived from the host strain *A. oryzae* BECh2, was tested in the same assay for mutagenicity in bacteria in vitro.

The Committee noted that the materials added to the phospholipase A1 liquid enzyme concentrate for stabilization, formulation and standardization have either been evaluated previously by the Committee or are common food constituents and do not raise safety concerns.

Assessment of dietary exposure

When phospholipase A1 is used as a processing aid in the production of cheese, most of the enzyme is drained off with the whey, and only a small amount remains in cheese. Although whey derivatives are known to be used as ingredients in processed foods, it is difficult to assess potential dietary exposure because of the wide variety of uses. On the basis of a conservative estimate of daily consumption of 375 g of cheese by a 60-kg adult and on the assumption that the enzyme is used at the recommended dosage and all total organic solids originating from enzyme preparation remain in the cheese, the dietary exposure would be to 0.22 mg of total organic solids per kg bw per day.

Evaluation

The Committee concluded that the information provided on the enzyme phospholipase A1 was too limited to allow an assessment of its safety. Only two test batches of the enzyme preparation were analysed for secondary metabolites, and, of the two toxicological studies provided, one, the assay for mutagenicity in bacteria in vitro, could not be interpreted owing to considerable cytotoxicity. Given that cytotoxicity was not observed when xylanase, an enzyme derived from the same host strain *A. oryzae* BECh2, was tested in the same assay, the Committee decided not to use the toxicological data provided on xylanase to assess the safety of phospholipase A1. The Committee concluded that, in order to make a proper safety assessment, the results of two adequate studies of genotoxicity (including a test for chromosomal aberration in mammalian cells in vitro) and a study of toxicity in vivo would be needed. Alternatives to toxicity testing in vivo would be the demonstration that no unintended compounds are present in the enzyme preparation or better molecular characterization of the genetically modified microorganism.

A toxicological monograph and a chemical and technical assessment were prepared, and new specifications were established.

3.1.5 ***Pullulan***

Explanation

Pullulan is a naturally occurring, fungal polysaccharide produced by fermentation of liquefied corn starch by *Aureobasidium pullulans*, a ubiquitous yeast-like fungus. It has a linear structure, consisting predominantly of repeating maltotriose units, which are made up of three α-1,4-linked glucose molecules, linked by α-1,6-glycosidic bonds. The

maltotriose units are interspersed with some maltotetraose units (about 6%) consisting of four α-1,4-linked glucose molecules; occasionally, there are branch points at which poly-maltotriosyl side-chains are attached to the main chain by a 1,3-glycosidic bond.

Pullulan is used as a glazing agent, as a film-forming agent, as a thickener or as a carrier for additives in the production of capsules for dietary supplements (substitute for gelatin), coatings for coated tablets (dietary supplements), edible flavoured films (breath fresheners), jams and jellies, confectionery and some meat and fruit products. It is also used as a texturizer in chewing-gum and as a foaming agent in milk-based desserts.

The Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*) requested that pullulan be reviewed by the Committee, which has not evaluated the substance previously.

Chemical and technical considerations

Pullulan is produced by fermentation from a food-grade hydrolysed starch with a non-toxin-producing strain of *A. pullulans*. After completion of the fermentation, the fungal biomass is removed by microfiltration, the filtrate is heat-sterilized, and pigments and other impurities are removed by adsorption and ion-exchange chromatography. The product contains not less than 90% glucan on a dried basis. The main impurities are mono-, di- and oligosaccharides from the starting material. The average relative molecular mass of pullulan can vary considerably, depending on culture conditions; a commercially available product has an average relative molecular mass of 200 000 Da.

Pullulan is stable in aqueous solution over a wide pH range (pH 3–8). Upon dry heating, pullulan decomposes and carbonizes at 250–280 °C. It dissolves readily in water but is insoluble in organic solvents. Aqueous solutions of pullulan are viscous but do not form gels. Upon drying, pullulan forms transparent, water-soluble, fat-resistant, odourless, anti-static, flavourless films.

Toxicological data

Pullulan is largely resistant to digestion in the gastrointestinal tract as a result of the occasional presence of 1,3-glycosidic linkages and the high percentage of α-1,6-glycosidic linkages, which are resistant to hydrolysis by salivary and pancreatic amylases. The degree of digestion appears to depend on the relative molecular mass. A commercially available pullulan (relative molecular mass, 200 000 Da) releases only a small amount of reducing sugar after salivary amylase treatment but is converted to a

substance with a lower relative molecular mass (about 70 000 Da) after treatment with an intestinal enzyme preparation.

Pullulan is fermented in the colon in vitro and in vivo by intestinal microflora, to produce short-chain fatty acids, although the degree of fermentation depends on the degree of polymerization of the pullulan. In humans, pullulan (relative molecular mass, 50 000 Da) could not be detected in faeces after daily consumption of 10 g for 14 days, suggesting that it was completely fermented. In contrast to maltodextrin, pullulan reduced the glycaemic response in healthy non-diabetic persons.

Although no studies were conducted to examine the effect of pullulan on the bioavailability of vitamins and minerals, there is no evidence from the published literature that similar polysaccharides of high relative molecular mass have adverse effects on vitamin or mineral bioavailability. When fed to rats at 20% in the diet, pullulan reduced intestinal calcium absorption but did not affect serum calcium levels.

The oral LD_{50} of *A. pullulans* was reported to be > 24 g/kg bw. In rats, a single oral dose of *A. pullulans* lysate at 10 or 20 g/kg bw caused no signs of toxicity. Other studies indicate that *A. pullulans* does not produce toxins and is not toxic when fed to rats.

The oral LD_{50} of pullulan was reported to be > 14 g/kg bw in mice. Short-term studies in rats showed that pullulan has little toxicity. In a 13-week study in rats given diets containing up to 10% pullulan (relative molecular mass, 200 000 Da), no evidence of treatment-related toxicity was found. The study showed a dose-dependent increase in caecum weight (full and empty) as a result of an increased level of poorly digested polysaccharide in the diet. This effect is considered to be a physiological response common to indigestible polysaccharides and of no toxicological significance. The NOEL was 10% in the diet, equal to 7900 mg/kg bw per day, on the basis of the highest dose used in this study. The results of other short-term studies in rats (9 and 62 weeks) support these conclusions. No long-term studies of toxicity or of reproductive toxicity were available on pullulan. Assays for genotoxicity with pullulan in vitro and in vivo gave negative results.

In a 14-day study in humans, daily consumption of 10 g of pullulan (relative molecular mass, 50 000 Da) had no adverse effects. The faecal *Bifidobacteria* population and short-chain fatty acid concentration increased, but no other clinical changes were observed. Abdominal fullness was the only clinical symptom reported. After a single dose of 50 g pullulan (relative molecular mass, 100 000 Da), the frequency of flatulence was increased for 24 h.

Assessment of dietary exposure

Pullulan is used as a substitute for gelatin in the production of capsule shells, as an ingredient of coated tablets and in edible, flavoured films (breath fresheners). The amount of pullulan ingested from one unit of each of these products is 135 mg per capsule, 30 mg per tablet and 29 mg per film.

Data on consumption of food supplements were available from France and the United Kingdom. For consumers at the 97.5th percentile, intake of seven capsules per day was reported to correspond to a dietary exposure to pullulan of 950 mg/day. As dietary supplements for children are usually formulated as tablets, the consumption of pullulan by children was estimated to be lower than that of adults and typically not to exceed 90 mg/day on the basis of intake of three tablets per day, as reported in the United Kingdom. If regular maximum daily consumption of seven capsules (950 mg/day of pullulan) and of one standard packet of breath-freshening films (700 mg/day of pullulan) is assumed, the maximum daily exposure would be 1.65 g.

Pullulan is used in Japan in various foodstuffs, at levels ranging from 2 g/kg in ham and sausages to 30 g/kg in various processed products; use of 50 g/kg was reported in hard sweets. A conservative estimate of dietary exposure from various foods by the budget method, assuming the presence of pullulan at the maximum reported level in a limited fraction of the diet (30 g/kg in 1/16 of the diet, corresponding to 187 g/day), resulted in a value of about 6 g/day. Consumption of sweets by children was considered separately, with consumption figures for France and the USA, resulting in an estimate of about 2.5 g/day.

The Committee recognized that the conservative estimates should not be summed.

Evaluation

The Committee concluded that the current uses of pullulan as a food additive and the studies on its safety provided sufficient information to allocate an ADI ‘not specified’.

A toxicological monograph, new specifications and a chemical and technical assessment were prepared.

3.1.6 ***Quillaia extract type 2***

Explanation

Quillaia extracts (synonyms: quillaja extracts, bois de Panama, Panama bark extracts, quillaia extracts, Quillay bark extracts, soapbark extracts; CAS

No. 68990-67-0, INS No. 999) are obtained by aqueous extraction of the milled inner bark or whole wood of *Quillaja saponaria* Molina (family Rosaceae), which is a large evergreen tree with shiny, leathery leaves and a thick bark, native to China and several South American countries, particularly Bolivia, Chile and Peru. Quillaia extracts (types 1 and 2) are used in foods, primarily for their foaming and emulsifying properties, which are attributed to their saponin content.

Quillaia extracts were considered previously by the Committee, at its twenty-sixth, twenty-ninth, fifty-seventh and sixty-first meetings (Annex 1, references *59*, *70*, *154* and *166*) At its fifty-seventh meeting, the Committee assessed all relevant information on toxicity and dietary exposure, as requested by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (*14*). At that time, the Committee gave temporary status to the ADI of 0–5 mg/kg bw allocated previously to the unpurified extract, pending further clarification of the specifications. The existing specifications were revised and designated 'tentative'.

At its sixty-first meeting, the Committee reviewed new information relating to the chemical characterization of quillaia extracts and further information for specifications. The Committee agreed that separate specifications were needed for the two forms of quillaia extract, type 1 ('unpurified') and type 2 ('semi-purified'). Specifications for the total saponin content of type-1 extract were set at 20–26% (dried weight basis), and the Committee agreed that, while there was some variation in saponin content, the material tested toxicologically was representative of the material specified as type-1 extract. The 'temporary' assignment to the ADI of 0–5 mg/kg bw was therefore removed. Specifications for the total saponin content of type-2 extract after a concentration step of ultra-filtration or chromatography were set at 75–90% (dried weight basis). The saponin profile of the type-2 extract when prepared by membrane ultrafiltration was similar to that of the extract assayed according to the specifications. No information was available on the saponin profile of the extract prepared by chromatography. Limited information was available on the composition of the non-saponin fraction. Although the Committee established separate specifications for type-1 and type-2 quillaia extracts, it was unable to establish an ADI for the type-2 extract owing to the limited information on the qualitative and quantitative composition of this extract. A decision about which additional toxicological studies were needed on the type-2 extract remained suspended in the absence of further data on composition.

Further evaluation of quillaia extract type 2 was requested by the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*). At its present meeting, the Committee considered additional information

on the production and composition of type-2 extract prepared by membrane ultrafiltration in Chile and by chromatography in Japan. The Committee also considered data on the acute toxicity in rats of preparations of quillaia type-1 and type-2 extracts.

Chemical and technical considerations

Quillaia extracts (types 1 and 2) can contain over 100 tri-terpenoid saponins, the glycosides of the aglycone quillaic acid. Other constituents are polyphenols, tannins, oxalate salts, simple sugars and trace amounts of fats and nitrogen compounds. Quillaia extract type 2 is derived from type 1 extract subjected to chromatography or ultrafiltration to reduce the amount of non-saponin soluble solids, such as polyphenols and tannins. Type 2 extract is used in food, primarily for its foaming and emulsifying properties, which are attributed to the saponin content. The chemical compositon and manufacture of type-1 extract were discussed by the Committee at its sixty-first meeting.

Chromatographic analysis indicated that the saponin profile of type-2 extract prepared by membrane ultrafiltration or chromatography is similar to that of type-1 extract. The new information indicated that the range of saponin contents is broader than established in the specifications at the sixty-first meeting; therefore, the specification for total saponin content of type-2 extract was revised to 65–90% on a dried basis at the present meeting.

Toxicological data

The Committee considered data on the acute oral toxicity in rats of two preparations of quillaia extract from Chile: a type-2 extract prepared by ultrafiltration and a type-1 extract. Groups of five Sprague-Dawley rats of each sex were given single oral doses ranging from 3000 to 20 000 mg/kg bw and were observed for clinical signs of toxicity for 14 days. The LD_{50} for the type-2 extract was 6600 mg/kg bw; when expressed in relation to the 14% saponin content of the standardized test material (72% on dry matter basis), the LD_{50} was 900 mg/kg bw. The LD_{50} for the type-1 extract was 11 400 mg/kg bw; when expressed in relation to the 8.8% saponin content of the standardized test material (20% w/w on dry matter basis), the LD_{50} was 1000 mg/kg bw. On the basis of the saponin content, the LD_{50}s for the two extracts were the same: about 900 mg/kg bw. No other new studies of toxicity were available.

Assessment of dietary exposure

Quillaia extracts are used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their major food use is in soft drinks. If a

similar technological function based on saponin content and the interchangeability of type-1 and type-2 extracts are assumed, the estimated dietary exposure to saponins from type-1 and type-2 extracts would be the same.

Evaluation

The previous requirement of the Committee for information on the qualitative and quantitative composition of the type-2 extract was considered to have been met, and the existing specifications for type-2 extract were revised. On the basis of the new information on composition, the Committee assessed the need for additional toxicological studies on this extract, as it had decided at its previous meeting, and concluded that no additional studies were necessary.

The Committee noted that there was no difference between type-1 and type-2 extracts with respect to acute toxicity when expressed in relation to the quillaia saponin content of the extracts.

On the basis of the conclusion of the Committee at its previous meeting that the material tested toxicologically was representative of the material specified as type-1 extract, and assuming that the toxicity is due to the saponin content, the Committee agreed that the ADI for quillaia type-1 extract could be changed to an ADI based on the saponin content and incorporated into a group ADI for type-1 and type-2 extracts. On the basis of the lower end of the range of the saponin content (20%), the ADI for type-1 extract would be 0–1 mg/kg bw expressed as quillaia saponins.

The Committee established a group ADI of 0–1 mg/kg bw for type-1 and type-2 extract expressed as quillaia saponins. The previously established ADI of 0–5 mg/kg bw for type-1 extract was withdrawn.

No toxicological monograph was prepared. The existing specifications and the chemical and technical assessment were revised.

3.1.7 *Quillaia extract type 1: Assessment of dietary exposure*

Explanation

Quillaia extracts were considered previously by the Committee, at its twenty-sixth, twenty-ninth, fifty-seventh and sixty-first meetings (Annex 1, references *59*, *70*, *154* and *166*). At its fifty-seventh meeting, the Committee assessed all relevant information on toxicity and dietary exposure and allocated a temporary ADI of 0–5 mg/kg bw to the unpurified extract, pending clarification of the specifications. At its sixty-first meeting, the

Committee reviewed new information relating to the chemical characterization of quillaia extracts and further information for specifications. The Committee agreed that separate specifications were needed for the two forms of quillaia extract, type 1 ('unpurified') and type 2 ('semi-purified') and also concluded that the data submitted for toxicological and dietary exposure assessment were specific to the material described as type-1 extract. The 'temporary' assignment to the ADI of 0–5 mg/kg bw for quillaia extract type 1 was therefore removed.

At its present meeting, the Committee decided to express the ADI on the basis of the saponin content. Taking the lower end of the specified range, a group ADI of 0–1 mg/kg bw, expressed as quillaia saponins, was established (see section 3.1.6).

Quillaia extracts can be used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their main food use is in soft drinks. The Codex Alimentarius Commission adopted a provision for the use of quillaia extract as a foaming agent at a level of 100 mg/kg in food category 14.1.4, 'water-based flavoured drinks', of the Codex General Standard for Food Additives.

At its fifty-seventh meeting, the Committee estimated dietary exposure to quillaia extracts by a stepwise procedure, assuming a quillaia concentration of 500 mg/kg in all water-based flavoured drinks. On the basis of this use level, the Committee concluded that consumption at the 95th percentile of the distribution of consumption of soft drinks, particularly by children, could exceed the ADI. Estimates of exposure based on consumption of soft drinks in the USA that are likely to contain quillaia at a level of 100 mg/kg were below the ADI.

The previous exposure estimates considered by JECFA did not explicitly include use of quillaia extract in semi-frozen carbonated and non-carbonated beverages. The Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*) therefore requested further information on dietary exposure to quillaia extract type 1 at levels up to 500 mg/kg in these products.

The composition of semi-frozen carbonated and non-carbonated beverages is similar to that of the corresponding unfrozen beverages. They differ in the content of foaming agents and carbonation or addition of air to expand their volume up to 180% of the original. Therefore, a use level of 500 mg/kg in unexpanded semi-frozen carbonated and non-carbonated beverages corresponds to 295 mg/l as consumed, expressed as quillaia extract.

Assessment of dietary exposure based on model diets

Dietary exposure to quillaia extracts was estimated after assuming the presence of the additive at 295 mg/l in all water-based flavoured drinks. High-percentile consumption of soft drinks is 446–1600 ml/day, resulting in a level of exposure to quillaia extracts of 130–470 mg per person per day or 44–160% of the group ADI of 0–1 mg/kg bw expressed as quillaia saponins (see section 3.1.6).

The Committee noted that this assessment assumes that semi-frozen carbonated and non-carbonated beverages are the only source of quillaia extracts. This assumption is based on the fact that no data were submitted about levels of quillaia extracts in solid foods and that it is unlikely that a person who consumes semi-frozen carbonated and non-carbonated beverages would also consume other beverages potentially containing the same additive.

Assessment of dietary exposure based on a probabilistic approach

A probabilistic exposure assessment was submitted, combining the frequency of consumption of semi-frozen carbonated and non-carbonated beverages with the amounts consumed per eating occasion. The amounts were estimated from the size of the containers available and on the consumption of 'frozen novelties' in the USA, used as a surrogate for semi-frozen carbonated and non-carbonated beverages. Assuming that the frequency and the amount per eating occasion are independent variables, dietary exposure to quillaia extracts is below the group ADI of 0–1 mg/kg bw, expressed as quillaia saponins, at the 90th percentile.

The hypothesis of independence between the amount consumed and the frequency of consumption could not be verified from the available information. Therefore, the possibility that a consumer of large amounts of semi-frozen carbonated and non-carbonated beverages is also a frequent consumer cannot be excluded. Assuming 100% dependence between the frequency and the amount of consumption, it is possible to estimate the number of consumers who potentially exceed the group ADI of 0–1 mg/kg bw, as follows: semi-frozen carbonated and non-carbonated beverages are consumed in the USA by 1–7% of the total population, which corresponds to 10 000–70 000 consumers per million. Of those consumers, 15% consume semi-frozen carbonated and non-carbonated beverages at least once a day, corresponding to 1500–10 500 individuals per million, and 1% drink > 1 l/day. Thus, the consumption of 15–100 individuals per million in the whole population could exceed the group ADI under these conditions.

An addendum to the monograph was prepared.

3.2 Revision of specifications

3.2.1 *Aspartame-acesulfame salt*

Aspartame-acesulfame salt was placed on the agenda for revision of specifications at the request of the Codex Committee on Food Additives and Contaminants (*14*). The Committee agreed to delete the text 'after adjusting the pH to alkalinity' in the definition section of the monograph, to change the text under solubility to 'sparingly soluble in water, slightly soluble in ethanol' and to correct the value for specific rotation.

3.2.2 *Hexanes*

The Committee was asked to review the specifications for hexanes in view of a submission that indicated that the present provision for the refractive index was not consistent with the major components present in the current article of commerce. As used in the food industry, hexanes, obtained by fractionation of petroleum, are a mixture of components ranging in chain length from C5 to C7, with a preponderance of C6 components. Recent changes in environmental regulations have led to a reduction of the n-hexane content of hexanes since the original specifications were prepared. In addition, the composition of hexanes depends on the region of production, the source of the raw material and the site of production. Therefore, the Committee concluded that the present articles of commerce differ from those previously evaluated and that the composition of the residues and their levels in foods might not be the same as those evaluated originally.

The Committee decided there was insufficient information available to change the current specifications and recommended a re-evaluation of hexanes at a future meeting.

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

Laccase was reviewed by the Committee at its sixty-first meeting and was placed on the agenda of the present meeting at the request of the Codex Committee on Food Additives and Contaminants at its Thirty-sixth Session (*14*) for further revision of the specifications. In response to the call for data, the Committee received a request to revise the text in the 'sources' section by removing reference to the ancestral strain number. The Committee agreed to this request and also removed the phrase 'non-pathogenic and non-toxigenic', as these requirements are described in the *General specifications and considerations for enzyme preparations used in food processing* (*3*). Furthermore, the description of the production and isolation of the enzyme was amplified.

3.2.4 *Monomagnesium phosphate and trisodium diphosphate*

At its sixty-first meeting (Annex 1, reference *166*), the Committee recommended that the tentative specifications for these two substances should be withdrawn if details of the methods and levels for 'loss on drying' and 'loss on ignition' were not received by the end of 2004. As the Committee received no additional information, it withdrew the specifications.

3.2.5 *Sucrose esters of fatty acids*

In response to a request from the Codex Committee on Food Additives and Contaminants (*14*), the Committee replaced the test methods in the specifications for the determination of free sucrose and propylene glycol by new methods. The Committee noted, however, that the method for determining free sucrose is still unsatisfactory and decided that it should be replaced by either a capillary gas chromatography or a high-performance liquid chromatography method. Furthermore, as the methods for free sucrose and propylene glycol currently require the use of pyridine, the Committee decided to request information on substitution of pyridine by a less toxic solvent.

The Committee also noted that the method for residual dimethyl sulfoxide called for use of a packed gas chromatography column and decided to request an updated method that does not have this requirement. The Committee assigned a tentative designation to the specifications and agreed to withdraw them if the requested information was not received by the end of 2006.

4 Flavouring agents

The Committee was asked to evaluate seven groups of flavouring agents at its present meeting. It agreed to evaluate six by the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*) but decided that it could not evaluate a seventh group with the Procedure because of concerns about the data on safety for some members of the group. The Committee also reviewed the specifications for 135 substances submitted for evaluation and reviewed and revised the existing 'tentative' specifications for four flavouring agents evaluated at previous meetings. Details are given in sections 4.1 and 4.2, and the conclusions and details of further information required are summarized in Annexes 2–6.

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

4.1.1 *Maltol and related substances*

The Committee evaluated a group of seven flavouring agents (see Table 1) comprising maltol and related substances. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). The Committee has evaluated two members of the group previously. Maltol (No. 1480) was evaluated at the eleventh meeting (Annex 1, reference *15*), when a temporary ADI of 0–1 mg/kg bw was established because no results of long-term studies were available. At the eighteenth meeting (Annex 1, reference *35*), the Committee withdrew the temporary ADI because the results of the long-term studies of toxicity that had been requested at the previous meeting had not been made available. At the twenty-second meeting (Annex 1, reference *47*), the Committee evaluated new data on toxicity and established a temporary ADI of 0–0.5 mg/kg bw. At its twenty-fifth meeting (Annex 1, reference *56*), the Committee evaluated additional data and assigned an ADI of 0–1 mg/kg bw. Ethyl maltol (No. 1481) was evaluated at the fourteenth meeting (Annex 1, reference *22*), when the Committee established an ADI of 0–2 mg/kg bw. At its eighteenth meeting (Annex 1, reference *35*), the Committee re-evaluated ethyl maltol and confirmed the previous ADI of 0–2 mg/kg bw.

One of the seven substances, maltol (No. 1480), has been reported to occur naturally in a wide variety of foods, including wheaten and rye bread, milk, butter, uncured pork, beer, cocoa, coffee, peanuts, soya proteins, beans, and clams. Under conditions of baking (e.g. bread, beans) and roasting (cocoa, coffee, peanuts), simple sugars are partly converted to maltol.

Estimated daily per capita *exposure*

Annual volumes of production have been reported for six of the seven flavouring agents in the group (Nos 1480, 1481, 1482, 1484, 1485 and 1486). With respect to the remaining substance (No. 1483), anticipated annual volumes of production have been given for its proposed use as a flavouring agent. The total reported and anticipated annual volumes of production of the seven flavouring agents in this group is about 38 000 kg in Europe and 73 000 kg in the USA. More than 99% of the total reported and anticipated annual volumes of production in Europe and the USA is accounted for by maltol and ethyl maltol. The per capita exposure to maltol in Europe and the USA is approximately 3600 and 2900 μg/day, respectively, and that of ethyl maltol in Europe and the USA is approximately 1900 and 6700 μg/day, respectively. The per capita exposure to the remainder of the

Table 1. Summary of results of safety evaluations of maltol and related substances used or proposed for use as flavouring agents

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class II							
Maltol	1480	118-71-8	Yes Europe: 3585 USA: 2898	No	Yes. The NOEL of 100 mg/kg bw per day (Annex 1, reference 56) is > 1600 times the estimated daily intake of maltol when used as a flavouring agent.	Note 1	At its 25th meeting, JECFA established an ADI of 0–1 mg/kg bw (Annex 1, reference 56).
Ethyl maltol	1481	4940-11-8	Yes Europe: 1851 USA: 6692	No	Yes. The NOEL of 200 mg/kg bw per day for ethyl maltol in rats (Annex 1, reference 35) is > 1800 times the estimated daily intake of ethyl maltol when used as a flavouring agent.	Note 1	At its 18th meeting, JECFA established an ADI of 0–2 mg/kg bw (Annex 1, reference 35).

Table 1 (contd)

Flavouring agent	No.	CAS no. and structure exceed the	Step A3[a] Does intake agent or are its threshold for human intake?[b]	Step A4 Is the flavouring substance or metabolites endogenous?	Step A5 Adequate NOEL for related substance?	Comments	Conclusion based on current intake
Maltyl isobutyrate	1482	65416-14-0	No Europe: 23 USA: 38	NR	NR	Note 2	No safety concern
2-Methyl-3-(1-oxo-propoxy)-4*H*-pyran-4-one	1483	68555-63-5	No Europe: ND USA: 26b	NR	NR	Note 2	No safety concern (conditional)
Structural class III							
2-Amyl-5 or 6-keto-1,4-dioxane	1485	65504-96-3	No Europe: ND USA: 0.2	NR	NR	Note 3	No safety concern

Table 1 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?[b]	Step A4 Is the flavouring agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class III							
2-Butyl-5- or -6-keto-1,4-dioxane	1484	65504-95-2	No Europe: ND	NR USA: 0.5	NR	Note 3	No safety concern
2-Hexyl-5 or 6-keto-1,4-dioxane	1486	65504-97-4	No Europe: ND	NR USA: 0.5	NR	Note 3	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation because intake of the substance was determined to be of no safety concern at Step A3 of the procedure.
Step 2: All the agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure.

[a] The thresholds for human intake for structural classes II and III are 540 μg/day and 90 μg/day, respectively. All intake values are expressed in μg/day. The combined per capita intakes of flavouring agents in structural class II are 5459 μg/day in Europe and 9655 μg/day in the USA. The combined per capita intake of flavouring agents in structural class III is 1.2 μg/day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Notes:
1. Conjugation with glucuronic acid or sulfate followed by excretion in urine
2. Hydrolysis to maltol and the corresponding carboxylic acid, followed by conjugation with glucuronic acid or sulfate and excretion in urine
3. Hydrolysis to a hydroxycarboxylic acid, followed by excretion as the glucuronic acid

flavouring agents in the group is 0–23 μg/day in Europe and 0.2–38 μg/day in the USA, most of the values being at the low end of these ranges. Per capita exposure to each agent is reported in Table 1.

Absorption, distribution, metabolism and elimination

Chemically, maltol is classified as a γ-pyrone. It is a hydroxyl-substituted 4*H*-pyran-4-one and is expected to be metabolized similarly to phenol, primarily undergoing phase II conjugation of the free hydroxy substituent. Maltol (2-methyl-3-hydroxy-4*H*-pyran-4-one) and ethyl maltol (2-ethyl-3-hydroxy-4*H*-pyran-4-one) are predominantly metabolized to sulfate and glucuronic acid conjugates, which are then eliminated in the urine. Maltol esters (Nos 1482 and 1483) are predicted to be hydrolysed to ethyl maltol and the corresponding simple aliphatic carboxylic acid (propionic acid or isobutyric acid) and to undergo further metabolism similar to that of maltol and ethyl maltol.

The remaining three substances (Nos 1484, 1485, and 1486) in the group are α-pyrone derivatives and contain a saturated 3*H*-pyranone nucleus. These three substances are lactones and are readily hydrolysed to yield the corresponding ring-opened hydroxy acid derivatives. In nature, lactones are formed by acid-catalysed intramolecular cyclization of four- or five-carbon hydroxycarboxylic acids to yield five- (γ-) or six- (δ-) membered lactone rings, respectively. The stability of the lactone ring in an aqueous environment is pH-dependent. In basic media such as blood, lactones hydrolyse rapidly to the open-chain hydroxycarboxylic acid. Studies of structurally related lactones indicate that the aliphatic lactones would be hydrolysed to yield the corresponding hydroxycarboxylic acid. These acids can undergo further oxidation to yield polar, excretable metabolites or enter the fatty acid pathway and undergo β-oxidative cleavage to yield polar metabolites of lower relative molecular mass, which are also excreted either unchanged or conjugated in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure

to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure to this group of flavouring agents, the Committee assigned four of the seven agents (Nos 1480, 1481, 1482 and 1483) to structural class II and the remaining three agents (Nos 1484, 1485 and 1486) 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 Procedure.

Step A3. The estimated daily per capita exposure to two of the four agents in structural class II (Nos 1482 and 1483) and of all three agents in structural class III is below the threshold of concern for their respective class (i.e. class II, 540 μg/day; class III, 90 μg/day). Four of these five substances (Nos 1482, 1484, 1485 and 1486) are reported to be used as flavouring agents. According to the Procedure, use of these four agents would not raise concern about safety at estimated daily exposure. The other substance (No. 1483) is proposed for use as a flavour. Although the Procedure indicates no safety concern with use of this flavouring agent at the estimated daily exposure derived from the anticipated annual volume of production, more reliable exposure estimates are needed. Estimated daily exposure to the remaining two agents in structural class II, maltol (No. 1480) and ethyl maltol (No. 1481), exceed the threshold of concern for structural class II. Exposure to maltol is 3600 μg per person per day in Europe and 2900 μg per person per day in the USA. Exposure to ethyl maltol is 1900 μg per person per day in Europe and 6700 μg per person per day in the USA. Accordingly, the evaluation of these two agents proceeded to step A4.

Step A4. Maltol (No. 1480) and ethyl maltol (No. 1481) are not endogenous. Therefore, their evaluation proceeded to step A5.

Step A5. At its twenty-fifth meeting, the Committee established an ADI of 0–1 mg/kg bw for maltol (No. 1480) on the basis of a NOEL of 100 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference *56*). This NOEL is more than 1800 times the estimated daily exposure to this agent from its use as a flavouring agent in Europe or the USA. At its eighteenth meeting, the Committee established an ADI of 0–2 mg/kg bw for ethyl maltol (No. 1481) on the basis of a NOEL of 200 mg/kg bw per day in a 2-year dietary study in rats (Annex 1, reference *35*). This NOEL is more than 1800 times the estimated daily exposure to this substance from its use as a flavouring agent in Europe or the USA. The Committee therefore concluded that exposure to flavouring agents in this group would not raise concern about safety.

The exposure considerations and other information used to evaluate maltol and six related derivatives according to the Procedure are summarized in Table 1.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all four agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold for class II (540 μg per person per day). All four agents in this group are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Their safety is also indicated by the results of studies on the toxicity of maltol and ethyl maltol. An evaluation of all the data indicates that combined exposure would not raise concern about safety.

In the unlikely event that all three agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human exposure threshold for class III (90 μg per person per day). Their safety is also indicated by the results of studies of toxicity. An evaluation of all the data indicates that combined exposure would not raise concern about safety.

Conclusions

The Committee maintained the previously established ADIs of 0–1 mg/kg bw for maltol and 0–2 mg/kg bw for ethyl maltol. The Committee concluded that use of the flavouring agents in this group of maltol and related substances would not present a safety concern at estimated daily exposure. For one agent (No. 1483), the evaluation was conditional, because the estimated daily exposure was based on the anticipated annual volume of production. The conclusion about the safety of this substance will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of the maltol derivatives were consistent with the results of the safety evaluation made with the Procedure.

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

4.1.2 *Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers*

A group of 40 furan-substituted substances was considered by the Committee (see Table 2). The Committee took note of the extensive evidence

Table 2. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

Flavouring agent	No.	CAS No. and structure
Structural class II		
2-Methylfuran	1487	
		534-22-5
2,5-Dimethylfuran	1488	
		625-86-5
2-Ethylfuran	1489	
		3208-16-0
2-Butylfuran	1490	
		4466-24-4
2-Pentylfuran	1491	
		3777-69-3
2-Heptylfuran	1492	
		3777-71-7
2-Decylfuran	1493	
		83469-85-6

Table 2 (contd)

Flavouring agent	No.	CAS No. and structure
3-Methyl-2-(3-methylbut-2-enyl) furan	1494	15186-51-3
3-(2-Furyl)acrolein	1497	623-30-3
3-(5-Methyl-2-furyl)prop-2-enal	1499	5555-90-8
2-Furyl methyl ketone	1503	1192-62-7
2-Acetyl-5-methylfuran	1504	1193-79-9
2-Acetyl-3,5-dimethylfuran	1505	22940-86-9

Table 2 (contd)

Flavouring agent	No.	CAS No. and structure
2-Butyrylfuran	1507	
		4208-57-5
(2-Furyl)-2-propanone	1508	
		6975-60-6
2-Pentanoylfuran	1509	
		3194-17-0
1-(2-Furyl)butan-3-one	1510	
		699-17-2
4-(2-Furyl)-3-buten-2-one	1511	
		623-15-4
Ethyl 3-(2-furyl)propanoate	1513	
		10031-90-0
Isobutyl 3-(2-furan)propionate	1514	
		105-01-1

Table 2 (contd)

Flavouring agent	No.	CAS No. and structure
Isoamyl 3-(2-furan)propionate	1515	7779-67-1
Isoamyl 4-(2-furan)butyrate	1516	7779-66-0
Phenethyl 2-furoate	1517	7149-32-8
Furfuryl methyl ether	1520	13679-46-4
Ethyl furfuryl ether	1521	6270-56-0
Difurfuryl ether	1522	4437-22-3
2,5-Dimethyl-3-furanthiol acetate	1523	55764-22-2

Table 2 (contd)

Flavouring agent	No.	CAS No. and structure
Furfuryl 2-methyl-3-furyl disulfide	1524	109537-55-5
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	61295-44-1
O-Ethyl *S*-(2-furylmethyl)thiocarbonate	1526	376595-42-5
Structural class III		
2,3-Dimethylbenzofuran	1495	3782-00-1
2,4-Difurfurylfuran	1496	64280-32-6
2-Methyl-3-(2-furyl)acrolein	1498	874-66-8
3-(5-Methyl-2-furyl)-butanal	1500	31704-80-0

Table 2 (contd)

Flavouring agent	No.	CAS No. and structure
2-Furfurylidenebutyraldehyde	1501	770-27-4
2-Phenyl-3-(2-furyl)prop-2-enal	1502	65545-81-5
3-Acetyl-2,5-dimethylfuran	1506	10599-70-9
Pentyl 2-furyl ketone	1512	14360-50-0
Propyl 2-furanacrylate	1518	623-22-3
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	114099-96-6

CAS, Chemical Abstracts Service

for the genotoxicity of several members of this group of flavouring agents related to furan, including the clastogenicity of 2-furyl methyl ketone (No. 1503) in mouse bone marrow. Furan, which is carcinogenic, is known to undergo epoxidation and ring opening to form a reactive 2-ene-1,4-dicarbonyl intermediate. Accordingly, there is concern that the observed genotoxicity might be due to formation of a reactive metabolite. No data were available on the potential of members of this group to form reactive metabolites, and no role of metabolism has been identified in the observed genotoxicity. Moreover, there were few data on genotoxicity in vivo, and specific in-vivo assays to address potential carcinogenicity were lacking.

The Committee concluded that the Procedure could not be applied to this group, because of the above concerns. Studies that would assist in resolving the concerns include studies of metabolism and assays for DNA reactivity, mutagenicity and carcinogenic potential in vivo of members of this group with representative structures.

4.1.3 *Eugenol and related hydroxyallylbenzene derivatives*

The Committee evaluated a group of seven hydroxyallylbenzene flavouring agents (Table 3), including eugenol (No. 1529). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). The Committee has evaluated one member of the group previously: eugenol (No. 1529) was evaluated at the twenty-sixth meeting (Annex 1, reference *59*), and an ADI of 0–2.5 mg/kg bw was assigned.

Three of the seven flavouring agents (Nos 1527, 1529 and 1531) have been reported to occur naturally in various foods. They have been detected in wheaten bread, clove buds, leaves and stems, oregano, tarragon, dill, basil, rosemary, pimento leaf and berry, cinnamon bark and leaf, laurel, apples, cherries, whisky and red and white wine.

Estimated daily per capita *exposure*

Annual volumes of production have been reported for four of the seven flavouring agents in this group (Nos 1529–1531 and 1533). For the remaining three substances (Nos 1527, 1528 and 1532), anticipated annual volumes of production have been given for their proposed use as flavouring agents. The total reported and anticipated annual volumes of production of eugenol and the six related hydroxyallylbenzenes are about 7900 kg in Europe and 26 000 kg in the USA. Approximately 98% of the total reported and anticipated annual volume of production in Europe and approximately 97% of that in the USA is accounted for by eugenol (No. 1529). Estimated

Table 3. Summary of results of safety evaluations of eugenol and related hydroxyallylbenzene derivatives

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Structural class I							
4-Allylphenol	1527	501-92-8	No Europe: 0.09[a] USA: 0.2[a]	NR	NR	See notes 1, 2 and 3	No safety concern (conditional)
2-Methoxy-6-(2-propenyl)phenol	1528	579-60-2	No Europe: 0.1[a] USA: 0.2[a]	NR	NR	See notes 1, 2 and 3	No safety concern (conditional)
Eugenol	1529	97-53-0	Yes Europe: 1107 USA: 3364	No	Yes. The NOEL of 300 mg/kg bw per day (National Toxicology Program, 1983) is > 16 000 and 5000 times the estimated daily intakes of 18 μg/kg bw in Europe and 56 μg/kg bw in the USA, respectively, when eugenol is used as a flavouring agent.	See notes 1, 2 and 3	An ADI of 2.5 mg/kg bw was established for eugenol (Annex 1, reference 59), which was maintained at
Eugenyl formate	1530	10031-96-6	No Europe: 0.01 USA: 0.05	NR	NR	See notes 1, 2, 3 and 4	No safety concern

Table 3 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the substance or its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on current intake
Eugenyl acetate	1531	93-28-7	No Europe: 23 USA: 90	NR	NR	See notes 1, 2, 3 and 4	No safety concern
Eugenyl isovalerate	1532	61114-24-7	No Europe: 0.4[a] USA: 0.5[a]	NR	NR	See notes 1, 2, 3 and 4	No safety concern (conditional)
Eugenyl benzoate	1533	531-26-0	No Europe: 0.003 USA: 0.9	NR	NR	See notes 1, 2, 3 and 4	No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.

Step 1: All the agents in this group are in structural class I (Cramer et al., 1978).

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

[a] The threshold for human intake for structural class I is 1800 μg/day. All intake values are expressed in μg/day. The combined per capita intake of the flavouring agents in structural class I is 1130 μg per day in Europe and 3456 μg per day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Table 3 (contd)

Notes:
1. The phenolic hydroxyl group forms a conjugate with glucuronic acid and is readily excreted in the urine.
2. Minor amounts of epoxide are formed on the allyl moiety, which undergoes hydrolysis, followed by conjugation and subsequent excretion.
3. Formation of quinone methide may occur, followed by conjugation with glutathione.
4. The ester group is hydrolysed by carboxyl esterases.

per capita exposure to eugenol is approximately 1100 μg/day in Europe and 3400 μg/day in the USA. Estimated per capita exposure to all the other flavouring agents in the group (Nos 1527, 1528, 1530–1533), on the basis of reported or anticipated annual volumes of production, are 0.003–23 μg/day in Europe, and 0.05–90 μg/day in the USA, most of the values being at the lower end of the ranges. The estimated per capita exposure to each flavouring agent is reported in Table 3.

Absorption, distribution, metabolism and elimination

In humans and rodents, orally administered eugenol and related allylhydroxyphenol derivatives are rapidly absorbed from the gastrointestinal tract and efficiently extracted by the liver, where they mainly undergo phase II conjugation. The resulting glucuronide and sulfate conjugates are subsequently excreted in the urine. To a lesser extent, eugenol is metabolized to polar products, some of which are more reactive than the parent molecule. These products are also conjugated and eliminated, primarily in the urine. Minute amounts (< 1%) of eugenol are excreted unchanged. The primary urinary metabolites of the other agents containing an unsubstituted phenolic group also form glucuronic acid and sulfate conjugates. Eugenyl esters are hydrolysed to eugenol and the corresponding carboxylic acid. These metabolites are readily excreted, primarily in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production, and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure, the Committee assigned all seven agents (Nos 1527–1533) to structural class I.

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

Step A3. Estimated daily per capita exposure to six of the seven flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/day for class I). Three of these six substances (Nos 1530, 1531 and 1533) are reported to be used as flavouring agents; according to the Procedure, use of these three agents raises no safety concern at estimated daily exposure. The other three substances (Nos 1527, 1528 and 1532) are proposed for use as flavouring agents. Although, according to the Procedure, use of these three flavouring agents raises no safety concern at the exposure levels estimated on the basis of the anticipated annual volumes of production, less uncertain exposure estimates are needed. Estimated daily exposure to the remaining agent in this group, eugenol (No. 1529), which is 1107 μg/day in Europe and 3364 μg/day in the USA, exceeds the threshold of concern for class I. Accordingly, the evaluation of eugenol proceeded to step A4.

Step A4. Eugenol and its metabolites are not endogenous. Accordingly, the evaluation of this agent proceeded to step A5.

Step A5. At its twenty-sixth meeting, the Committee established an ADI of 0–2.5 mg/kg bw per day for eugenol on the basis of the results of a 19-week study in rats (Annex 1, reference *59*). At its current meeting, the Committee considered the results of a bioassay in rodents, in which the NOEL was 300 mg/kg bw per day. This NOEL for eugenol, which is consistent with the previous evaluation leading to the ADI, is more than 16 000 and 5000 times the estimated daily exposure to eugenol from its use as a flavouring agent in Europe (18 μg/kg bw) and the USA (56 μg/kg bw), respectively. The Committee therefore concluded that eugenol would not present a safety concern at the estimated daily exposure.

Considerations on exposure and other information used to evaluate eugenol and the six related hydroxyallylbenzene derivatives according to the Procedure are summarized in Table 3.

Consideration of secondary components

One member of this group of flavouring agents, eugenyl formate (No. 1530), has an assay value of < 95%. The secondary component in No. 1530, eugenyl formate, is eugenol (No. 1529), which was evaluated at the present meeting and considered not to present a safety concern at estimated current levels of exposure. The Committee also concluded that the flavouring agent as

specified would not present a safety concern at the estimated levels of exposure.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all seven agents in structural class I were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold for class I (i.e. 1800 μg per person per day). All seven agents in this group are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Moreover, the combined exposure to all seven agents would be well below the ADI of 0–2.5 mg/kg bw for eugenol. Overall evaluation of the data indicates that combined exposure would not raise concerns about safety.

Conclusions

The Committee maintained the previously established ADI of 0–2.5 mg/kg bw for eugenol. It concluded that use of the flavouring agents in the group of eugenol and related hydroxyallylbenzene derivatives would not present a safety concern at the estimated exposure levels. For three flavouring agents (Nos 1527, 1528 and 1532), the evaluation was conditional because the estimated exposure was based on anticipated annual volumes of production. The conclusion of the safety evaluation of these three agents will be revoked if use levels or poundage data are not provided by December 2007. The Committee noted that the available data on the toxicity and metabolism of the hydroxyallylbenzenes are consistent with the results of the safety evaluation with the Procedure.

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

4.1.4 *Anthranilate derivatives*

The Committee evaluated a group of 19 anthranilate derivatives (Table 4) by the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1, Annex 1, references *116*, *122* and *131*). The Committee had previously evaluated two members of this group. Methyl anthranilate (No. 1534) was evaluated at the eleventh meeting (Annex 1, reference *14*) and was assigned a conditional ADI[1] of 0–1.5 mg/kg bw. At its twenty-third meeting (Annex 1, reference *50*), the Committee re-evaluated the conditional ADI of methyl anthranilate and recommended that it be converted to an

[1] 'Conditional ADI', which signifies an ADI with special considerations, is a term no longer used by JECFA.

Table 4. Summary of results of safety evaluations of anthranilate derivatives used or proposed to be used as flavouring agents

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
Structural class I							
Methyl anthranilate	1534	134-20-3	Yes Europe: 804 USA: 3764	No	Yes. The NOEL of 150 mg/kg bw per day (Annex 1, reference 50) is > 11 000 and > 2300 times the estimated daily intakes of 13 and 63 μg/kg bw in Europe and the USA, respectively, when used as a flavouring agent.	See note 1	An ADI of 0–1.5 mg/kg bw was established for methyl anthranilate by the Committee at its twenty-third meeting (Annex 1, reference 50), which was maintained at the present meeting.
Ethyl anthranilate	1535	87-25-2	No Europe: 14 USA: 39	NR	NR	See note 1	No safety concern
Butyl anthranilate	1536	7756-96-9	No Europe: 0.003 USA: 14	NR	NR	See note 1	No safety concern

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
Isobutyl anthranilate	1537	7779-77-3	No Europe: 1 USA: 0.4	NR	NR	See note 1	No safety concern
cis-3-Hexenyl anthranilate	1538	65405-76-7	No Europe: ND USA: 53[b]	NR	NR	See note 1	No safety concern (conditional)
Citronellyl anthranilate	1539	68555-57-7	No Europe: 7[b] USA: 9[b]	NR	NR	See note 1	No safety concern (conditional)
Linalyl anthranilate	1540	7149-26-0	No Europe: 0.04 USA: 0.07	NR	NR	See note 1	No safety concern

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
Cyclohexyl anthranilate	1541	7779-16-0	No Europe: ND USA: 0.007	NR	NR	See note 1	No safety concern
β-Terpinyl anthranilate	1542	14481-52-8	No Europe: 0.004 USA: 1	NR	NR	See note 1	No safety concern
Phenylethyl anthranilate	1543	133-18-6	No Europe: 2 USA: 7	NR	NR	See note 1	No safety concern
β-Naphthyl anthranilate	1544	63449-68-3	No Europe: ND USA: 2	NR	NR	See note 1	No safety concern

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
Methyl *N*-methyl-anthranilate	1545	85-91-6	No Europe: 60 USA: 120	NR	NR	See note 2	An ADI of 0–0.2 mg/kg bw was established for methyl *N*-methyl-anthranilate by the Committee at its twenty-third meeting (Annex 1, reference 50), which was maintained at the present meeting.
Ethyl *N*-methyl-anthranilate	1546	35472-56-1	No Europe: 0.03[b] USA: 0.04[b]	NR	NR	See note 2	No safety concern (conditional)
Ethyl *N*-ethyl-anthranilate	1547	38446-21-8	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 3	No safety concern (conditional)

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
Isobutyl *N*-methyl-anthranilate	1548	65505-24-0	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 2	No safety concern (conditional)
Methyl *N*-formyl-anthranilate	1549	41270-80-8	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 4	No safety concern (conditional)
Methyl *N*-acetyl-anthranilate	1550	2719-08-6	No Europe: 0.05[b] USA: 0.06[b]	NR	NR	See note 5	No safety concern (conditional)
Methyl *N*,*N*-dimethyl-anthranilate	1551	10072-05-6	No Europe: 15[b] USA: 18[b]	NR	NR	See note 6	No safety concern (conditional)

Table 4 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3[a] 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 estimated daily intake
N-Benzoylanthranilic acid	1552	579-93-1	No Europe: 1[b] USA: 2[b]	NR	NR	See note 7	No safety concern (conditional)

CAS: Chemical Abstracts Service; ND: no intake data reported; N/R: not required for evaluation because intake of the substance was determined to be of no safety concern at Step A3 of the procedure.

Step 2: All the agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure.

[a] The threshold for human intake for structural class I is 1800 μg/day. The combined per capita intake of flavouring agents in this group are 904 μg/day in Europe and 4030 μg/day in the USA.

[b] Intake estimate based on anticipated annual volume of production

Notes:

1. Hydrolysed to anthranilic acid, followed by rapid excretion in the urine in conjugated form with glycine (as ortho-aminohippuric acid) or glucuronic acid. The alcohols formed on hydrolysis would be oxidized or conjugated with glucuronic acid or sulfate, followed by excretion in the urine.
2. Hydrolysed to N-methylanthranilic acid, followed by excretion in the urine
3. Hydrolysed to N-ethylanthranilic acid, followed by excretion in the urine
4. Hydrolysed to N-formylanthranilic acid, followed by excretion in the urine
5. Hydrolysed to N-acetylanthranilic acid, followed by excretion in the urine
6. Hydrolyzed to N,N-dimethylanthranilic acid, followed by excretion in the urine
7. Conjugated at the carboxylic acid group to glycine and acyl-glucuronic acid conjugates, followed by excretion in the urine

(unconditional) ADI of 0–1.5 mg/kg bw. Methyl *N*-methylanthranilate (No. 1545) was evaluated at the twenty-third meeting (Annex 1, reference *50*) and was assigned an ADI of 0–0.2 mg/kg bw.

Four of the 19 flavouring agents (Nos 1534, 1535, 1545 and 1546) have been reported to occur naturally in foods. They have been detected in, for example, starfruit, orange juice, grapefruit juice, strawberries and orange, mandarin and tangerine peel oils. The substance that occurs naturally most frequently is methyl anthranilate (No. 1534).

Estimated daily per capita *exposure*

Annual volumes of production have been reported for 10 of the 19 flavouring agents in this group (Nos 1534–1537 and 1540–1545). For the remaining nine substances (Nos 1538, 1539, 1546–1552), anticipated annual volumes of production were given for their proposed use as flavouring agents. The total reported and anticipated annual volume of production of the 19 flavouring agents in this group is about 6300 kg in Europe and 30 000 kg in the USA. Methyl anthranilate (No. 1534) accounts for approximately 89% of the total reported and anticipated annual volume of production in Europe and 94% in the USA. Estimated daily exposure to methyl anthranilate in Europe and the USA is approximately 800 and 3800 μg per person, respectively. Ethyl anthranilate (No. 1535) and methyl *N*-methylanthranilate (No. 1545) account for most of the remaining total reported and anticipated annual volumes of production (approximately 8% in Europe and 4% in the USA). Estimated daily per capita exposure to ethyl anthranilate is 14 μg in Europe and 39 μg in the USA; that to methyl *N*-methylanthranilate is 60 μg in Europe and 120 μg in the USA; and that to the remaining flavouring agents in this group is 0.003–15 μg in Europe and 0.007–53 μg in the USA. The estimated daily per capita exposure to each agent is reported in Table 4.

Absorption, distribution, metabolism and elimination

The 11 anthranilic acid esters (Nos 1534–1544) and the five *N*-alkyl anthranilic acid esters (Nos 1545–1548 and 1551) in this group are expected to be readily absorbed, either unchanged or in hydrolysed form. Once absorbed, the unchanged esters are hydrolysed in the liver to their corresponding alcohols and carboxylic acids (anthranilic acid, *N*-methyl-anthranilic acid, *N*-ethylanthranilic acid or *N*,*N*-dimethylanthranilic acid). These anthranilic acid derivatives are then rapidly excreted in the urine.

Given the relative resistance of the amide bond to hydrolysis, the two combined amides–esters in this group (Nos 1549 and 1550) are expected to be hydrolysed at the methyl ester bond, with rapid excretion of the

corresponding carboxylic acids (*N*-formylanthranilic acid or *N*-acetylanthranilic acid) in the urine, either unchanged or in conjugated form. Rather than undergoing hydrolysis at the amide bond, *N*-benzoylanthranilic acid (No. 1552) will be conjugated with glycine or glucuronic acid at the free carboxylic acid group, before excretion in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure, the Committee assigned all 19 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 the agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily exposure to 18 of the 19 flavouring agents (Nos 1535–1552) is below the threshold of concern (i.e. 1800 μg per person per day for class I). Nine of these 18 substances (Nos 1535–1537 and 1540–1545) are reported to be used as flavouring agents; according to the Procedure, use of these nine flavouring agents raises no safety concern at estimated current exposure. The other nine substances (Nos 1538, 1539 and 1546–1552) are proposed for use as flavouring agents. Although, according to the Procedure, use of these nine flavouring agents raises no safety concern at estimated exposure on the basis of anticipated annual volumes of production, less uncertain estimates are needed. Estimated daily exposure to the remaining agent in this group, methyl anthranilate (No. 1534), which is 804 μg per person in Europe and 3764 μg per person in the USA, exceeds the threshold of concern for class I. Accordingly, the evaluation of methyl anthranilate proceeded to step A4.

Step A4. Methyl anthranilate is not endogenous in humans. Therefore, its evaluation proceeded to step A5.

Step A5. At its twenty-third meeting, the Committee established an ADI of 0–1.5 mg/kg bw for methyl anthranilate on the basis of a NOEL of 150 mg/kg bw per day in a short-term study in rats (Annex 1, reference *50*). This NOEL is > 11 000 and > 2300 times greater than the estimated daily exposure to methyl anthranilate from its use as a flavouring agent in Europe (13 μg/kg bw) and the USA (63 μg/kg bw), respectively. The Committee therefore concluded that methyl anthranilate would not present a safety concern at the estimated daily exposure.

The exposure considerations and other information used to evaluate the 19 anthranilate derivatives in this group according to the Procedure are summarized in Table 4.

Consideration of secondary components

All 19 flavouring agents in this group have minimum assay values of ≥ 95%. Hence, it is not necessary to consider secondary components.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all 19 agents in this group were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold of 1800 μg per person per day for class I. All these agents are, however, expected to be efficiently metabolized and would not saturate metabolic pathways. Overall evaluation of the data indicated that combined exposure to these agents would not raise a safety concern.

Conclusions

The Committee maintained the previously established ADIs of 0–1.5 mg/kg bw for methyl anthranilate and 0–0.2 mg/kg bw for methyl *N*-methylanthranilate (Annex 1, reference *50*). The Committee concluded that use of the flavouring agents in this group of anthranilate derivatives would not present a safety concern at the estimated exposure levels. For nine flavouring agents (Nos 1538, 1539 and 1546–1552), the evaluation was conditional because the estimated exposure was based on anticipated annual volumes of production. The conclusions of the safety evaluations of these agents will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of the anthranilate derivatives were consistent with the results of the safety evaluation conducted with the Procedure.

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

4.1.5 *Miscellaneous nitrogen-containing substances*

The Committee evaluated a group of 16 flavouring agents (Table 5) by the Procedure for the Safety Evaluation of flavouring Agents (Annex 1, reference *131*). This group comprised five structurally related isothiocyanates (Nos 1560–1564) that included allyl isothiocyanate (No. 1560); six alkylated oxazole analogues (Nos 1553–1557 and 1569); two methylated oxazoline analogues (Nos 1558–1559); two pyrimidines (Nos 1565–1566); and one pyrazole (No. 1568).

None of these agents has previously been evaluated by the Committee. Fourteen of the 16 substances (Nos 1553–1565 and 1569) have been reported to occur naturally in foods and have been detected in a variety of vegetables, cooked meats, cocoa, coffee, pineapple and papaya.

Estimated daily per capita exposure

Annual volumes of production have been reported for six of the 16 flavouring agents in this group (Nos 1559, 1560, 1564–1566 and 1568). For the remaining 10 substances (Nos 1553–1558, 1561–1563 and 1569), anticipated annual volumes of production have been given for their proposed use as flavouring agents. The total reported and anticipated annual volume of production of the 16 flavouring agents in this group is about 10 600 kg in Europe and 1400 kg in the USA. More than 98% of the total reported and anticipated annual volume of production in Europe and more than 70% in the USA is accounted for by one substance in this group, allyl isothiocyanate (No. 1560). Estimated per capita exposure to this substance is approximately 1500 μg/day in Europe and 130 μg/day in the USA. Per capita exposure to all the other flavouring agents in the group is 0.03–13 μg/day in Europe and 0.01–52 μg/day in the USA, most of the values being at the lower end of the range (Table 5).

Absorption, distribution, metabolism and elimination

Data on structurally related substances indicate that oxazoles, oxazolines, pyrimidines and pyrazoles will be rapidly absorbed, metabolized and excreted in the urine. The metabolism of oxazoles involves two pathways: oxazole ring cleavage and, if the ring is properly substituted, ring hydroxylation. The presence of a substituent at the 2-position tends to stabilize the oxazole ring.

Isothiocyanates are readily absorbed and distributed to all the main tissues in rodents, peak concentrations in the tissues being achieved 2–8 h after dosing. At comparable doses, there are clear sex- and species-specific differences in the distribution, metabolism and excretion of substituted isothiocyanates.

Table 5. Summary of results of safety evaluations of miscellaneous nitrogen-containing substances

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Structural class II							
Trimethyloxazole	1553	20662-84-4	No Europe: 1[b] USA: 1[b]	Yes. The NOEL of 2.3 mg/kg bw per day for the related substance 2-ethyl-4,5-dimethyl-oxazole (Griffiths et al., 1979) is 115 000 times the estimated daily intake of trimethyloxazole of 0.02 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 3	No safety concern (conditional)
2,5-Dimethyl-4-ethyloxazole	1554	30408-61-8	No Europe: 0.2[b] USA: 0.2[b]	Yes. The NOEL of 2.3 mg/kg bw per day for the related substance 2-ethyl-4,5-dimethyloxazole (Griffiths et al., 1979) is 575 000 times the estimated daily intake 2,5-dimethyl-4-ethyloxazole of 0.004 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 3	No safety concern (conditional)

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Ethyl-4,5-dimethyloxazole	1555	53833-30-0	No Europe: 0.03 USA: 0.7[b]	Yes. The NOEL of 2.3 mg/kg bw per day (Griffiths et al., 1979) is 4 600 000 and 230 000 times the estimated daily intake of 2-ethyl-4,5-dimethyloxazole of 0.0005 μg/kg bw in Europe and 0.01 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 3 (conditional)	No safety concern
2-Isobutyl-4,5-dimethyloxazole	1556	26131-91-9	No Europe: 0.2[b] USA: 0.2[b]	Yes. The NOEL of 2.3 mg/kg bw per day for the related substance 2-ethyl-4,5-dimethyloxazole (Griffiths et al., 1979) is 575 000 times the estimated daily intake of 2-isobutyl-4,5-dimethyloxazole of 0.004 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 3	No safety concern (conditional)

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Methyl-4,5-benzo-oxazole	1557	95-21-6	No Europe: 0.1[b] USA: ND	Yes. The NOEL of 2.3 mg/kg bw per day for the related substance 2-ethyl-4,5-dimethyloxazole (Griffiths et al., 1979) is 1 150 000 times the estimated daily intake of 2-methyl-4,5-benzo-oxazole of 0.002 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 1,2	No safety concern (conditional)
2,4-Dimethyl-3-oxazoline	1558	77311-02-5	No Europe: 0.07[b] USA: ND	Yes. The NOEL of 41 mg/kg bw per day for the related substance 2,4,5-trimethyl-Δ-3-oxazoline (Morgareidge,1972) is 41 000 000 times the estimated daily intake of 2,4-dimethyl-3-oxazoline of 0.001 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 3	No safety concern (conditional)

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2,4,5-Trimethyl-Δ-3-oxazoline	1559	22694-96-8	No Europe: 0.04 USA: 0.01	Yes. The NOEL of 41 mg/kg bw per day (Morgareidge, 1972) is approximately 60 000 000 and 205 000 times the estimated daily intake of 2,4,5-trimethyl-Δ-3-oxazoline of 0.0007 μg/kg bw in Europe and 0.0002 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 3	No safety concern
Allyl isothiocyanate	1560	57-06-7	Yes Europe: 1502 USA: 133		Yes. The NOEL of 12 mg/kg bw per day (National Toxicology Program, 1982) is > 400 and > 5000 times the estimated daily intake of allyl isothiocyanate of 25 μg/kg bw in Europe and 2.2 μg/kg bw in the USA, when used as a flavouring agent.	See notes 4 and 5	No safety concern

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Butyl isothiocyanate	1561	592-82-5	No Europe: 2[b] USA: ND	Yes. The NOEL of 12 mg/kg bw per day for the related substance allyl isothiocyanate (National Toxicology Program, 1982) is 400 000 times the estimated daily intake of butyl isothiocyanate of 0.03 μg/kg bw in Europe, when used as a flavouring agent.	NR	See notes 4 and 5	No safety concern (conditional)
Benzyl isothiocyanate	1562	622-78-6	No Europe: 1[b] USA: 0.4[b]	Yes. The NOEL of 5 mg/kg bw per day for the related substance phenethyl isothiocyanate (Ogawa et al., 2001) is 250 000 and about 700 000 times the estimated daily intake of benzyl isothiocyanate of 0.02 μg/kg bw in Europe and 0.007 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 4	No safety concern (conditional)

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Phenethyl isothiocyanate	1563	2257-09-2	No Europe: 0.4[b] USA: 0.5[b]	Yes. The NOEL of 5 mg/kg bw per day (Ogawa et al., 2001) is 700 000 and about 600 000 times the estimated daily intake of phenethyl isothiocyanate of 0.007 μg/kg bw in Europe and 0.008 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 4	No safety concern (conditional)
3-Methylthiopropyl isothiocyanate	1564	505-79-3	No Europe: 13 USA: 52	Yes. The NOEL of 30 mg/kg bw per day (Harper et al., 1961) is 150 000 and 30 000 times the estimated daily intake of 3-methylthiopropyl isothiocyanate of 0.2 μg/kg bw in Europe and 0.9 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 4	No safety concern

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
4-Acetyl-2-methyl-pyrimidine	1565	67860-38-2	No Europe: ND USA: 0.01	Yes. The NOEL of 1 mg/kg bw per day (Peano, 1981) is 5 000 000 times the estimated daily intake of 4-acetyl-2-methylpyrimidine of 0.0002 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 4	No safety concern
4,5-Dimethyl-2-propyloxazole	1569	53833-32-2	No Europe: 0.1b USA: 0.1b	Yes. The NOEL of 2.3 mg/kg bw per day for the related substance 2-ethyl-4,5-dimethyloxazole (Griffiths et al., 1979) is 1 150 000 times the estimated daily intake of 4,5-dimethyl-2-propyloxazole of 0.002 μg/kg bw in both Europe and the USA, when used as a flavouring agent.	NR	See note 3	No safety concern (conditional)

Table 5 (contd)

Flavouring agent	No.	CAS no. and structure	Step B3[a] Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Structural class III							
5,7-Dihydro-2-methylthieno(3,4-*d*)-pyrimidine	1566	36267-71-7	No Europe: ND USA: 0.4	Yes. The NOEL of 6.6 mg/kg bw per day (Shellenberger, 1970) is 1 100 000 times the estimated daily intake of 5,7-dihydro-2-methylthieno-(3,4-*d*)pyrimidine of 0.006 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 2	No safety concern
1-Phenyl-3- or -5-propylpyrazole	1568	65504-93-0	No Europe: ND USA: 0.2	Yes. The NOEL of 25 mg/kg bw per day (Posternak et al., 1969) is about 6 000 000 times the estimated daily intake of 1-phenyl-3- or -5-propyl-pyrazole of 0.004 μg/kg bw in the USA, when used as a flavouring agent.	NR	See note 2	No safety concern

Table 5 (contd)

CAS, Chemical Abstracts Service; ND, no intake data reported; NR, not required for evaluation because consumption of the substance was determined not to exceed the threshold of concern at Step B3 of the Procedure
Step 1: Fourteen flavouring agents are in structural class II (Nos 1553–1565 and 1569), and two are in structural class III (Nos 1566 and 1568).
Step 2: None of the miscellaneous nitrogen derivatives (Nos 1553–1566, 1568 and 1569) can be predicted to be metabolized to innocuous products.

[a] The thresholds for concern for structural classes II and III are 540 and 90 μg/day, respectively. All intake values are expressed in μg per day. The combined per capita intake of the flavouring agents in structural class II is 1520 μg per day in Europe and 188 μg per day in the USA. The combined per capita intake of the flavouring agents in structural class III is 0.6 μg per day in the USA (no intake data reported for Europe).
[b] Intake estimate based on anticipated annual volume of production

Notes:
1. Predicted to be absorbed rapidly, followed by ring cleavage and excretion in the urine
2. Predicted to be readily absorbed, followed by ring hydroxylation and excretion in the urine
3. Predicted to be readily absorbed, followed by side-chain oxidation and excretion in the urine
4. Readily absorbed, principally conjugated with glutathione, followed by formation of mercapturic acid conjugate and excretion in the urine
5. Hydrolysis followed by excretion in the urine

Metabolic studies in humans, mice and rats indicate that isothiocyanates react readily with reduced glutathione (GSH) to form a conjugate as the principal metabolite and that the reaction is catalysed enzymatically by glutathione *S*-transferase enzymes and non-enzymatically (at a slower rate), both reactions occurring in a pH-dependent equilibrium. The isothiocyanate–GSH conjugates formed are subsequently excreted into bile, and corresponding *N*-acetylcysteine adducts appear as the major metabolite in urine. A key element of isothiocyanate metabolism is the highly electrophilic, reactive central carbon in the group, as it drives Michael addition reactions with *N*-, *O*- or *S*-based nucleophiles (e.g. GSH), giving rise to the relatively stable but reversible conjugates implicated in its toxicity. In humans and rats, aromatic isothiocyanates are metabolized mainly to the corresponding mercapturic acid conjugates, which subsequently hydrolyse to the corresponding cysteine conjugates as the major urinary metabolites. The lability of glutathione conjugates under the conditions in the rodent bladder can lead to formation of unconjugated, 'free' isothiocyanate and GSH. The presence of free isothiocyanates can increase irritation of the rat bladder epithelium. In rabbits, mice and guinea-pigs, however, the cysteine conjugate is hydrolysed and then undergoes transamination and cyclization to form a substituted thiazolidine-2-thione as the main urinary metabolite.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure, the Committee assigned 14 agents in this group (Nos 1553–1565 and 1569) to structural class II and the remaining two agents (Nos 1566 and 1568) to structural class III.

Step 2. None of the flavouring agents in this group can be predicted to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the B-side of the Procedure.

Step B3. Estimated daily per capita exposure in Europe and the USA of 13 of the flavouring agents in structural class II (Nos 1553–1559, 1561–1565 and 1569) and both of the flavouring agents in structural class III (Nos 1566 and 1568) is below the threshold of concern for their respective class (i.e. class II, 540 μg/day; class III, 90 μg/day). Accordingly, the evaluation of these 15 agents proceeded to Step B4. The estimated per capita exposure to one of the agents in structural class II, allyl isothiocyanate (No. 1560), which is 1502 μg/day in Europe and 133 μg/day in the USA, exceeds the threshold of concern (540 μg per day) for its class. In accordance with the Procedure, more extensive data are needed to evaluate the safety of flavouring agents the exposure to which exceeds the threshold of concern for their structural class at Step B3. Additional data on allyl isothiocyanate were therefore considered.

Step B4. For 2-ethyl-4,5-dimethyloxazole (No. 1555), the NOEL of 2.3 mg/kg bw per day in a 91-day study in rats treated by gavage is 4 600 000 times the estimated exposure from its use as flavouring agent in Europe (0.0005 μg/kg bw per day) and 230 000 times the estimated exposure from its proposed use in the USA (0.01 μg/kg bw per day).

The NOEL for 2-ethyl-4,5-dimethyloxazole is also appropriate for the structurally related agents trimethyloxazole (No. 1553), 2,5-dimethyl-4-ethyloxazole (No. 1554), 2-isobutyl-4,5-dimethyloxazole (No. 1556), 2-methyl-4,5-benzo-oxazole (No. 1557) and 4,5-dimethyl-2-propyloxazole (No. 1569), all of which are oxazole analogues and as such are expected to be metabolized via similar metabolic pathways. The NOEL of 2.3 mg/kg bw per day is 115 000 times the estimated exposure to trimethyloxazole from its proposed use as a flavouring agent in both Europe and the USA (0.02 μg/kg bw per day), 575 000 times the estimated exposure to 2,5-dimethyl-4-ethyloxazole and 2-isobutyl-4,5-dimethyloxazole from their proposed use as flavouring agents in both Europe and the USA (0.004 μg/kg bw per day) and 1 500 000 times the estimated exposure to 2-methyl-4,5-benzo-oxazole and 4,5-dimethyl-2-propyloxazole from their proposed use as flavouring agents in both Europe and the USA (0.002 μg/kg bw per day).

The NOEL of 41 mg/kg bw per day for 2,4,5-trimethyl-Δ-3-oxazoline (No. 1559) in a 90-day feeding study in rats is approximately 60 000 000 times the estimated exposure to this substance from its use as flavouring agent in Europe (0.0007 μg/kg bw per day) and 205 000 000 times that in the USA (0.0002 μg/kg bw per day).

The NOEL for 2,4,5-trimethyl-Δ-3-oxazoline is also appropriate for the structurally related agent 2,4-dimethyl-3-oxazoline (No. 1558), as this is an

oxazoline analogue and is therefore expected to be metabolized via similar metabolic pathways. The NOEL of 41 mg/kg bw per day is 41 000 000 times the estimated exposure to 2,4-dimethyl-3-oxazoline from its proposed use as a flavouring agent in Europe (0.001 μg/kg bw per day).

Although no NOEL is available for butyl isothiocyanate (No. 1561), the NOEL of 12 mg/kg bw per day for the structurally related agent allyl isothiocyanate (No. 1560; see below) is also appropriate for butyl isothiocyanate, as they are both isothiocyanates, which will be metabolized via similar metabolic pathways. This NOEL is 400 000 times the estimated exposure to butyl isothiocyanate from its proposed use as a flavouring agent in Europe (0.03 μg/kg bw per day).

Although no NOEL is available for benzyl isothiocyanate (No. 1562), the NOEL of 5 mg/kg bw per day for the structurally related agent phenethyl isothiocyanate is also appropriate for benzyl isothiocyanate, as both are isothiocyanates, which will be metabolized via similar metabolic pathways. This NOEL is 250 000 times the estimated exposure to this substance from its proposed use as a flavouring agent in Europe (0.02 μg/kg bw per day) and about 700 000 times that in the USA (0.007 μg/kg bw per day).

For phenethyl isothiocyanate (No. 1563), the NOEL of 5 mg/kg bw per day in a 91-day feeding study in rats is approximately 700 000 times the estimated exposure to this substance from its proposed use as a flavouring agent in Europe (0.007 μg/kg bw per day) and about 600 000 times that in the USA (0.008 μg/kg bw per day).

For 3-methylthiopropyl isothiocyanate (No. 1564), the NOEL of 30 mg/kg bw per day in an 84-day feeding study in rats is 150 000 times the estimated exposure to this substance from its use as a flavouring agent in Europe (0.2 μg/kg bw per day) and about 30 000 times that in the USA (0.9 μg/kg bw per day).

For 4-acetyl-2-methylpyrimidine (No. 1565), the NOEL of 1 mg/kg bw per day in a 91-day study in rats treated by gavage is 5 000 000 times the estimated exposure to this substance from its use as flavouring agent in the USA (0.0002 μg/kg bw per day).

For 5,7-dihydro-2-methylthieno(3,4-*d*)pyrimidine (No. 1566), the NOEL of 6.6 mg/kg bw per day in a 90-day feeding study in rats is 1 100 000 times the estimated exposure to this substance from its use as a flavouring agent in the USA (0.006 μg/kg bw per day).

For 1-phenyl-3- or -5-propylpyrazole (No. 1568), the NOEL of 25 mg/kg bw per day in a 90-day feeding study in rats is about 6 000 000 times the

estimated exposure to this substance from its use as a flavouring agent in the USA (0.004 μg/kg bw per day).

The Committee concluded that the margins between the estimated daily exposure to the five substances reported to be used as flavouring agents (Nos 1559, 1564–1566 and 1568) and the NOELs for these agents were adequate, and that their use would not present a safety concern. The Committee also concluded that the margins between the exposure estimated from anticipated annual volumes of production for the other 10 substances proposed for use as flavouring agents (Nos 1553–1558, 1561–1563 and 1569) and the NOELs for these agents were adequate. Although their use would raise no safety concern at estimated exposure levels, more accurate estimates of exposure are required.

The exposure considerations and other information used to evaluate these miscellaneous nitrogen-containing flavouring agents are summarized in Table 5.

Consideration of flavouring agents with high exposure evaluated on the B-side of the Procedure

As stipulated in the Procedure, more extensive data on metabolism and toxicity were considered to complete the safety evaluation of allyl isothiocyanate (No. 1560), as the exposure level estimated from use of this compound as a flavouring agent in Europe exceeded the threshold of concern for structural class II (540 μg per person per day).

Short-term and long-term studies

Several short-term of studies of toxicity were conducted in rats and mice given allyl isothiocyanate orally. Furthermore, allyl isothiocyanate was tested in a series of short-term studies of toxicity and long-term studies of carcinogenicity in both laboratory species.

In a 20-day study, groups of five weanling Osborne-Mendel rats of each sex were given allyl isothiocyanate in corn oil at a dose of 0 (vehicle control), 20 or 50 mg/kg bw per day. Macroscopically, the non-glandular part of the stomach was thickened, with occasional roughening of the lining, at both doses. Minor inflammatory foci were reported in the livers of rats at the higher dose.

Groups of male outbred Shoe:WIST rats (number of animals per group not specified) were given allyl isothiocyanate at a dose of 0 (paraffin oil vehicle control), 10, 20 or 40 mg/kg bw per day, 5 days per week for up to 6 weeks by gavage. Hepatohistopathology showed diffuse ballooning of centrilobular hepatocytes in some rats at the highest dose. The kidneys of rats at this dose

and at the middle dose showed dilatation of distal tubules and increased desquamation.

In a 9-week study conducted to examine the possible effects of allyl isothiocyanate on growth, groups of four weanling rats (strain not specified) were fed basal diet (control) or basal diet with 0.1% allyl isothiocyanate for 5 weeks. This dietary level was calculated to provide an average daily exposure to 100 mg/kg bw. During weeks 6–9, the rats were given allyl isothiocyanate by gavage daily at the dose in food (about 100 mg/kg bw). Only animals treated by gavage had lower body-weight gains than controls (statistics not reported).

A dose-range-finding study was conducted in mice. Groups of five $B6C3F_1$ mice of each sex were given allyl isothiocyanate in corn oil by gavage at a dose of 3, 6, 12, 25 or 50 mg/kg bw per day for 14 days. One male at 50 mg/kg bw per day died. No dose-dependent change in body-weight gain was found. Four of the five males and all females at the highest dose showed thickened areas of mucosa in the non-glandular region of the stomach, and four males and one female had a thickened urinary bladder wall.

In a 13-week study, groups of 10 $B6C3F_1$ mice of each sex were given allyl isothiocyanate by gavage at a dose of 0 (vehicle control), 1.5, 3, 6, 12 or 25 mg/kg bw per day on 5 days per week. The mean body weights of treated and control animals were comparable, and no gross or histological changes were reported at any dose.

In a study of carcinogenicity, groups of 50 male and 50 female $B6C3F_1$ mice received allyl isothiocyanate by gavage at a dose of 0 (vehicle control), 12 or 25 mg/kg bw per day, 5 days per week for 103 weeks. Many of the female mice that died before week 104 had suppurative inflammation of the peritoneum, uterus or multiple organs, suggesting generalized infection. The final mean body weights of treated and control animals were comparable (statistics not reported). The incidence of primary tumours was not increased in treated mice. Male mice showed a statistically significant, dose-related increase in cytoplasmic vacuolization in the liver (control, 2/49; low dose, 8/49; high dose, 13/50). The severity of this lesion was similar in the three groups. Most of the vacuoles were centrilobular and all contained fat. The authors concluded that allyl isothiocyanate was not carcinogenic under the conditions of this study.

A dose-range finding study was conducted in groups of five Fischer 344/N rats of each sex given allyl isothiocyanate in corn oil by gavage at a dose of 25, 50, 100, 200 or 400 mg/kg bw per day for 14 days. Clinical signs, including inactivity and ruffled fur, were observed at all doses, the severity increasing with dose. All animals at the two highest doses died before the

end of the study. Reduced body weights were observed at 100 mg/kg bw per day. Gross necropsy revealed a thickened mucosal surface of the stomach and adhesion of the stomach to the peritoneum in treated animals.

Groups of 10 Fischer 344/N rats of each sex were given allyl isothiocyanate by gavage at a dose of 0 (vehicle control), 1.5, 3, 6, 12 or 25 mg/kg bw per day, 5 days per week for 13 weeks. All animals survived to scheduled termination with no clinical signs of toxicity. No significant changes in body weight and no gross or histological changes were reported at any dose.

In a carcinogenesis bioassay, groups of 50 male and 50 female Fischer 344/N rats received allyl isothiocyanate by gavage at a dose of 0 (vehicle control), 12 or 25 mg/kg bw per day, 5 days per week for 103 weeks. The mean body weights of male rats at the higher dose were lower than those of controls throughout the study. No clinical signs of toxicity were reported. Survival (58–74%) was comparable in all groups, including controls. The incidence of subcutaneous fibrosarcomas was increased in females at the higher dose, with a significant positive trend. The incidence of undifferentiated leukaemia was increased over that in controls in treated male rats at both doses: control, 2/50; low dose, 6/50; high dose, 8/50. Males at the higher dose also had a significantly increased incidence of transitional-cell papillomas of the urinary bladder: control, 0/49; high dose, 4/49. Epithelial hyperplasia of the urinary bladder also occurred in males, with a significant overall trend at the higher dose. Hyperplasia was not observed in animals with papillomas. The authors concluded that, under the conditions of this bioassay, allyl isothiocyanate was carcinogenic in male rats, causing transitional-cell papillomas of the urinary bladder, but that the evidence of an association with subcutaneous fibrosarcomas in female rats was equivocal.

Genotoxicity

Numerous tests for genotoxicity were reported. The studies indicated mixed results in vitro, but mainly negative results (8 of 10 studies) in vivo. The two studies with positive results showed only weak activity.

Mode of action

In rats given allyl isothiocyanate at an LD_{50} dose of 112 mg/kg bw by stomach tube, marked irritation of the lungs and gastrointestinal tract was reported. In mice, the sensitizing effects of allyl isothiocyanates on the skin correlated with a perturbation in the ratio of skin GSH to glutathione disulfide, suggesting that the substance might induce oxidative stress in mouse skin epithelia.

An underlying concept in the hypothesis for the mode of toxic action of allyl isothiocyanate is the chemical reactivity of the highly electrophilic central carbon in the isothiocyanate group (–N=C=S). It can efficiently undergo Michael addition reactions with *N*-, *O*- or *S*-based nucleophiles such as the thiol of GSH. The reaction gives rise to relatively stable but reversible GSH adducts, which have been implicated in its cytotoxicity in hepatocytes in vitro. Free and conjugated forms of allyl isothiocyanate are in equilibrium, allowing for the presence of as much as 15–20% free allyl isothiocyanate. The position of the equilibrium can be shifted in favour of the free form under conditions of low GSH concentration and alkaline pH, which may exist in the urinary bladder. Therefore, increased absorption of free allyl isothiocyanate might occur in the bladder epithelium.

Data on the metabolism and disposition of allyl isothiocyanate show clear sex- and species-specific differences. Mice excrete essentially all orally administered allyl ^{14}C-isothiocyanate within 96 h, while rats retain up to 20% in the carcass over the same interval. Blood concentrations of radioactivity returned to background levels within 96 h in mice but persisted up to 240 h in rats. In a separate experiment, mice excreted more than 80% of an administered dose of allyl ^{14}C-isothiocyanate in the urine within 3 days, while rats excreted only 55%, indicating that rats selectively retain thiocyanate ion. Studies in rats showed that females produce twice as much urine as males, so that the male rat bladder is exposed to higher concentrations of allyl isothiocyanate and its metabolites than the female rat bladder.

The histopathological evidence of male bladder epithelial hyperplasia reported in the long-term study in rats correlates with the biochemical evidence that the male rat bladder is subjected to longer exposure to high concentrations of possible irritants, such as allyl isothiocyanate or its metabolites.

Both the qualitative and the quantitative aspects of the molecular disposition of allyl isothiocyanate and its associated toxicological sequelae have been relatively well defined in studies in mammals and are similar to those reported for other irritating substances in the urinary bladder. Epithelial-cell papillomas are benign lesions on luminal surfaces.

On the basis of the observations that allyl isothiocyanate has strong irritant and cytotoxic properties, is considered not to be genotoxic in vivo and induces sex- and species-specific benign transitional-cell papillomas only in male rats, it is highly probable that allyl isothiocyanate operates through a secondary non-genotoxic mechanism, like other irritating bladder carcinogens, such as sodium saccharin.

The two factors that distinguish human exposure to allyl isothiocyanate from that of rats are daily exposure and bladder function. Rats in the 2-year study were given 25 000 μg/kg bw daily, while human exposure from use of allyl isothiocyanate as a flavouring agent is approximately 1000 times less (25 μg/kg bw per day). Furthermore, humans excrete about 1500 ml of urine per day. Therefore, the potential concentration of allyl isothiocyanate or its metabolites in the human bladder is orders of magnitude lower than that required to induce hyperplasia and papillomas in rats. As no toxicity was observed in the bladder at a dose of 12 000 μg/kg bw per day in the study in rats, it is highly unlikely that this mode of action of carcinogenicity would operate in humans.

The NOEL for allyl isothiocyanate in the 2-year study in rats was 12 mg/kg bw per day. This NOEL is more than 400 times the estimated daily exposure to allyl isothiocyanate when used as a flavouring agent in Europe (25 μg/kg bw per day) and more than 5000 times that in the USA (2.2 μg/kg bw per day). Therefore, on the basis of the additional data on toxicity, the Committee concluded that allyl isothiocyanate (No. 1560) would not be expected to present a safety concern at estimated current exposure (Table 5).

Consideration of secondary components

One member of this group of flavouring agents, 2,4,5-trimethyl-Δ-3-oxazoline (No. 1559), has an assay value of < 95%. The secondary component in this substance, trimethyloxazole (No. 1553), was evaluated at the present meeting, where present levels of exposure were considered to present no safety concern. The Committee also concluded that the flavouring agent as specified would not present a safety concern at the estimated levels of exposure.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all 14 agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold for class II (540 μg per person per day). More than 98% of the total combined estimated exposure in Europe (1520 μg/kg bw per day) is accounted for by allyl isothiocyanate (No. 1560), for which toxicity data are available that adequately support the safety of this substance at the exposure level estimated from its use as a flavouring agent. In the unlikely event that both agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human exposure threshold for class III (90 μg per person per day). Overall evaluation of the data indicates that combined exposure would not raise concerns about safety.

Conclusions

The Committee concluded that use of the miscellaneous nitrogen-containing substances would not present a safety concern at the estimated daily exposure. For 10 flavouring agents (Nos 1553–1558, 1561–1563 and 1569), the evaluation was conditional because the estimated exposure was based on anticipated annual volumes of production. The conclusions of the safety evaluations of these agents will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of these miscellaneous nitrogen-containing substances were consistent with the results of the safety evaluation.

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

4.1.6 *Epoxides*

The Committee evaluated a group of nine epoxide flavouring agents, including ethyl methylphenylglycidate (No. 1577) (Table 6). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). The Committee previously evaluated two members of the group. Ethyl 3-phenylglycidate (No. 1576) was evaluated at the twenty-fifth meeting (Annex 1, reference *56*), when no ADI was assigned; ethyl methylphenylglycidate (No. 1577) was evaluated at the twenty-eighth meeting, when an ADI of 0–0.5 mg/kg bw was assigned (Annex 1, reference *66*).

Five of the nine flavouring agents (Nos 1570–1572, 1574 and 1575) have been reported to occur naturally in various foods and have been detected in fruits (e.g. citrus fruit, currants, mango and guava), beverages (beer) and a wide variety of spices and essential oils (e.g. scotch spearmint oil, celery seed, cinnamon bark and leaf oil, clove stem oil, ginger, peppermint oil, cornmint oil, pepper, thyme, hop oil, calamus, basil, rosemary, lemon balm, sage, pimento leaf, winter savoury, angelica seed oil, German camomile oil, and mastic gum oil).

Estimated daily per capita *exposure*

Annual volumes of production have been reported for six of the nine flavouring agents in this group (Nos 1572 and 1574–1578). For the remaining three substances (1570, 1571 and 1573), anticipated annual volumes of production were given for their proposed use as flavouring agents. The total reported and anticipated annual volume of production of the nine epoxides

Table 6. Summary of results of safety evaluations of epoxides used or proposed for use as flavouring agents

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on estimated daily intake
Structural class III							
4,5-Epoxy-(E)-2-decenal	1570	188590-62-7	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 1	No safety concern (conditional)
β-Ionone epoxide	1571	23267-57-4	No Europe: 0.09[b] USA: 0.1[b]	NR	NR	See note 1	No safety concern (conditional)
trans-Carvone-5,6-oxide	1572	18383-49-8	No Europe: 0.01 USA: 0.2	NR	NR	See note 1	No safety concern
Epoxyoxophorone	1573	38284-11-6	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 1	No safety concern (conditional)

Table 6 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on estimated daily intake
Piperitenone oxide	1574	35178-55-3	No Europe: 0.01 USA: 0.2	NR	NR	See note 1	No safety concern
β-Caryophyllene oxide	1575	1139-30-6	No Europe: 0.01 USA: 0.1	NR	NR	See note 1	No safety concern
Ethyl 3-phenylglycidate	1576	121-39-1	Yes Europe: 114 USA: 96	No	Yes. The NOEL for the related compound, ethyl methylphenylglycidate, is 35 mg/kg bw per day (Dunnington et al., 1981), which is > 17 000 times the estimated daily intake of ethyl 3-phenylglycidate of 2 μg/kg bw in Europe and the USA when used as a flavouring agent.	See note 2	No safety concern

Table 6 (contd)

Flavouring agent	No.	CAS no. and structure	Step A3[a] Does intake exceed the threshold for human intake?	Step A4 Is the agent or are its metabolites endogenous?	Step A5 Adequate NOEL for substance or related substance?	Comments	Conclusion based on estimated daily intake
Ethyl methylphenylglycidate	1577	77-83-8	Yes Europe: 240 USA: 1840	No	Yes. The NOEL is 35 mg/kg bw per day (Dunnington et al., 1981), which is > 8000 and > 1000 times the estimated daily intakes of 4 μg/kg bw in Europe and 31 μg/kg bw in the USA from use as a flavouring agent.	See note 3	An ADI of 0–0.5 mg/kg bw was established for ethyl methylphenylglycidate by the Committee at its 28th meeting (Annex 1, reference 66), which was maintained at the present meeting.
Ethyl methyl-*para*-tolylglycidate	1578	74367-97-8	No Europe: 23 USA: 0.009	NR	NR	See note 4	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.
Step 1: All the agents in this group are in structural class III (Cramer et al., 1978).
Step 2: All the agents in this group are expected to be metabolized to innocuous products.

Table 6 (contd)

[a] The threshold for human intake for structural class III is 90 μg/day. All intake values are expressed in μg/day. The combined per capita intakes of flavouring agents in structural class III are 377 μg per day in Europe and 1937 μg per day in the USA.
[b] Intake estimate based on anticipated annual volume of production

Notes:
1. Epoxide hydrolysed via epoxide hydrolase to form vicinal diol, which forms glucuronic acid conjugate and is eliminated in the urine,or the epoxide is directly conjugated with glutathione by glutathione transferase and is eliminated in the urine.
2. The ester group is hydrolysed by carboxyl esterases followed by loss of carbon dioxide and rearrangement to phenacetaldehyde.
3. The ester group is hydrolysed by carboxyl esterases followed by loss of carbon dioxide and rearrangement to 2-phenylpropanal.
4. The ester group is hydrolysed by carboxyl esterases followed by loss of carbon dioxide and rearrangement to para-methyl-2-phenylpropanal.

is about 2600 kg in Europe and 14 800 kg in the USA. About 95% of the total annual reported and anticipated volume in Europe and about 99% of that in the USA are accounted for by ethyl methylphenylglycidate (No. 1577) and ethyl 3-phenylglycidate (No. 1576). Estimated per capita exposure to ethyl methylphenylglycidate are 240 μg/day in Europe and 1800 μg/day in the USA, and those of ethyl 3-phenylglycidate are 114 and 96 μg/day, respectively. Estimated exposure to all the other flavouring agents in the group is 0.01–23 μg/day in Europe and 0.009–0.2 μg/day in the USA. The estimated daily per capita exposure to each agent is reported in Table 6.

Absorption, distribution, metabolism and elimination

Epoxides are characterized by an oxygen-containing three-membered ring. The inherent strain and polarity of the C–O bond in the epoxide ring are factors that promote its cleavage in the presence of suitable nucleophiles. They undergo chemical hydrolysis in gastrointestinal fluids. In vivo, epoxide hydrolase, which has been identified in the cytosol, endoplasmic reticulum (microsomes), mitochondria and nuclei of liver and to some extent kidney cells, catalyses epoxide ring cleavage by water to yield vicinal *trans*-diols. The diols are then excreted primarily in the urine unchanged or as glucuronic acid or sulfate conjugates. Alternatively, epoxides can be conjugated with glutathione, mediated by glutathione *S*-transferase, to yield the corresponding mercapturic acid conjugates, which also are excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated

from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure, the Committee assigned all nine flavouring agents (Nos 1570–1578) to structural class III.

Step 2. All the flavouring agents in this group can be predicted to be metabolized to innocuous products. The evaluation of all the agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. Estimated daily exposure to seven of the nine flavouring agents (Nos 1570–1575 and 1578) are below the threshold of concern for structural class III (90 mg per day). One substance (No. 1578) is reported to be used as a flavouring agent in Europe and the USA, and three others (Nos 1572, 1574 and 1575) are reported to be used in one region only. The remaining three substances (Nos 1570, 1571 and 1573) are only proposed for use as flavouring agents. According to the Procedure, the use of these seven flavouring agents and the estimated exposureraise no safety concern; however, less uncertain exposure estimates are needed for those flavouring agents for which only anticipated volume data were available (Nos 1570, 1571 and 1573).

Estimated daily per capita exposure to the remaining two substances, ethyl 3-phenylglycidate (No. 1576) and ethyl methylphenylglycidate (No. 1577), for which annual volumes of production were reported, exceed the threshold of concern for structural class III (90 μg per day). The per capita exposure to ethyl 3-phenylglycidate (No. 1576) is 114 μg/day in Europe and 96 μg/day in the USA, and that of ethyl methylphenylglycidate (No. 1577) is 240 μg/day in Europe and 1840 μg/day in the USA. Accordingly, the evaluation of these agents proceeded to step A4 of the Procedure.

Step A4. These two agents and their metabolites are not endogenous. Accordingly, the evaluation of this agent proceeded to step A5.

Step A5. At its twenty-eighth meeting, the Committee established an ADI of 0–0.5 mg/kg bw for ethyl methylphenylglycidate on the basis of the results of a long-term study, in which the NOEL was 35 mg/kg bw per day. This NOEL is more than 8000 times the estimated daily exposure of 4 μg/kg

bw in Europe and more than 1000 times that of 31 μg/kg bw in the USA. This NOEL is more than 17 000 times the estimated exposure to the related substance, ethyl 3-phenylglycidate, from its use as a flavouring agent in Europe and in the USA (both 2 mg/kg bw per day). The Committee therefore concluded that these flavouring agents would not present a safety concern at estimated daily exposure levels.

The exposure considerations and other information used to evaluate the nine epoxides according to the Procedure are summarized in Table 6.

Consideration of secondary components

One member of this group of flavouring agents, 4,5-epoxy-(E)-2-decenal (No. 1570), has an assay value of < 95%. The secondary component, 4-5-epoxy-(Z)-2-decenal, is expected to have the same metabolic fate as the E isomer. It was therefore considered not to present a safety concern at the estimated levels of exposure.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all nine flavouring agents in this group were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold for class III (90 μg per person per day); however, all nine agents are expected to be efficiently metabolized at the exposure levels estimated from their use as flavouring agents. Specifically, epoxides primarily undergo epoxide hydrolase-catalysed ring cleavage, resulting in the production of vicinal trans-diols, which are subsequently excreted predominantly in the urine unchanged or as glucuronic acid or sulfate conjugates. In an alternative pathway of metabolism, epoxides can undergo conjugation with GSH to yield the corresponding mercapturic acid conjugates, which are also excreted in urine. Theoretically, therefore, simultaneous consumption of the epoxides (especially trans-epoxides) at sufficiently high concentrations could result in depletion of GSH; however, under normal conditions, intracellular GSH concentrations (1–10 mmol/l) can be replenished and are sufficient to detoxify the concentrations of epoxides resulting from their use as flavouring agents. Moreover, additional cytoprotection is provided by the hydrolytic activity of epoxide hydrolase. Therefore, at the exposure levels resulting from use of the nine epoxides evaluated in this group as flavouring agents and due to the constant replenishment of GSH by biosynthesis, the combined exposure to these flavouring agents would not present a safety concern.

Conclusions

The Committee maintained the previously established ADI of 0–0.5 mg/kg bw for ethyl methylphenylglycidate (No. 1577). It concluded that use of the

flavouring agents in this group of epoxides would not present a safety concern at estimated exposure. For three flavouring agents (Nos 1570, 1571 and 1573), the evaluation was made conditional because estimated daily exposure was based on anticipated annual volumes of production. The conclusions of the safety evaluations of these three agents will be revoked if use levels or poundage data are not provided by December 2007. The Committee noted that the available data on the toxicity and metabolism of these epoxides are consistent with the safety evaluation made with the Procedure.

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

4.1.7 *Aliphatic and aromatic amines and amides*

The Committee evaluated the group of 37 aliphatic and aromatic amine and amide flavouring agents shown in Table 7. The group comprised 13 primary aliphatic and aromatic amines (Nos 1579–1591), five tertiary aliphatic and aromatic amines (Nos 1610–1614), four alicyclic amines (Nos 1607–1609 and 1615), four aliphatic and alicyclic imines (Nos 1603–1606) and 11 amides (Nos 1592–1602). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Annex 1, reference *131*). None of these flavouring agents has been evaluated previously by the Committee.

The Committee noted that the available data on one of the compounds in the group, acetamide (No. 1592), indicated that it was clearly carcinogenic in both mice and rats; although the mechanism of tumour formation is unknown, the possibility of a genotoxic mechanism cannot be discounted. The Committee considered it inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose, and agreed that acetamide would not be evaluated according to the Procedure.

Twenty-eight of the 36 remaining flavouring agents (Nos 1579–1591, 1593, 1598, 1600, 1603, 1604 and 1607–1615) have been reported to occur naturally in various foods. They have been detected in apple, banana, cabbage, carrot, lettuce, rutabaga, tomato, radish, sweet corn, potato, kale, celery, cauliflower, beetroot, rhubarb, sauerkraut, jackfruit, truffle, pepper, laurel, garlic, blue cheeses, Cheddar, Swiss, Camembert, Limburger, Manchengo, provolone, Russian and Tilset cheeses, caviar, fatty fish (raw, smoked, tinned or salted), lean fish (raw, processed or cooked), clam, squid, shrimp, oyster, crab, scallop, beef, pork, chicken, mutton, beer, red and white wine, sherry, sake, cider, cocoa, coffee, black and green tea, barley, oats, popcorn, rice, and wheat and rye breads.

Table 7. Summary of results of safety evaluations of aliphatic and aromatic amines and amides used or proposed for use as flavouring agents

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Structural class I							
Ethylamine	1579	75-04-7	No Europe: 0.1[b] USA: 0.2 [b]	NR	NR	See note 1	No safety concern (conditional)
Propylamine	1580	107-10-8	No Europe: 0.01[b] USA: 0.02[b]	NR	NR	See note 1	No safety concern (conditional)
Isopropylamine	1581	75-31-0	No Europe: 0.0[b] USA: 0.02[b]	NR	NR	See note 1	No safety concern (conditional)
Butylamine	1582	109-73-9	No Europe: 104 USA: 0.01	NR	NR	See note 1	No safety concern
Isobutylamine	1583	78-81-9	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 1	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
sec-Butylamine	1584	13952-84-6	No Europe: 2[b] USA: 2[b]	NR	NR	See note 1	No safety concern (conditional)
Pentylamine	1585	110-58-7	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 1	No safety concern (conditional)
2-Methylbutylamine	1586	96-15-1	No Europe: 0.01[b] USA: 0.02[b]	NR	NR	See note 1	No safety concern (conditional)
Isopentylamine	1587	107-85-7	No Europe: 28 USA: 0.07	NR	NR	See note 1	No safety concern
Hexylamine	1588	111-26-2	No Europe: 0.006[b] USA: 0.007[b]	NR	NR	See note 1	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
1-Amino-2-propanol	1591	78-96-6	No Europe: ND USA: 16[b]	NR	NR	See note 1	No safety concern (conditional)
(+/–)-*N*,*N*-Dimethyl-menthyl succinamide	1602	544714-08-1	No Europe: 71[b] USA: 88[b]	NR	NR	See notes 2 and 3	No safety concern (conditional)
Trimethylamine	1610	75-50-3	No Europe: 153 USA: 70	NR	NR	See note 4	No safety concern
Triethylamine	1611	121-44-8	No Europe: 0.7[b] USA: 0.9[b]	NR	NR	See note 4	No safety concern (conditional)
Tripropylamine	1612	102-69-2	No Europe: 0.01[b] USA: 0.02[b]	NR	NR	See note 4	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Trimethylamine oxide	1614	1184-78-7	No Europe: 0.07[b] USA: 0.09[b]	NR	NR	See note 5	No safety concern (conditional)
Structural class II							
Phenethylamine	1589	64-04-0	No Europe: ND USA: 0.05	NR	NR	See note 6	No safety concern
2-(4-Hydroxyphenyl)-ethylamine	1590	51-67-2	No Europe: 0.01[b] USA: 0.02[b]	NR	NR	See note 7	No safety concern (conditional)
Butyramide	1593	541-35-5	No Europe: 0.001[b] USA: 0.002[b]	NR	NR	See notes 2 and 8	No safety concern (conditional)
1-Pyrroline	1603	5724-81-2	No Europe: ND USA: 0.4[b]	NR	NR	See note 9	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Acetyl-1-pyrroline	1604	99583-29-6	No Europe: 0.09[b] USA: 0.1[b]	NR	NR	See note 10	No safety concern (conditional)
2-Propionylpyrroline	1605	133447-37-7	No Europe: 0.1[b] USA: 0.2[b]	NR	NR	See note 10	No safety concern (conditional)
Piperidine	1607	110-89-4	No Europe: 103 USA: 96	NR	NR	See note 11	No safety concern
2-Methylpiperidine	1608	109-05-7	No Europe: 0.001[b] USA: 0.002[b]	NR	NR	See note 11	No safety concern (conditional)
Pyrrolidine	1609	123-75-1	No Europe: 0.2 USA: 2	NR	NR	See note 11	No safety concern

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Piperazine	1615	110-85-0	No Europe: 0.001[b] USA: 0.002[b]	NR	NR	See note 11	No safety concern (conditional)
Structural class III							
1,6-Hexalactam	1594	105-60-2	No Europe: 0.001[b] USA: 0.002[b]	Yes. The NOEL of 750 mg/kg bw per day (National Toxicology Program, 1982) is at least 2.5 x 1010 times the estimated daily intake of 0.00002 μg/kg bw in Europe and 0.00003 μg/kg bw in the USA) from its proposed use as a flavouring agent.		See notes 2 and 8	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
2-Isopropyl-*N*,2,3-trimethylbutyramide	1595	51115-67-4	Yes Europe: ND USA: 1054[b]	Yes. There is a 14-day study in rats (Nixon & Alden, 1978) and two 14-week studies in rats (Pence, 1980a; Cheng, 1982), as well as a study of reproduction and teratogenicity in rats (Pence, 1980b). The NOEL of 5 mg/kg bw per day in these studies is 280 times the estimated daily intake of 18 μg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)
N-Ethyl (E)-2,(Z)-6-nonadienamide	1596	608514-56-3	No Europe: ND USA: 88[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide is 600 000 times the estimated daily intake of N-ethyl(E)-2,(Z)-6-nonadienamide of 1 μg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
N-Cyclopropyl (E)-2, (Z)-6-nonadienamide	1597	608514-55-2	No Europe: ND USA: 40[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide is > 800 000 times the estimated daily intake of *N*-cyclopropyl-(E)-2,(Z)-6-nonadienamide of 0.7 μg/kg bw from its proposed use as a flavouring agent in the USA.		See notes 2 and 8	No safety concern (conditional)
N-Isobutyl (E,E)-2,4-decadienamide	1598	18836-52-7	No Europe: 67[b] USA: 83[b]	Yes. The NOEL of 572 mg/kg bw per day for the structurally related substance *N*-isobutyl-2,6,8-decatrienamide is at least 600 000 times the estimated daily intake of *N*-isobutyl-(E,E)-2,4-decadienamide of 1 μg/kg bw from its proposed use as a flavouring agent in both Europe and the USA.		See notes 2 and 8	No safety concern (conditional)

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Nonanoyl 4-hydroxy-3-methoxybenzylamide	1599	2444-46-4	No Europe: 7 USA: 0.07[b]	Yes. The NOEL of 8.4 mg/kg bw per day (Posternak et al., 1969) is at least 70 000 times the estimated daily intake from its reported use as a flavouring agent in Europe (0.12 μg/kg bw) and 8 400 000 times that in the USA (0.001 μg/kg bw).		See note 12	No safety concern
Piperine	1600	94-62-2	No Europe: 23 USA: 0.07	Yes. The NOEL of 20 mg/kg bw per day (Bhat & Chandrasekhara, 1986b) is 50 000 times the estimated daily intake from its reported use as a flavouring agent in Europe (0.4 μg/kg bw) and 20 000 000 times that in the USA (0.001 μg/kg bw).		See note 13	No safety concern

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
N-Ethyl-2-isopropyl-5-methylcyclohexane-carboxamide	1601	39711-79-0	Yes Europe: 0.5 USA: 127	Yes. There is a 28-day (Miyata, 1995) and a 22-week study in rats (Hunter et al., 1975) and a 28-day and a 52-week study in dogs (James, 1974). The NOEL of 8 mg/kg bw per day in the studies in rats (Miyata, 1995) is 1 000 000 times the estimated daily intake of *N*-ethyl 2-isopropyl-5-methylcyclohexanecar-boxamide from its reported use as a flavouring agent in Europe (0.008 μg/kg bw) and 4000 times that in the USA (2 μg/kg bw).		See notes 2 and 8	No safety concern

Table 7 (contd)

Flavouring agent	No.	CAS No. and structure	Step A3/B3[a] Does the estimated intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for the flavouring agent or related substance?	Are additional data available to perform a safety evaluation for substances with an estimated intake exceeding the threshold of concern?	Comments	Conclusion based on estimated daily intake
Isopentylidene isopentylamine	1606	35448-31-8	No Europe: 0.009[b] USA: 0.01[b]	Yes. The NOEL of 115 mg/kg bw per day for the related substance *sec*-butylamine (No. 1584) (Gage, 1970) is at least 5.75 x 10^8 times the estimated daily intake of isopentylidene isopentylamine from its proposed use as a flavouring agent in Europe (0.0001 μg/kg bw) and in the USA (0.0002 μg/kg bw).		See note 14	No safety concern (conditional)
N,N-Dimethylphenethylamine	1613	19342-01-9	No Europe: 0.0[b] USA: 0.09[b]	Yes. The NOEL of 247 ppm by inhalation (equivalent to 157 mg/kg bw per day) for the related substance phenethyl alcohol (No. 987) (Lynch et al., 1990) is at least 1 x 108 times the estimated daily intake of *N,N*-dimethylphenethylamine from its proposed use as a flavouring agent in Europe and the USA (0.001 μg/kg bw).		See note 4	No safety concern (conditional)

Table 7 (contd)

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.
Step 1: Sixteen flavouring agents in this group are in structural class I, 11 are in structural class II and 10 are in structural class III (Cramer et al., 1978).
Step 2: Twenty-seven of the agents in this group (Nos 1579–1593, 1602–1605, 1607–1612, 1614 and 1615) are expected to be metabolized to innocuous products. The remaining 10 agents (Nos 1594-1601, 1606 and 1613) are not expected to be metabolized to innocuous agents.

[a] The thresholds for human intake of structural classes I, II and III are 1800, 540 and 90 μg/person per day, respectively. All intake values are expressed in μg/person per day. The combined per capita intakes of the flavouring agents in structural class I is 359 μg/person per day in Europe and 178 μg/person per day in the USA, that of the flavouring agents in structural class II is 103 μg/person per day in Europe and 99 μg/person per day in the USA, and that of the flavouring agents in structural class III is 98 μg/person per day in Europe and 1392 μg per day in the USA.
[b] Intake estimate based on anticipated annual volume of production

Notes:
1. Aliphatic primary amines readily undergo oxidative deamination, and the resulting aldehydes and ketones enter existing pathways of metabolism and excretion.
2. Amides undergo limited hydrolysis with the corresponding ammonium ion or amines and enter known pathways of metabolism and excretion.
3. Anticipated to undergo hydrolysis at the ester moiety, followed by conjugate formation and subsequent elimination in the urine.
4. Tertiary amines primarily undergo *N*-oxidation to form the corresponding N-oxide, which is readily excreted in the urine.
5. Trimethylamine oxide is expected to be readily excreted in the urine.
6. Phenethylamine undergoes oxidative deamination and further oxidation to form phenylacetic acid, which is readily excreted in the urine in conjugate form.
7. Tyramine undergoes rapid deamination by monoamine oxidase and is excreted as acidic metabolites.
8. Amides are expected to undergo oxidation and enter known pathways of metabolism.
9. Pyrroline, an imine, is anticipated to undergo hydrolysis to the corresponding iminoketone, which will be reduced to the corresponding alcohol.
10. The ketone moiety can be anticipated to be reduced to the corresponding alcohol, which will form glucuronic acid conjugates, which are excreted in the urine.
11. Alicyclic amines undergo both *N*- and *C*-oxidation, followed by excretion of the polar metabolites in the urine.
12. This phenolic substance is anticipated readily to form glucuronic acid conjugates, which are excreted in the urine.
13. Hydrolysis of the amide group of piperine and subsequent oxidation of metabolites to form conjugates of piperonylic acid and vanillic acid are expected.
14. This imine is expected to undergo hydrolysis to form isoamylamine and isoamyl aldehyde, which will enter known pathways of metabolism and excretion.

Estimated daily per capita *exposure*

Annual volumes of production were reported for nine of the 36 flavouring agents in this group (Nos 1582, 1587, 1589, 1599–1601, 1607, 1609 and 1610). For the remaining 27 substances, anticipated annual volumes were given for their proposed use as flavouring agents. The total reported and anticipated annual volume of the 36 aliphatic and aromatic amines and amides is about 3900 kg in Europe and 9900 kg in the USA. About 64% of the total reported and anticipated annual volume in Europe is accounted for by butylamine (No. 1582), piperidine (No. 1607) and trimethylamine (No. 1610), and about 78% in the USA is accounted for by 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595), *N*-ethyl-2-isopropyl-5-methylcyclohexane carboxamide (No. 1601) and piperidine (No. 1607). Estimated per capita exposure in Europe to butylamine, piperidine and trimethylamine are 104, 103 and 153 μg/day, respectively. Estimated per capita exposure in the USA to 2-isopropyl-*N*,2,3-trimethylbutyramide, *N*-ethyl-2-isopropyl-5-methyl-cyclohexane carboxamide and piperidine is 1054, 127 and 96 μg per person per day, respectively. Estimated per capita exposure to all the other flavouring agents in the group is 0.001–71 μg/day in Europe and 0.002–88 μg/day in the USA, most of the values being at the lower end of the ranges. The estimated per capita exposure to each agent is reported in Table 7.

Absorption, distribution, metabolism and elimination

A number of the amines in this group are endogenous and have been identified as normal constituents of urine from healthy individuals, as a result of the catabolism of sarcosine, creatine and choline. These include trimethylamine (No. 1610), ethylamine (No. 1579), isopentylamine (No. 1587), piperidine (No. 1607), pyrrolidine (No. 1609), phenethylamine (No. 1589) and trimethylamine oxide (No. 1614).

Aliphatic amines are metabolized primarily by flavin-containing monooxygenases, monoamine oxidases or amine oxidases by a process known as oxidative deamination. The initial step is hydroxylation of the carbon adjacent to the nitrogen (C-oxidation), followed by formation of an imine, with concomitant reduction of molecular oxygen to hydrogen peroxide. The resulting imine is rapidly hydrolysed to the corresponding aldehyde, which is oxidized to the corresponding carboxylic acid. Representative primary aliphatic and aromatic amines in this group are readily absorbed and are rapidly metabolized to carboxylic acids, which are excreted in the urine.

Alicyclic secondary amines (Nos 1607–1609 and 1615) also undergo C-oxidation at the α-carbon, but oxidation can also occur at other carbons on

the ring. The alicyclic imines in this group (Nos 1603–1606) are readily absorbed and rapidly hydrolysed in aqueous solution to yield the corresponding aminoaldehyde or iminoketone, both of which are further metabolized.

Primary, secondary and tertiary amines can also undergo *N*-oxidation by cytochrome P450 enzymes. Primary aliphatic amines with an accessible α-substituted carbon atom can be *N*-oxidized to nitroso groups and subsequently to oximes, which are labile and readily hydrolysed. Secondary amines can be *N*-oxidized to reactive hydroxylamines, which are further oxidized to form nitrones, which are readily hydrolysed. For tertiary amines, *N*-oxidation by flavin-dependent monooxygenases is the primary route of metabolism, resulting in the formation of stable *N*-oxides. Tertiary aliphatic amines can also be metabolized by C-oxidation, leading to dealkylation and formation of the corresponding primary and secondary amines and an aliphatic aldehyde or ketone.

The aliphatic amides in this group are reported to undergo limited hydrolysis, the extent of which depends partly on the chain length. They are well absorbed and metabolized to polar metabolites, although there are limited data on the actual metabolic routes of the amides in this group; a variety of polar metabolites are detected in the urine of animals after an oral dose.

The available data on the aliphatic and aromatic amines in this group indicate that they are likely to be rapidly absorbed in the gastrointestinal tract and transformed by well-understood metabolic pathways to polar metabolites, which are rapidly eliminated in the urine. The information on the amides in this group is more limited.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

In applying the Procedure to flavouring agents for which both a reported and an anticipated volume of production were given, the Committee based its evaluation on the reported volume of production if the exposure estimated from it exceeded the exposure estimated from the anticipated volume of production and applied no conditions to its decision on safety. If the exposure estimated from the anticipated volume of production exceeded the exposure estimated from the reported volume of production, the Committee based its evaluation on the anticipated volume of production but considered its decision on safety to be 'conditional', pending receipt of information on use levels or poundage data by December 2007. In applying the Procedure to flavouring agents for which only anticipated volumes of production were given, the decision was likewise made conditional.

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these flavouring agents, the Committee assigned 16 agents (Nos

1579–1588, 1591, 1602, 1610–1612 and 1614) to structural class I, 10 flavouring agents (Nos 1589, 1590, 1593, 1603–1605, 1607–1609 and 1615) to structural class II and the remaining 10 flavouring agents (Nos 1594–1601, 1606 and 1613) to structural class III.

Step 2. Twenty-six flavouring agents in this group, namely all those in structural classes I and II (Nos 1579–1591, 1593, 1602–1605, 1607–1612, 1614 and 1615), are predicted to be metabolized to innocuous products. The evaluation of these agents therefore proceeded via the A-side of the Procedure. For the 10 flavouring agents in structural class III, namely the medium chain saturated and unsaturated aliphatic and alicyclic amides (Nos 1594–1601 and 1606) and *N*,*N*-dimethylphenethylamine (No. 1613), limited metabolic data were available, and evaluation of these agents therefore proceeded via the B-side of the Procedure.

Step A3. Estimated daily per capita exposure to all 16 flavouring agents in structural class I are below the threshold of concern (1800 μg/day for class I). Three of these 16 substances (Nos 1582, 1587 and 1610) are reported to be used as flavouring agents, and, according to the Procedure, use of these three agents and estimated current exposure raise no safety concern. The other 13 substances (Nos 1579–1581, 1583–1586, 1588–1602 and 1611–1614) are proposed for use as flavouring agents. Although, according to the Procedure, use of these 13 flavouring agents raises no safety concern at the exposure levels estimated from anticipated volumes of production, less uncertain estimates are needed. Estimated daily per capita exposure to all 10 flavouring agents in structural class II are below the threshold of concern (540 μg/day). Three of these 10 substances (Nos 1589, 1607 and 1609) are reported to be used as flavouring agents, and, according to the Procedure, their use raises no safety concern at estimated current exposure. The other seven substances (Nos 1590, 1593, 1603–1605, 1608 and 1615) are proposed for use as flavouring agents. Although, according to the Procedure, use of these seven agents raises no safety concern at the exposure levels estimated from anticipated volumes of production, less uncertain exposure estimates are needed.

Step B3. Estimated per capita exposure to eight of the flavouring agents in structural class III (Nos 1594, 1596–1600, 1606 and 1613) are below the threshold of concern (90 μg/day). One of these substances (No. 1600) is reported to be used as a flavouring agent in Europe and the USA, one (No. 1599) is reported to be used in Europe and to be proposed for use in the USA, and six (Nos 1594, 1596–1598, 1606 and 1613) are proposed for use in both regions. For those seven substances proposed for use in flavours in one or more region (Nos 1594, 1596–1599, 1606 and 1613), less uncertain exposure estimates are needed. In accordance with the Procedure, evaluation

of these eight flavouring agents proceeded to Step B4. Per capita exposure in the USA of the two remaining flavouring agents in structural class III, 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595; exposure, 1054 μg/day) and *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601; exposure, 127 μg/day), exceed the threshold of concern for their class. In accordance with the Procedure, data must be available on these substances or closely related substances for an evaluation of safety. For No. 1595, which is proposed for use as a flavouring agent, a less uncertain exposure estimate is needed.

Step B4. The NOEL of 750 mg/kg bw per day for 1,6-hexalactam (No. 1594) is at least 2.5 x 10^{10} times higher than the estimated exposure from its proposed use as a flavouring agent in Europe (0.00002 μg/kg bw per day) and in the USA (0.00003 μg/kg bw per day).

The NOEL of 572 mg/kg bw per day for the structurally related substance, *N*-isobutyl-2,6,8-decatrienamide, is applicable to *N*-ethyl(E)-2,(Z)-6-nonadienamide (No. 1596), to *N*-cyclopropyl(E)-2,(Z)-6-nonadienamide (No. 1597) and to *N*-isobutyl(E,E)-2,4-decadienamide (No. 1598), as they follow similar pathways of metabolism. This NOEL is 600 000 times the estimated exposure to *N*-ethyl(E)-2,(Z)-6-nonadienamide (No. 1596) from its proposed use as a flavouring agent in the USA (1 μg/kg bw per day) and is more than 800 000 times the estimated exposure to *N*-cyclopropyl(E)-2,(Z)-6-nonadienamide (No. 1597) from its proposed use as flavouring agent in the USA (0.7 μg/kg bw per day) and at least 600 000 times the estimated exposure to *N*-isobutyl(E,E)-2,4-decadienamide (No. 1598) from its proposed use as flavouring agent in Europe and in the USA (both 1 μg/kg bw per day).

The NOEL of 8.4 mg/kg bw per day for nonanoyl 4-hydroxy-3-methoxy-benzylamide (No. 1599) is 70 000 times the estimated exposure from its proposed use as a flavouring agent in Europe (0.12 μg/kg bw per day) and 8 400 000 times that in the USA (0.001 μg/kg bw per day).

The NOEL of 20 mg/kg bw per day for piperine (No. 1600) is 50 000 times the estimated exposure to piperine from its reported use as a flavouring agent in Europe (0.4 μg/kg bw per day) and 20 000 000 times that in the USA (0.001 μg/kg bw per day).

The NOEL of 115 mg/kg bw per day for the structurally related substance *sec*-butylamine (No. 1584) is applicable to isopentylidene isopentylamine (No. 1606) and is at least 5.75 × 10^{8} times the estimated exposure to isopentylidene isopentylamine from its proposed use as flavouring agent in Europe (0.0001 μg/kg bw per day) and in the USA (0.0002 μg/kg bw per day).

The NOEL of 247 ppm in a study in rats treated by inhalation (equivalent to an oral dose of 157 mg/kg bw per day) for the structurally related substance triethylamine (No. 1611) is applicable to *N*,*N*-dimethylphenethylamine (No. 1613) and is at least 1 x 10^8 times the estimated exposure to *N*,*N*-dimethylphenethylamine from its proposed use as flavouring agent in Europe (0.001 μg/kg bw per day) and in the USA (0.001 μg/kg bw per day).

The Committee concluded that the margin between the estimated current exposure to piperine (No. 1600), which is reported to be used as a flavouring agent, and the NOEL for this agent was adequate, and its use would not present a safety concern. The Committee also concluded that the margins between estimated exposure to the other seven substances proposed for use as flavouring agents in one or more regions (Nos 1594, 1596–1599, 1606 and 1613) based on the anticipated annual volumes of production, and the NOELs for these agents were adequate. Although their use would raise no safety concern at estimated exposure levels, less uncertain exposure estimates are needed.

Consideration of flavouring agents with high exposure evaluated on the B-side of the Procedure

In accordance with the Procedure, more data on toxicity were considered to evaluate the safety of 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595) and *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601), as the estimated exposure levels from proposed use (No. 1595) and reported use (No. 1601) as flavouring agents were determined to exceed the threshold of concern for structural class III (90 μg per person per day).

The results of three studies in Sprague-Dawley (CD®) rats treated by gavage were available on 2-isopropyl-*N*-2,3-trimethylbutyramide: a 14-day study in groups of six rats of each sex at a dose of 0, 5, 25 or 50 mg/kg bw in corn oil twice daily; a 14-week study in groups of 30 rats of each sex at a dose of 0, 10, 50 or 100 mg/kg bw in corn oil once daily; and a 14-week study in groups of 30 rats of each sex at a dose of 0, 1, 2, 5, 10 or 50 mg/kg bw in corn oil once daily. The studies showed treatment-related hepatic and renal toxicity at doses of 10 mg/kg bw and higher. The NOEL was 5 mg/kg bw per day, on the basis of histopathological lesions in the kidneys of male rats in the 14-week study. A study of reproductive and teratogenic toxicity in rats at a dose of 0, 10, 50 or 100 mg/kg bw showed no reproductive effects or fetal abnormalities. The NOEL of 5 mg/kg bw per day is 280 times the estimated daily exposure to 2-isopropyl-*N*-2,3-trimethylbutyramide when used as a flavouring agent in the USA (18 μg/kg bw per day).

Two studies were conducted on *N*-ethyl-2-isopropyl-5-methylcyclohexane-carboxamide in rats treated by gavage: a 28-day study in groups of six

Crj:CD(SD) rats of each sex at a dose of 0, 8, 40, 200 or 1000 mg/kg bw per day and a 22-week study in groups of 15 Sprague-Dawley (CFY) rats of each sex at a dose of 0, 100, 300 or 725 mg/kg bw per day. Mild toxicity in the liver and kidneys was observed at doses of 40 mg/kg bw and above. Two further studies were conducted in beagle dogs given gelatine capsules: a 28-day study in groups of one male and one female given a dose of 0, 600, 1000 or 1500 mg/kg bw per day and a 52-week study in groups of three animals of each sex given a dose of 0, 100, 300 or 1000 mg/kg bw per day. These studies showed mild toxic effects in the liver at all doses. The NOEL of 8 mg/kg bw per day in these studies is 1 000 000 times the estimated daily exposure to *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide when used as a flavouring agent in Europe (0.008 μg/kg bw per day) and 4000 times that in the USA (2 μg/kg bw per day).

The additional toxicity data indicate that 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595) and *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) would not be expected to raise safety concerns at their estimated levels of exposure when used as flavouring agents. For one of these agents (No. 1595), however, less uncertain exposure estimates are needed, as the existing estimate was based on anticipated poundage.

The stepwise evaluation of the 36 aliphatic and aromatic amines and amides evaluated according to the Procedure is summarized in Table 7.

Consideration of secondary components

One member of this group of flavouring agents, isopentylidene isopentylamine (No. 1606), has an assay value of < 95%. One of its secondary components, 3-methylbutyraldehyde (No. 258), was evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*) and considered to be of no concern at estimated levels of exposure. The other secondary component, diisopentylamine, has not been evaluated by the Committee; however, it is structurally related to the primary and secondary amines that were evaluated in this group of flavouring agents and is expected to have the same metabolic fate. These amines are primarily oxidized to imines by flavin-containing monooxygenases, monoamine oxidases or amine oxidases, and the resulting imine can be further oxidized to produce the corresponding aldehyde and ammonia. Moreover, the NOELs for the structurally related compounds piperidine (No. 1607) and trimethylamine (No. 1610) are 80 and 160 mg/kg bw per day, respectively. On this basis, diisopentylamine was considered not to present a safety concern at estimated levels of exposure. The Committee also concluded that the flavouring agent as specified would not present a safety concern at the estimated levels of exposure.

Consideration of combined exposure from use as flavouring agents

In the unlikely event that all 16 agents in structural class I were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human exposure threshold for class I (1800 μg per person per day). Likewise, in the unlikely event that all 10 agents in structural class II were to be consumed concurrently on a daily basis, the estimated combined exposure would not exceed the human exposure threshold for class II (540 μg per person per day). In the unlikely event that all 10 agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined exposure would exceed the human exposure threshold for class III (90 μg per person per day); however, the toxicity data available for these substances adequately supports their safety at the exposure levels estimated from their use as flavouring agents. Overall evaluation of the data indicates that combined exposure would not raise safety concerns.

Conclusions

On the basis of the available data on the toxicity of acetamide (No. 1592), the Committee concluded that its use as a flavouring agent or for any other food additive purpose would be inappropriate, and it was therefore not evaluated by the Procedure.

The Committee concluded that use of the remaining 36 flavouring agents in this group of aliphatic and aromatic amines and amides would not present a safety concern at the estimated exposure levels. For 27 flavouring agents (Nos 1579–1581, 1583–1586, 1588, 1590–1591, 1593–1598, 1602–1606, 1608 and 1611–1615), the evaluation was conditional because the exposure was estimated on the basis of an anticipated annual volume of production. The conclusions of the safety evaluations of these 27 flavouring agents will be revoked if use levels or poundage data are not provided before December 2007. The Committee noted that the available data on the toxicity and metabolism of these aliphatic and aromatic amines and amides were consistent with the results of the safety evaluations.

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

4.2 Specifications of identity and purity for flavouring agents

4.2.1 *Specifications for flavouring agents evaluated for the first time*

The Committee reviewed the specifications of 135 substances submitted for evaluation (see Annex 2).

The Committee agreed that acetamide (No. 1592) should not be added to food and decided not to include specifications for this substance. The Committee thus prepared specifications for 134 substances.

The safety evaluations of 53 flavouring agents were made 'conditional', and this decision is noted in the table of specifications for these agents.

Forty flavouring agents in the furan group (Nos 1487–1526) were not fully evaluated, pending further toxicological information. Nevertheless, the Committee decided to retain the specifications as a basis for future safety evaluations of these substances.

Specifications for three flavouring agents, maltyl isobutyrate (No. 1482), 3-acetyl-2,5-dimethylfuran (No. 1506) and 2,4,5-trimethyl-Δ-3-oxazoline (No. 1559), were designated 'tentative', pending receipt of additional data.

New specifications were prepared for 131 flavouring agents (see Annex 2). The Committee noted that seven agents had been evaluated previously as food additives and had food additive specifications. The Committee had already agreed that substances used as flavouring agents should comply with existing food additive specifications. Two of the substances, maltol (No. 1480) and ethyl maltol (No. 1481), were believed to have uses in addition to flavouring agents. Therefore, new specifications were prepared in the format for flavouring agents, and the existing food additive specifications were revised (see section 2.3.6). Five of the substances, eugenol (No. 1529), methyl anthranilate (No. 1534), methyl *N*-methylanthranilate (No. 1545), ethyl 3-phenylglycidate (No. 1576) and ethyl methylphenylglycidate (No. 1577), have no functional uses other than as flavouring agents; therefore, the Committee decided that the specifications presented in flavouring agent format should replace existing food additive specifications (see section 2.3.5).

4.2.2 *Revision of existing specifications for flavouring agents*

The existing 'tentative' specifications for four flavouring agents, sodium 3-methyl-2-oxobutanoate (No. 631.2), sodium 3-methyl-2-oxopentanoate (No. 632.2), sodium 4-methyl-2-oxopentanoate (No. 633.2) and sodium 2-oxo-3-phenylpropionate (No. 1479), were reviewed and revised to include new information on methods of assay. The 'tentative' designations of the specifications were nevertheless maintained, pending more detailed information on these methods.

5. Recommendations

1. 2006 will mark the fiftieth anniversary of the first meeting of the Committee. In recognition of this event, the Committee recommended that FAO and WHO take special note and consider plans for acknowledging this milestone during the 2006 meeting of the Committee.

2. The Committee recommended that all the chapters of the *General principles and methods for the risk assessment of chemicals in food* and the comments received during public review undergo external peer review before the principles and methods are considered and applied by JECFA.

3. The Committee reaffirmed use of the 'threshold of toxicological concern' approach for flavouring agents. It recommended that guidance be drawn up on application of the approach with regard to substances present in the diet in small amounts, such as certain residues of processing aids, packaging materials and contaminants, to provide advice on the risk assessment of substances for which full toxicological datasets are not available or are unnecessary. The Committee recommended that such guidance be developed by a special task group appointed by the Joint FAO and WHO secretaries and incorporated into the *General principles and methods for the risk assessment of chemicals in food*.

4. The *General specifications and considerations for enzyme preparations used in food processing* were revised by the Committee at its fifty-seventh meeting (Annex 1, reference *154*) and published in FAO Food and Nutrition Paper 52, Addendum 9 (Annex 1, reference *156*). Over the past few years, sections of these general specifications have become out of date. The Committee recommended that the document be revised at the next meeting.

5. The Committee noted that the current specifications and safety evaluation for hexanes are not appropriate for the article of commerce and recommended that they be re-evaluated at a future meeting.

6. The Committee recommended that the tentative general method for determining residual solvents by gas chromatography be revised to include more solvents, as part of a general review of the methods of analysis of solvents used in the preparation of food additives, during further revision of *Guide to specifications—general notices, general analytical techniques, identification tests, test solutions and other reference materials*(*3*).

7. To address concerns raised at the fifty-fifth meeting (Annex 1, reference 149), at a recent FAO/WHO workshop on dietary exposure assessment (see section 2.4) and in several recent publications, the Committee recommended that the Secretariat form a small group to consider all relevant aspects of the use of an additional screening method based on use levels, to complement the MSDI, the method used by JECFA for estimating dietary exposure to flavouring agents. The Committee also recommended that experts on intake work with the temporary advisers during preparation of monographs.

8. The Committee recommended that data on poundage be collected regularly for all flavouring agents, so that rolling averages of poundage can be calculated. This information should be collected with attention to adequate quality control.

9. The apparent discrepancy in dietary exposure to some flavouring agents between that estimated from reported poundage and that estimated from published use levels requires further investigation to ensure that the safety evaluations are based on exposure estimates that reflect current (and future) practice in the food and flavouring industries. The Committee recommended that studies be undertaken in this area, giving priority to substances of potential toxicological concern, for which there is only a low margin of safety between the potential exposure level and the NOEL in studies in experimental animals with the same compound or a structural analogue.

10. The Committee recommended that the JECFA Secretariat ensure that data on use levels are included in submissions from sponsors for safety evaluation of flavouring agents, as requested in the call for data. The Committee noted that such data were not submitted by the sponsors at the current meeting. Subsequent submissions that do not contain this information will not be evaluated by the Committee.

11. In view of a number of common inherited polymorphisms in folate metabolism, the Committee recommended that the health effects of folates be evaluated further when there is better understanding of the role of relevant genetic polymorphisms in the population.

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References

1. *Joint FAO/WHO Conference on Food Additives*. Rome, Food and Agriculture Organization of the United Nations, 1956 (FAO Nutrition Meetings Report Series, No. 11); Geneva, World Health Organization, 1956 (WHO Technical Report Series, No. 107).

2. Report of a Joint FAO/WHO Expert Consultation on Safety Assessment of Food Derived from Biotechnology. Geneva, World Health Organization, 2001 (WHO/SDE/PHE/FOS/01.3).

3. *Guide to specifications—general notices, general analytical techniques, identification tests, test solutions and other reference materials*. Rome, Food and Agriculture Organization of the United Nations, 1991 (FAO Food and Nutrition Paper 5, Rev. 2).

4. Report of a Joint FAO/WHO Consultation on Food Consumption and Exposure Assessment of Chemicals, Geneva, World Health Organization, 1997 (WHO/FSF/FOS/97.5).

5. Kroes B, Galli C, Munro I, Schilter B, Tran L-A, Walker R, Wurtzen F. Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. *Food and Chemical Toxicology*, 2000, 38:255–312.

6. Kroes R, Renwick AG, Cheeseman M, Kleiner J, Mangelsdorf I, Piersma A, Schilter B, Schlatter J, van Schothorst F, Vos JG, Wurtzen G. Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet. European branch of the International Life Sciences Institute. *Food and Chemical Toxicology*, 2004, 42;65–83.

7. Munro IC. Safety assessment procedures for indirect food additives: An overview. *Regulatory Toxicology and Pharmacology*, 1990, 12:1–11.

8. Munro IC. *A procedure for the safety evaluation of flavoring substances.* In: *Toxicological evaluation of certain food additives and contaminants* (WHO Food Additives Series 35), Geneva: World Health Organization, Annex 5, 1996.

9. Munro IC, Ford RA, Kennepohl E, Sprenger JG. Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern. *Food and Chemical Toxicology*, 1996 (in press).

10. Renwick AG. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents. *Toxicology Letters*, 2004, 149:223–224.

11. Rulis AM. De minimis and the threshold of regulation. In: Felix CW, ed., Food protection technology. Chelsea, Michigan: Lewis Publishers Inc., 1986, pp. 29–37.

12. Rulis AM. Establishing a threshold of concern. In: Bonin JJ, Stevenson DE, eds, Risk assessment in setting national priorities, New York: Plenum Press, 1986, Vol 7, pp. 271–278.

13. Rulis AM. Threshold of regulation: Options for handling minimal risk situations. In: Finley, JW, Robinson SF, Armstrong DJ, eds, *Food safety assessment* (ACS Symposium Series, No. 484), Washington DC, American Chemical Society, 1992, pp. 132–139.

14. *Report of the Thirty-sixth Session of the Codex Committee on Food Additives and Contaminants*, Rotterdam, Netherlands, 2004 (ALINORM 04/27/12).

15. European Food Safety Authority. *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to calcium L-methylfolate*. Question No. EFSA-Q-2004-007 adopted on 28 Octob er 2004. *EFSA J.* 2004, 135:1-20. Available at: http://www.efsa.eu.int/science/afc/afc_opinions/705_en.html.

16. Institute of Medicine. *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline*. Washington DC, National Academy Press, 1998.

17. FAO/WHO. *Human vitamin and mineral requirements*. Report of a joint FAO/WHP consultation, Bangkok, Thailand, September 1998. Rome: Food and Agricultural Organization of the United Nations. Available at: http://www.fao.org/es/esn/publications.

Annex 1

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

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents.* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.
24. *Specifications for the identity and purity of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.

25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances.* (Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.

40. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others.* FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants.* WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives.* FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.
57. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.
59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

60. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications General notices, general methods, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours.* FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives.* FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 37, 1986.
76. *Principles for the safety assessment of food additives and contaminants in food.* WHO Environmental Health Criteria, No. 70. Geneva, World Health Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.
77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987 and corrigendum.
78. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.

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.
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 25, 1990.
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.
89. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 26, 1990.
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.
95. *Toxicological evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the 37th meetings, 1956–1990*. Rome, Food and Agricultural Organization of the United Nations, 1992 (2 volumes).
97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
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.
100. *Guide to specifications—General notices, general analytical techniques, identification tests, test solutions, and other reference materials.* FAO Food and Nutrition Paper, No. 5, Ref. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.

102. *Toxicological evaluation of certain food additives and naturally occurring toxicants.* WHO Food Additive Series, No. 30, 1993.
103. *Compendium of food additive specifications: addendum 1.* FAO Food and Nutrition Paper, No. 52, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
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.
109. *Compendium of food additive specifications: addendum 2.* FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/7, 1995.
116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.
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.
119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/8, 1996.
122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 37, 1996.

124. *Compendium of food additive specifications, addendum 4.* FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/10, 1998.
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.
134. *Evaluation of certain veterinary drug residues in food* (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 41, 1998.
136. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/11, 1999.
137. *Evaluation of certain food additives* (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.
138. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 42, 1999.
139. *Compendium of food additive specifications, addendum 6.* FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. *Evaluation of certain veterinary drug residues in food* (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, in press.
141. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 43, 2000
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, in press.
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, in press.
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.
152. *Evaluation of certain mycotoxins in food* (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 906, 2002.
153. *Safety evaluation of certain mycotoxins in food.* WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74, 2001.
154. *Evaluation of certain food additives and contaminants* (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, in press.
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. **E***valuation 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* (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.
166. *Evaluation of certain food additives and contaminants* (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 52, 2004.
168. *Compendium of food additive specifications: addendum 11*. FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.

169. *Evaluation of certain veterinary drug residues in food* (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
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.
172. *Compendium of food additive specifications: addendum 12*. FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. *Evaluation of certain food additives* (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. *Safety evaluation of certain food additives* WHO Food Additives Series, No. 54, 2005.
175. *Evaluation of certain food contaminants* (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
176. *Safety evaluation of certain contaminants in food*. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.
177. *Evaluation of certain food additives* (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934 2006.
178. *Compendium of food additive specifications: addendum 13*. FAO Food and Nutrition Paper, No. 52, Add.13 (in prepration).

Annex 2

Acceptable daily intakes, other toxicological information and information on specifications

1. Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Beeswax	R	No safety concern at predicted dietary intake (< 650 mg per person per day), based on long history of use and lack of toxicity observed with major components
Candelilla wax	R	No safety concern at predicted dietary intake (< 650 mg per person per day)
Calcium L-5-methyltetrahydrofolate	N	No safety concern for proposed use in dry crystalline or microencapsulated form as alternative to folic acid used in dietary supplements, foods for special dietary uses and other foods. Safety of folate fortification and supplementation as such not evaluated.
Phospholipase A1 from *Fusarium venenatum* expressed in *Aspergillus oryzae*	N	Information provided too limited to assess safety
Pullulan	N	ADI 'not specified'[b]
Quillaia extract type 1	S	Previous ADI converted to an ADI based on saponin content from the lower end of specified saponin range and established as group ADI for quillaia extract type 1 and quillaia extract type 2. Assessment of dietary exposure included additional use of quillaia extract type 1 in semi-frozen carbonated and non-carbonated beverages (≤ 500 mg/kg product). In a model diet approach, high-percentile consumption estimated to lead to intake of 44–157% of ADI, assuming presence of quillaia extract type 1 at 295 mg/l in all water-based flavoured drinks. In a probabilistic exposure assessment and assuming that the frequency and amount per eating occasion are independent variables, the estimated dietary exposure was below the ADI at the 90th percentile. Assuming 100% dependence between frequency and amount consumed, estimated that 100–700 individuals per million in the entire population could exceed the ADI .

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Quillaia extract type 2	R	Previous ADI established for quillaia extract type 1 converted to an ADI based on saponin content from the lower end of the specified saponin range and established as a group ADI for quillaia extract type 1 and type 2.

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

[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.

2. Food additives considered for specifications only

Food additive	Specifications[a]
Aspartame acesulfame salt	R
Hexanes	See below
Laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*	R
Monomagnesium phosphate and trisodium diphosphate	Wb
Sucrose esters of fatty acids	R, T

[a] R: existing specifications revised; T: tentative specifications; W: existing specifications withdrawn.

[b] As no information was received on these substances, the existing tentative specifications were withdrawn.

Hexanes

As used in the food industry, 'hexane' is a mixture of hydrocarbons. Recent changes in environmental regulations have led to a change in composition of hexanes since the original specifications were established. In addition, the composition of hexanes depends on the region of production, the source of the raw material and the site of production. Therefore, the Committee concluded that the present articles of commerce differ from those previously evaluated by the Committee and that the composition of the residues and their levels in foods may not be the same as those evaluated in the original safety assessment. The Committee also concluded that there was insufficient information available to change the current specifications, and therefore recommended a re-evaluation of hexanes.

3. Flavouring agents evaluated with the Procedure for the Safety Evaluation of Flavouring Agents

See also the discussion on the safety evaluation of flavouring agents in Annexes 3 and 4.

A. Maltol and related substances

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Maltol	1480	N[b]	See footnote c
Ethyl maltol	1481	N[b]	See footnote d
Maltyl isobutyrate	1482	N, T	No safety concern
2-Methyl-3-(1-oxopropoxy)-4*H*-pyran-4-one	1483	N	No safety concern (conditional)[e]
2-Butyl-5- or 6-keto-1,4-dioxane	1484	N	No safety concern
2-Amyl-5 or 6-keto-1,4-dioxane	1485	N	No safety concern
2-Hexyl-5 or 6-keto-1,4-dioxane	1486	N	No safety concern

[a] N: new specifications prepared; T: tentative specifications.
[b] Revised specifications for these substances in the standard additive format were also prepared.
[c] The ADI of 0–1 mg/kg bw established at the twenty-fifth meeting was maintained.
[d] The ADI of 0–2 mg/kg bw established at the eighteenth meeting was maintained.
[e] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.

B. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

The Committee took note of the extensive positive genotoxicity data for several members of this group of flavouring agents related to furan. Furan, which is carcinogenic, is known to undergo epoxidation and ring opening to form a reactive 2-ene-1,4-dicarbonyl intermediate. Accordingly, concern arises that the observed genotoxicity may be due to formation of a reactive metabolite. Data on the potential of members of this group to form a reactive metabolite were not available and the role of metabolism in the observed genotoxicity has not been identified. Moreover, there was a paucity of in vivo genotoxicity data to allay concern. Also, specific in vivo assays to address potential carcinogenicity were lacking. Because of these concerns, the Committee concluded that the Procedure could not be applied to this group.

Flavouring agent	No.	Specifications[a]
2-Methylfuran	1487	N
2,5-Dimethylfuran	1488	N
2-Ethylfuran	1489	N
2-Butylfuran	1490	N
2-Pentylfuran	1491	N
2-Heptylfuran	1492	N
2-Decylfuran	1493	N
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	N
2,3-Dimethylbenzofuran	1495	N
2,4-Difurfurylfuran	1496	N
3-(2-Furyl)acrolein	1497	N
2-Methyl-3(2-furyl)acrolein	1498	N
3-(5-Methyl-2-furyl)prop-2-enal	1499	N
3-(5-Methyl-2-furyl)-butanal	1500	N
2-Furfurylidenebutyraldehyde	1501	N
2-Phenyl-3-(2-furyl)prop-2-enal	1502	N
2-Furyl methyl ketone	1503	N
2-Acetyl-5-methylfuran	1504	N
2-Acetyl-3,5-dimethylfuran	1505	N
3-Acetyl-2,5-dimethylfuran	1506	N,T
2-Butyrylfuran	1507	N
(2-Furyl)-2-propanone	1508	N
2-Pentanoylfuran	1509	N
1-(2-Furyl)butan-3-one	1510	N
4-(2-Furyl)-3-buten-2-one	1511	N
Pentyl 2-furyl ketone	1512	N
Ethyl 3-(2-furyl)propanoate	1513	N
Isobutyl 3-(2-furan)propionate	1514	N
Isoamyl 3-(2-furan)propionate	1515	N
Isoamyl 4-(2-furan)butyrate	1516	N
Phenethyl 2-furoate	1517	N
Propyl 2-furanacrylate	1518	N
2,5-Dimethyl-3-oxo-(2*H*)-fur-4-yl butyrate	1519	N
Furfuryl methyl ether	1520	N
Ethyl furfuryl ether	1521	N
Difurfuryl ether	1522	N
2,5-Dimethyl-3-furanthiol acetate	1523	N
Furfuryl 2-methyl-3-furyl disulfide	1524	N
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	N
O-Ethyl *S*-(2-furylmethyl)thiocarbonate	1526	N

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

C. Eugenol and related hydroxyallylbenzene derivatives

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
4-Allylphenol	1527	N	No safety concern (conditional)[b]
2-Methoxy-6-(2-propenyl)phenol	1528	N	No safety concern (conditional)[b]
Eugenol	1529	R[c]	See footnote d
Eugenyl formate	1530	N	No safety concern
Eugenyl acetate	1531	N	No safety concern
Eugenyl isovalerate	1532	N	No safety concern (conditional)[b]
Eugenyl benzoate	1533	N	No safety concern

[a] N: new specifications prepared; R: existing specifications revised.
[b] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.
[c] As this substance is used only as a flavouring agent, the Committee considered that the existing specifications in the standard food additive format should be deleted.
[d] The ADI of 0-2.5 mg/kg bw established at the twenty-sixth meeting was maintained.

D. Anthranilate derivatives

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Methyl anthranilate	1534	R[b]	See footnote c
Ethyl anthranilate	1535	N	No safety concern
Butyl anthranilate	1536	N	No safety concern
Isobutyl anthranilate	1537	N	No safety concern
cis-3-Hexenyl anthranilate	1538	N	No safety concern (conditional)[d]
Citronellyl anthranilate	1539	N	No safety concern (conditional)[d]
Linalyl anthranilate	1540	N	No safety concern
Cyclohexyl anthranilate	1541	N	No safety concern
β-Terpinyl anthranilate	1542	N	No safety concern
Phenylethyl anthranilate	1543	N	No safety concern
β-Naphthyl anthranilate	1544	N	No safety concern
Methyl *N*-methylanthranilate	1545	R[b]	See footnote e
Ethyl *N*-methylanthranilate	1546	N	No safety concern (conditional)[d]
Ethyl *N*-ethylanthranilate	1547	N	No safety concern (conditional)[d]
Isobutyl *N*-methylanthranilate	1548	N	No safety concern (conditional)[d]
Methyl *N*-formylanthranilate	1549	N	No safety concern (conditional)[d]
Methyl *N*-acetylanthranilate	1550	N	No safety concern (conditional)[d]

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Methyl *N*,*N*-dimethylanthranilate	1551	N	No safety concern (conditional)[d]
N-Benzoylanthranilic acid	1552	N	No safety concern (conditional)[d]

[a] N: new specifications prepared; R: existing specifications revised.
[b] As this substance is used only as a flavouring agent, the Committee decided that the existing specifications in the standard food additive format should be deleted.
[c] The ADI of 0–1.5 mg/kg bw established at the twenty-third meeting was maintained.
[d] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.
[e] The ADI of 0–0.2 mg/kg bw established at the twenty-third meeting was maintained.

E. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Trimethyloxazole	1553	N	No safety concern (conditional)[b]
2,5-Dimethyl-4-ethyloxazole	1554	N	No safety concern (conditional)[b]
2-Ethyl-4,5-dimethyloxazole	1555	N	No safety concern (conditional)[b]
2-Isobutyl-4,5-dimethyloxazole	1556	N	No safety concern (conditional)[b]
2-Methyl-4,5-benzo-oxazole	1557	N	No safety concern (conditional)[b]
2,4-Dimethyl-3-oxazoline	1558	N	No safety concern (conditional)[b]
2,4,5-Trimethyl-δ-3-oxazoline	1559	N,T	No safety concern
Allyl isothiocyanate	1560	N	No safety concern
Butyl isothiocyanate	1561	N	No safety concern (conditional)[b]
Benzyl isothiocyanate	1562	N	No safety concern (conditional)[b]
Phenethyl isothiocyanate	1563	N	No safety concern (conditional)[b]
3-Methylthiopropyl isothiocyanate	1564	N	No safety concern
4-Acetyl-2-methylpyrimidine	1565	N	No safety concern
5,7-Dihydro-2-methylthieno(3,4-*d*)pyrimidine	1566	N	No safety concern
1-Phenyl-3- or -5-propylpyrazole	1568	N	No safety concern
4,5-Dimethyl-2-propyloxazole	1569	N	No safety concern (conditional)[b]

[a] N: new specifications prepared; T: tentative specifications.
[b] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.

F. Epoxides

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
4,5-Epoxy-(E)-2-decenal	1570	N	No safety concern (conditional)[b]
β-Ionone epoxide	1571	N	No safety concern (conditional)[b]
trans-Carvone-5,6-oxide	1572	N	No safety concern
Epoxyoxophorone	1573	N	No safety concern (conditional)[b]
Piperitenone oxide	1574	N	No safety concern
β-Caryophyllene oxide	1575	N	No safety concern
Ethyl 3-phenylglycidate	1576	Rc	No safety concern
Ethyl methylphenylglycidate	1577	Rc	See footnote d
Ethyl methyl-*para*-tolylglycidate	1578	N	No safety concern

[a] N: new specifications prepared; R: existing specifications revised.
[b] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.
[c] As this substance is used only as a flavouring agent, the Committee decided that the existing specifications in the standard food additive format should be deleted.
[d] The ADI of 0–0.5 mg/kg bw established at the twenty-eighth meeting. was maintained.

G. Aliphatic and aromatic amines and amides

Acetamide (No. 1592)

The Committee noted that the available data on the toxicity of this substance indicate that it is clearly carcinogenic in both mice and rats, and, although the mechanism of tumour formation is unknown, the possibility of a genotoxic mechanism cannot be discounted. The Committee considered it inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose and agreed that acetamide would not be evaluated according to the Procedure. No specifications were prepared.

Other substances in this group

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Ethylamine	1579	N	No safety concern (conditional)[b]
Propylamine	1580	N	No safety concern (conditional)[b]
Isopropylamine	1581	N	No safety concern (conditional)[b]
Butylamine	1582	N	No safety concern
Isobutylamine	1583	N	No safety concern (conditional)[b]

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
sec-Butylamine	1584	N	No safety concern (conditional)[b]
Pentylamine	1585	N	No safety concern (conditional)[b]
2-Methylbutylamine	1586	N	No safety concern (conditional)[b]
Isopentylamine	1587	N	No safety concern
Hexylamine	1588	N	No safety concern (conditional)[b]
Phenethylamine	1589	N	No safety concern
2-(4-Hydroxyphenyl)ethylamine	1590	N	No safety concern (conditional)[b]
1-Amino-2-propanol	1591	N	No safety concern (conditional)[b]
Butyramide	1593	N	No safety concern (conditional)[b]
1,6-Hexalactam	1594	N	No safety concern (conditional)[b]
2-Isopropyl-*N*,2,3-trimethylbutyramide	1595	N	No safety concern (conditional)[b]
N-Ethyl (E)-2,(Z)-6-nonadienamide	1596	N	No safety concern (conditional)[b]
N-Cyclopropyl (E)-2,(Z)-6-nonadienamide	1597	N	No safety concern (conditional)[b]
N-Isobutyl (E,E)-2,4-decadienamide	1598	N	No safety concern (conditional)[b]
Nonanoyl 4-hydroxy-3-methoxybenzylamide	1599	N	No safety concern
Piperine	1600	N	No safety concern
N-Ethyl-2-isopropyl-5-methylcyclohexane carboxamide	1601	N	No safety concern
(+/–)-*N*,*N*-Dimethyl menthyl succinamide	1602	N	No safety concern (conditional)[b]
1-Pyrroline	1603	N	No safety concern (conditional)[b]
2-Acetyl-1-pyrroline	1604	N	No safety concern (conditional)[b]
2-Propionylpyrroline	1605	N	No safety concern (conditional)[b]
Isopentylidene isopentylamine	1606	N	No safety concern (conditional)[b]
Piperidine	1607	N	No safety concern
2-Methylpiperidine	1608	N	No safety concern (conditional)[b]
Pyrrolidine	1609	N	No safety concern
Trimethylamine	1610	N	No safety concern
Triethylamine	1611	N	No safety concern (conditional)[b]
Tripropylamine	1612	N	No safety concern (conditional)[b]
N,*N*-Dimethylphenethylamine	1613	N	No safety concern (conditional)[b]

Flavouring agent	No.	Specifications[a]	Conclusions based on current intake
Trimethylamine oxide	1614	N	No safety concern (conditional)[b]
Piperazine	1615	N	No safety concern (conditional)[b]

[a] N: new specifications prepared.
[b] Evaluation conditional because the estimated daily intake is based on the anticipated annual volume of production. The conclusion of the safety evaluation of this substance will be revoked if use levels or poundage data are not provided before the end of 2007.

4. Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications[a]
Sodium salt of 3-methyl-2-oxobutanoic acid	631.2	R,T
Sodium salt of 3-methyl-2-oxopentanoic acid	632.2	R,T
Sodium salt of 4-methyl-2-oxopentanoic acid	633.2	R,T
Sodium 2-oxo-3-phenylpropionate	1479	R,T

[a] R: existing specifications revised; T: tentative specifications

Annex 3
Further information required

1. Need for use levels and reported poundage data for flavouring agents

The evaluations of a number of flavouring agents were made conditional because the estimated daily intake was based on the anticipated annual volume of production. The safety evaluation of these substances will be revoked if use levels or poundage data are not provided before the end of 2007. The Committee also requested use levels or poundage data to be provided for the flavouring agents it had assessed previously on the basis of an MSDI that was calculated from anticipated poundage. These would include any substances for which the MSDI based on anticipated poundage for one region (European Union or USA) was higher than the MSDI based on recorded poundage in the other region.

The existing assessments will be revoked if such data are not forthcoming by the end of 2007.

The Committee emphasized that data on level of use are required for all flavouring agents listed in 'calls for data'. Subsequent submissions that do not contain this information will not be evaluated by the Committee.

Details of all the flavouring agents for which further data are required are given in Annex 4.

2. Information for specifications

2.1 Flavouring agents

2.1.1 Sodium 3-methyl-2-oxobutanoate (No. 631.2), sodium 3-methyl-2-oxopentanoate (No. 632.2), sodium 4-methyl-2-oxopentanoate (No. 633.2) and sodium 2-oxo-3-phenylpropionate (No. 1479)

The existing tentative specifications for these four flavouring agents were revised to include new information on methods of assay. Nevertheless, the tentative designations of the specifications were maintained, pending more detailed information on these methods. For the first three substances, information on an assay by high-performance liquid chromatography with an ion-exchange column are required; for flavouring agent No. 1479, information on an assay by high-performance liquid chromatography is required.

2.1.2 Maltol (No.1480) and ethyl maltol (No. 1481)

New specifications were prepared for these substances in the flavouring agent format. Both substances are, however, believed to have uses other than as flavouring agents, and the existing specifications in the standard food additive format were revised and made tentative. In both cases, information is required on functional uses other than for flavouring and on the method of assay.

2.1.3 Maltyl isobutyrate (No. 1482), 3-acetyl-2,5-dimethylfuran (No. 1506) and 2,4,5-trimethyl-δ-3-oxazoline (No. 1559)

New tentative specifications were prepared for these substances. In each case, information is required about why the quoted ranges for specific gravity are wider than would be expected, given the level of purity of the substances. In addition, further information is required on why the range of refractive indexes for flavouring agent No. 1559 is wider than would be expected, given the level of purity of the substance.

2.2 Sucrose esters of fatty acids

The specifications for sucrose esters of fatty acids were revised but maintained as tentative. Information is required on:

— a method of analysis for the determination of free sucrose by capillary gas chromatography or high-performance liquid chromatography;

— an alternative and less toxic solvent than pyridine for preparing the standard and sample solutions for determinations of free sucrose and propylene glycol; and

— a method of analysis for the determination of dimethyl sulfoxide that does not require a packed column.

The tentative specifications mentioned above will be withdrawn unless the requested information is received before the end of 2006.

Annex 4

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

No.	Name	Minimum assay value (%)	Other requirements	Comments on secondary components
Eugenol and related hydroxyallylbenzene derivatives				
1530	Eugenyl formate	94	2–3% eugenol	Eugenol (No. 1529) was evaluated at the current meeting (see monograph). It has been examined for toxicity in studies lasting from 30 days to 2 years. The NOELs in most of these studies were > 400 mg/kg bw per day (Trubek, 1958; Hagan et al., 1965; Bar & Griepentrog, 1967; Hagan et al., 1967; Miller et al., 1983; National Toxicology Program, 1983; Hirose et al., 1987). In a 2-year study, the NOEL was > 450 mg/kg per day in mice and 300 mg/kg per day in rats (National Toxicology Program, 1983).
Miscellaneous nitrogen-containing substances				
1559	2,4,5-Trimethyl-Δ-3-oxazoline	94	2–3% trimethyloxazole	Trimethyloxazole (No. 1553) was evaluated at the current JECFA meeting. It is expected to have a similar metabolic fate and similar toxicity as the primary material, 2,4,5-trimethyl-Δ-3-oxazoline, and the other oxazoles and oxazolines in this group. In a 90-day study with the primary material, the NOEL was > 41 mg/kg per day (Morgareidge, 1972).

Annex 4 (contd)

No.	Name	Minimum assay value (%)	Other requirements	Comments on secondary components
Epoxides				
1570	4,5-Epoxy-(E)-2-decenal	87	8–10% Z isomer	4,5-Epoxy-(Z)-2-decenal is expected to have the same metabolic fate as the E isomer and the other epoxides in this group. Epoxide hydrolase present in the cytosol (Gill et al., 1974), endo plasmic reticulum (Oesch et al., 1970) and nucleus (Bresnick et al., 1977) catalyses epoxide ring cleavage by water to yield vicinal *trans*-diols. Alternatively, glutathione transferase present in the cytosol catalyses ring cleavage by glutathione to yield *trans*-thioalcohol conjugates (Jakoby, 1978). In a 28-day study, the NOEL for the structurally related compound cyclohexane oxide was 100 mg/kg bw per day (Sauer et al., 1997).
Aliphatic and aromatic amines and amides				
1606	Isopentylidene isopentylamine	93	2–3% diisopentylamine; 1-2% 3-methylbutyr-aldehyde	Diisopentylamine is expected to have the same metabolic fate as the other primary, secondary and tertiary amines in this group. They are mainly oxidized to imines by flavin-containing mono-oxygenases, monoamine oxidases or amine oxidases. The resulting imine can be further oxidized to the corresponding aldehyde and ammonia (Kearney et al., 1971). In 90-day studies with structurally related materials, the NOELs were 80 mg/kg bw per day for piperidine and 160 mg/kg bw per day for trimethylamine (Amoore et al., 1978). 3-Methylbutyraldehyde (No. 258) was evaluated by the Committee at its forty-sixth meeting and found to be of no safety concern at current levels of intake.

Annex 4 (contd)

References

Amoore, J.E., Gumbmann, M.R., Booth, A.N. & Gould, D.H. (1978) Synthetic flavors: Efficiency and safety factors for sweaty and fishy odorants. *Chem. Senses Flavour*, **3**, 307.

Bär, V.F. & Griepentrog, F. (1967) [Where we stand concerning the evaluation of flavouring substances from the viewpoint of health]. *Med. Ernahr.*, **8**, 244–251.

Bresnick, E., Stoming, T.A., Vaught, J.B., Thakker, D.K. & Jerina, D.M. (1977) Nuclear metabolism of benzo(a)pyrene and of (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene—Comparative chromatographic analysis of alkylated DNA. *Arch. Biochem. Biophys.*, **183**, 31–37.

Gill, S.S., Hammock, B.D. & Casida, J.E. (1974) Mammalian metabolism and environmental degradation of juvenoid 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene and related compounds. *J. Agric. Food Chem.*, **22**, 386–395.

Hagan, E.C., Jenner, P.M., Jones, W.I., Fitzhugh, O.G., Long, E.L., Brouwer, J.G. & Webb, W.K. (1965) Toxic properties of compounds related to safrole. *Toxicol. Appl. Pharmacol.*, **7**, 18–24.

Hagan, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A. & Brouwer, J.B. (1967) Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. *Food Cosmet. Toxicol.*, **5**, 141–157.

Hirose, M., Masuda, A., Imaida, K., Kagawa, M., Tsuda, H. & Ito, N. (1987) Induction of forestomach lesions in rats by oral administrations of naturally occurring antioxidants for 4 weeks. *Jpn. J. Cancer Res. (Gann)*, **78**, 317–321.

Kearney, E.B., Salach, J.I., Walker, W.H., Seng, R. & Singer, T.P. (1971) Structure of the covalently bound flavin of monoamine oxidase. *Biochem. Biophys. Res Commun.*, **42**, 490–496.

Miller, E.C., Swanson, A.B., Phillips, D., Fletcher, L.T., Liem, A. & Miller, J.A. (1983) Structure–activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res.*, **43**, 1124–1134.

Morgareidge, K. (1972) 90-day feeding studies in rats with 2,4,5-trimethyl-Δ3-oxazoline (31202). Food and Drug Research Laboratories. Unpublished report submitted to WHO by the Food and Extract Manufacturers Association of the United States, Washington DC, USA.

National Toxicology Program (1983) *Carcinogenesis Studies of Eugenol (CAS No. 97-53-0) in F344/N Rats and B6C3F1 Mice (Feed Studies)* (NTP TR 223. NTP 84-1779), Research Triangle Park, North Carolina, USA, National Toxicology Program.

Oesch, F., Jerina, D.M. & Daly, J. (1970) A radiometric assay for hepatic epoxide hydrase activity with 7-^{3}H-styrene oxide. *Biochim. Biophys. Acta*, **227**, 685–691.

Sauer, J.-M., Bao, J., Smith, R.L., McClure, T.D., Mayersohn, M., Pillai, U., Cunningham, M.L. & Sipes, I.G. (1997) Absorption, disposition kinetics, and metabolic pathways of cyclohexane oxide in the male Fischer 244 rat and female B6C3F1 mouse. *Drug Metab. Disposition*, **25**, 371–378.

Trubek Laboratories, Inc. (1958) Toxicolgical screening of eugenol, p-methoxybenzaldehyde and piperonal in rats. Class IX. Aromatic aldehydes. Unpublished report submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Annex 5
Flavouring agents for which use level or reported poundage data are required

The safety assessments of these flavouring agents will be revoked if data on levels of use or reported poundage data are not provided before the end of 2007 (see Annex 3).

1. Flavouring agents evaluated at the present meeting that were assessed as of 'no safety concern' on a conditional basis

No.	Flavouring agent
1483	2-Methyl-3-(1-oxopropoxy)-4*H*-pyran-4-one
1527	4-Allylphenol
1528	2-Methoxy-6-(2-propenyl)phenol
1532	Eugenyl isovalerate
1538	*cis*-3-Hexenyl anthranilate
1539	Citronellyl anthranilate
1546	Ethyl *N*-methylanthranilate
1547	Ethyl *N*-ethylanthranilate
1548	Isobutyl *N*-methylanthranilate
1549	Methyl *N*-formylanthranilate
1550	Methyl *N*-acetylanthranilate
1551	Methyl *N*,*N*-dimethylanthranilate
1552	*N*-Benzoylanthranilic acid
1553	Trimethyloxazole
1554	2,5-Dimethyl-4-ethyloxazole
1555	2-Ethyl-4,5-dimethyloxazole
1556	2-Isobutyl-4,5-dimethyloxazole
1557	2-Methyl-4,5-benzo-oxazole
1558	2,4-Dimethyl-3-oxazoline
1561	Butyl isothiocyanate
1562	Benzyl isothiocyanate
1563	Phenethyl isothiocyanate
1569	4,5-Dimethyl-2-propyloxazole
1570	4,5-Epoxy-(E)-2-decenal
1571	β-Ionone epoxide
1573	Epoxyoxophorone
1579	Ethylamine
1580	Propylamine
1581	Isopropylamine
1583	Isobutylamine
1584	*sec*-Butylamine
1585	Pentylamine
1586	2-Methylbutylamine
1588	Hexylamine
1590	2-(4-Hydroxyphenyl)ethylamine
1591	1-Amino-2-propanol
1593	Butyramide
1594	1,6-Hexalactam

No.	Flavouring agent
1595	2-Isopropyl-*N*,2,3-trimethylbutyramide
1596	*N*-Ethyl (E)-2(Z)-6-nonadienamide
1597	*N*-Cyclopropyl (E)-2(Z)-6-nonadienamide
1598	*N*-Isobutyl (E,E)-2,4-decadienamide
1602	(±)-*N*,*N*-Dimethyl menthyl succinamide
1603	1-Pyrroline
1604	2-Acetyl-1-pyrroline
1605	2-Propionylpyrroline
1606	Isopentylidene isopentylamine
1608	2-Methylpiperidine
1611	Triethylamine
1612	Tripropylamine
1613	*N*,*N*-Dimethylphenethylamine
1614	Trimethylamine oxide
1615	Piperazine

2. Flavouring agents evaluated at the 59th (2002), 61st (2003) and 63rd (2004) meetings of JECFA, for which only anticipated poundage data were available or for which the MSDI derived from anticipated poundage data from one region (European Union or USA) was greater than the MSDI derived from recorded poundage data for the other region

No.	Flavouring agent	Year	Note
963	Ethyl cyclohexanecarboxylate	2002	a
986	10-Hydroxymethylene-2-pinene	2002	a
1063	2,5-Dimethyl-3-furanthiol	2002	b
1065	Propyl 2-methyl-3-furyl disulfide	2002	a
1066	Bis(2-methyl-3-furyl) disulfide	2002	b
1067	Bis(2,5-dimethyl-3-furyl) disulfide	2002	b
1068	Bis(2-methyl-3-furyl) tetrasulfide	2002	a
1070	2,5-Dimethyl-3-furan thioisovalerate	2002	a
1077	Furfuryl isopropyl sulfide	2002	b
1082	2-Methyl-3,5- or -6-(furfurylthio)pyrazine	2002	b
1085	3-[(2-Methyl-3-furyl)thio]-4-heptanone	2002	a
1086	2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	2002	a
1087	4-[(2-Methyl-3-furyl)thio]-5-nonanone	2002	a
1089	2-Methyl-3-thioacetoxy-4,5-dihydrofuran	2002	a
1157	4-Hydroxy-4-methyl-5-hexenoic acid γ-lactone	2003	a
1158	(±) 3-Methyl-γ-decalactone	2003	a
1159	4-Hydroxy-4-methyl-7-*cis*-decenoic acid γ-lactone	2003	a
1160	Tuberose lactone	2003	a
1161	Dihydromintlactone	2003	a
1162	Mintlactone	2003	b
1163	Dehydromenthofurolactone	2003	b
1164	(±)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid γ-lactone	2003	a
1167	2-(4-Methyl-2-hydroxyphenyl)propionic acid γ-lactone	2003	a
1174	2,4-Hexadien-1-ol	2003	a
1176	(E,E)-2,4-Hexadienoic acid	2003	a

No.	Flavouring agent	Year	Note
1180	(E,E)-2,4-Octadien-1-ol	2003	a
1183	2,4-Nonadien-1-ol	2003	a
1188	(E,Z)-2,6-Nonadien-1-ol acetate	2003	a
1189	(E,E)-2,4-Decadien-1-ol	2003	a
1191	Methyl (E)-2-(Z)-4-decadienoate	2003	a
1193	Ethyl 2,4,7-decatrienoate	2003	a
1199	(±)-2-Methyl-1-butanol	2003	a
1217	2-Methyl-2-octenal	2003	a
1218	4-Ethyloctanoic acid	2003	a
1226	8-Ocimenyl acetate	2003	a
1228	3,7,11-Trimethyl-2,6,10-dodecatrienal	2003	a
1229	12-Methyltridecanal	2003	a
1232	1-Ethoxy-3-methyl-2-butene	2003	b
1236	2,2,6-Trimethyl-6-vinyltetrahydropyran	2003	b
1239	Cycloionone	2003	a
1245	2,4-Dimethylanisole	2003	a
1248	1,2-Dimethoxybenzene	2003	a
1265	4-Propenyl-2,6-dimethoxyphenol	2003	a
1289	Erythro- and threo-3-mercapto-2-methylbutan-1-ol	2003	b
1290	(±)-2-Mercaptomethylpentan-1-ol	2003	b
1292	3-Mercapto-2-methylpentanal	2003	b
1293	4-Mercapto-4-methyl-2-pentanone	2003	b
1296	*spiro*[2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3´-(1´-oxa-2´-methyl)-cyclopentane]	2003	a
1299	2,3,5-Trithiahexane	2003	b
1300	Diisopropyl trisulfide	2003	b
1311	2-(2-Methylpropyl)pyridine	2004	a
1319	2-Propionylpyrrole	2004	b
1322	2-Propylpyridine	2004	a
1334	4-Methylbiphenyl	2004	b
1342	δ-3-Carene	2004	a
1343	α-Farnesene	2004	a
1344	1-Methyl-1,3-cyclohexadiene	2004	a
1367	*trans*-2-Octen-1-yl acetate	2004	b
1368	*trans*-2-Octen-1-yl butanoate	2004	b
1369	*cis*-2-Nonen-1-ol	2004	b
1370	(E)-2-Octen-1-ol	2004	a
1371	(E)-2-Butenoic acid	2004	a
1372	(E)-2-Decenoic acid	2004	a
1373	(E)-2-Heptenoic acid	2004	a
1374	(Z)-2-Hexen-1-ol	2004	a
1375	*trans*-2-Hexenyl butyrate	2004	a
1376	(E)-2-Hexenyl formate	2004	a
1377	*trans*-2-Hexenyl isovalerate	2004	a
1378	*trans*-2-Hexenyl propionate	2004	a
1379	*trans*-2-Hexenyl pentanoate	2004	a
1380	(E)-2-Nonenoic acid	2004	a
1381	(E)-2-Hexenyl hexanoate	2004	a
1382	(Z)-3- and (E)-2-Hexenyl propionate	2004	a
1384	2-Undecen-1-ol	2004	a
1407	Dihydronootkatone	2004	b
1409	β-Ionyl acetate	2004	a

No.	Flavouring agent	Year	Note
1410	α-Isomethylionyl acetate	2004	a
1411	3-(l-Menthoxy)-2-methylpropane-1,2-diol	2004	a
1412	Bornyl butyrate	2004	a
1413	DL-Menthol(±)propylene glycol carbonate	2004	a
1414	L-Monomenthyl glutarate	2004	a
1415	L-Menthyl methyl ether	2004	a
1416	*para*-Menthane-3,8-diol	2004	a
1435	Taurine	2004	a
1438	L-Arginine	2004	a
1439	L-Lysine	2004	a
1447	Tetrahydrofurfuryl cinnamate	2004	a
1457	(±)-2-(5-Methyl-5-vinyltetrahydrofuran-2-yl)propionaldehyde	2004	a
1475	Ethyl 2-ethyl-3-phenylpropanoate	2004	a
1478	2-Oxo-3-phenylpropionic acid	2004	a

[a] Flavourings for which only anticipated poundage data were available

[b] Flavourings for which the MSDI derived from anticipated poundage data from the USA was greater than the MSDI derived from recorded poundage data from the European Union

Annex 6

Divergent opinion on safety assessment of flavouring substances

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JECFA has adopted part of the concept of 'threshold of toxicological concern' for evaluating flavouring agents. The concept is based on the assumption that, if the level of exposure is low, risk assessment can be based on data for structurally related compounds. The data include those on absorption, distribution, metabolism, excretion and toxicity for compounds of the same structural class. The threshold of toxicological concern is defined as the level of human exposure below which it can be anticipated there are no significant risk for health even in the absence of data on the compound itself.

The quality of the estimate of dietary exposure is therefore crucial for reaching a conclusion about the safety of flavouring agents evaluated by this Procedure.

The estimated dietary intake used by the Committee is based on the amount of the flavouring agent produced per year by industry (also called poundage data) divided by the number of consumers, assumed to be 10% of the population.

During the sixty-fifth meeting, 135 flavouring substances were submitted for safety assessment. Production figures were not available for 60 of them, and industry provided the Committee with 'anticipated production data', corresponding to volumes that might be produced in the future. The Committee agreed that these data were not adequate for use in its procedure for evaluating the safety of flavouring substances. Nevertheless, the Committee came to conclusions about the safety of these substances by applying the normal procedure, although making the conclusions conditional.

The minority opinion is that the safety of the 60 flavouring substances without reported poundage data should not be evaluated by the normal procedure, even on a conditional basis.