WHO Technical Report Series

974

Evaluation of certain food additives

Seventy-sixth report of the
Joint FAO/WHO Expert Committee on
Food Additives

Food and Agriculture Organization of the United Nations

World Health Organization

World Health Organization

WHO Technical Report Series
974

Evaluation of certain food additives

Seventy-sixth report of the
Joint FAO/WHO Expert Committee on
Food Additives

Food and Agriculture Organization of the United Nations

World Health Organization

World Health Organization

WHO Library Cataloguing-in-Publication Data:

Evaluation of certain food additives: seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives.

(WHO technical report series ; no. 974)

1.Food additives - analysis. 2.Food additives - toxicity. 3.Flavouring agents. 4.Diet - adverse effects. 5.Risk assessment. I.World Health Organization. II.Food and Agriculture Organization of the United Nations. III.Joint FAO/WHO Expert Committee on Food Additives. Meeting (76th: 2012, Geneva, Switzerland). IV.Series.

ISBN 978 92 4 120974 8 (NLM classification: WA 712)

ISSN 0512-3054

Typeset in India

Printed in Malta

Contents

Seventy-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Geneva, 5–14 June 2012

Members

Professor J. Alexander, Norwegian Institute of Public Health, Oslo, Norway

Dr M. DiNovi, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, United States of America (USA)

Dr Y. Kawamura, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan

Dr Madduri Veerabhadra Rao, Department of the President's Affairs, Al Ain, United Arab Emirates

Dr A. Mattia, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*Chairperson*)

Mrs I. Meyland, Birkerød, Denmark (*Vice-Chairperson*)

Dr U. Mueller, Food Standards Australia New Zealand, Barton, ACT, Australia

Professor A. Renwick, Emeritus Professor, Faculty of Medicine, University of Southampton, Southampton, England (*Joint Rapporteur*)

Dr J. Schlatter, Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health, Zurich, Switzerland

Dr P. Sinhaseni, Community Risk Analysis Research and Development Center, Bangkok, Thailand

Mrs H. Wallin, Finnish Food Safety Authority (Evira), Helsinki, Finland (*Joint Rapporteur*)

Secretariat

Dr D. Benford, Food Standards Agency, London, England (*WHO Expert*)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr S. Choudhuri, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Expert*)

Professor L.R.M. dos Santos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, São Paulo, Brazil (*FAO Expert*)

Dr R. Ellis, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

Dr B. Fields, Food Standards Australia New Zealand, Canberra, ACT, Australia (*WHO Expert*)

Dr D. Folmer, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*FAO Expert*)

Dr S.M.F. Jeurissen, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment, Bilthoven, the Netherlands (*WHO Expert*)

Dr F. Kayama, School of Medicine, Jichi Medical University, Tochigi, Japan (*WHO Expert*)

Dr J.-C. Leblanc, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France (*FAO Expert*)

Dr S.Y. Lee, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Staff Member*)

Professor S.M. Mahungu, Department of Dairy, Food Science and Technology, Egerton University, Egerton, Kenya (*FAO Expert*)

Professor S. Rath, Department of Analytical Chemistry, University of Campinas, Campinas, São Paulo, Brazil (*FAO Expert*)

Ms M. Sheffer, Ottawa, Canada (*WHO Editor*)

Professor I.G. Sipes, College of Medicine, University of Arizona, Tucson, AZ, USA (*WHO Expert*)

Dr J.R. Srinivasan, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*FAO Expert*)

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)

Dr T. Umemura, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (*WHO Expert*)

Professor G.M. Williams, Department of Pathology, New York Medical College, Valhalla, NY, USA (*WHO Expert*)

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

Safety evaluation of certain food additives. WHO Food Additives Series, No. 67, 2012.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 13, 2012.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Geneva from 5 to 14 June 2012. The meeting was opened by Dr Angelika Tritscher, Acting Director of the Department of Food Safety and Zoonoses of the World Health Organization (WHO), on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Tritscher noted the long history of the Committee and the importance of its assessments, which serve as the basis for Codex Alimentarius Commission standards, in the context of international food safety.

Dr Tritscher informed the Committee about the recent World Health Assembly and meeting of the Executive Board, at which Member States expressed strong support for the importance of food safety and their appreciation and recognition of the importance of the Committee's work. Sincere appreciation was expressed to the experts for putting their time and expertise at the service of FAO and WHO. Dr Tritscher then briefly informed the Committee on the current reform process at WHO and the ongoing process for programme planning and prioritization. Food safety is considered to be an important component of global public health and is closely linked to food security and sustainable development. As such, it is a priority for the Organization in the context of global health security.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-sixth meeting had completed declaration of interest forms. The following declared interests and potential conflicts were discussed by the Committee. Professor Glenn Sipes serves on a scientific expert panel of the Research Institute of Fragrance Materials, and the University of Arizona, his previous employer, had received research funds from the flavours industry to support generic hydrolysis studies of flavours. Since none of this work included flavours on the agenda of this meeting, this was not considered a conflict of interest. Professor Andrew Renwick consulted for several food manufacturers on sweeteners, but none of the consultancies were related to any of the compounds evaluated at this meeting. Professor Gary Williams received a

research grant to study the genotoxicity of a specific flavour compound. Since this study was submitted to support the evaluation of one of the flavour groups to be considered at this meeting, this was considered a conflict of interest, and Professor Williams did not participate in the discussion and evaluation of this group.

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 75 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-third meeting (Annex 1, reference *202*).

The tasks before the Committee were:

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

— to review and prepare specifications for certain food additives and flavouring agents (section 3 and Annex 2);

— to undertake safety evaluations of certain food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

For the revision of the specification for paprika extract, no data were received, and, based on the commitment by the sponsor to provide data for a future meeting, this evaluation was postponed. The following compounds that were scheduled to be evaluated as flavouring agents were removed from the agenda:

— No. 2124 ((3*R*)-4-[[(1*S*)-1-benzyl-2-methoxy-2-oxo-ethyl]amino]-3-[3-(3-hydroxy-4-methoxy-phenyl)propylamino]-4-oxo-butanoic acid hydrate) is a high-intensity sweetener called Advantame. Therefore, it was considered inappropriate to evaluate this compound as a flavouring agent. Evaluation as a food additive has been requested by the Codex Committee on Food Additives (CCFA) and will be scheduled for a future JECFA meeting.

— No. 2168 (rebaudioside A) and No. 2169 (rebaudioside C) are naturally occurring sweeteners and are included in the previous evaluation of the food additive steviol glycosides, for which an acceptable daily intake (ADI) of 0–4 mg/kg body weight (bw), expressed as steviol, has been

established (Annex 1, reference *190*). The Committee did not consider it appropriate to evaluate these substances as flavouring agents. Specifications for Nos 2168 and 2169 were not prepared, as these additives are covered under the existing specifications for steviol glycosides.

Seven flavouring agents (Nos 2099–2105) were proposed for evaluation as additions to the previously evaluated group of furfuryl alcohol and related substances. However, three of these compounds (Nos 2103–2105) are more appropriately considered in the group of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution and were considered within this group.

Thirteen flavouring agents (Nos 2150–2162) were proposed for evaluation as additions to the previously evaluated group of pyridine, pyrrole and quinoline derivatives. However, Nos 2161 and 2162 are more appropriately considered as additions to the group of miscellaneous nitrogen-containing substances. This additional group evaluation was added to this meeting.

One flavouring agent, No. 2069, was scheduled for evaluation in the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters. The Committee decided that this compound did not belong to this group, and therefore it was not further considered.

The Committee agreed to consider amending the test on the degree of substitution of starch sodium octenylsuccinate (International Numbering System for Food Additives [INS] No. 1450) in the specifications monograph for modified starches. The requests to make specific edits regarding monosodium glutamate (INS No. 621), dimethyl dicarbonate (INS No. 242) and 4-hexylresorcinol (INS No. 586) were considered as minimal editorial amendments that will be done for the online *Combined Compendium of Food Additive Specifications* database.

2.2 Report from the Forty-fourth Session of the Codex Committee on Food Additives (CCFA)

The Codex Secretariat informed the Committee about the principal achievements and outputs of the Forty-fourth Session of CCFA.

The Forty-fourth Session of CCFA had forwarded 160 food additive provisions of the Codex *General Standard for Food Additives* (GSFA) to the Thirty-fifth Session of the Codex Alimentarius Commission for adoption, with amendments to the name and descriptors of food category 16.0 GSFA, which was renamed "Prepared foods". In addition, the Forty-fourth Session of CCFA recommended the adoption of 22 new, revised or corrected specifications for the identity and purity of food additives, prepared by the seventy-fourth meeting of JECFA; amendments to the INS; and the *Risk Analysis*

Principles Applied by the Codex Committee on Food Additives. CCFA also requested the Commission to revoke a number of food additive provisions of the GSFA, the specifications for potassium bromate and the Codex *Information on the Use of Food Additives in Foods* (CAC/MISC 1-1989).

The Forty-fourth Session of CCFA continued working on the provisions for aluminium-containing food additives in the GSFA with a view to reduce their uses and use levels and seek alternatives to these food additives.

The Forty-fourth Session of CCFA agreed on a revised priority list of compounds for evaluation (or re-evaluation) by JECFA, to prepare a prioritized list of 107 food colours by applying the prioritization tool, as discussed at the Session, for re-evaluation by JECFA and to compile information from members and observers on these colours.

2.3 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, the Committee took into consideration the principles established and contained in the publication, Environmental Health Criteria, No. 240, *Principles and Methods for the Risk Assessment of Chemicals in Food*, published in 2009 (*2*).

2.4 Statement on the inclusion of secondary additives in a specifications monograph

Some food additives may require the addition of one or more secondary additives to ensure their stability and effective use in foods. Examples may include, but are not limited to, the use of antioxidants or preservatives to promote the stability of a primary additive or anti-caking agents, diluents or emulsifiers to ensure its technological function. In cases where the Committee has considered the use of a secondary additive or class of additives with a particular technological purpose to be appropriate, a short statement allowing for the addition of secondary additives will be included in the definition section of the specifications monograph.

Accordingly, any secondary additive must have been determined to be safe for use in food by the Committee. They should be of food-grade quality and used at the minimum level required to achieve the intended technological function.

2.5 Food additive specifications

2.5.1 *Analytical method for the determination of phosphorus as phosphorus pentoxide*

The Committee at its current meeting noted that the titrimetric and gravimetric methods in the *Combined Compendium of Food Additive Specifications*,

Volume 4 (Annex 1, reference *180*), are not reliable for the determination of phosphorus as phosphorus pentoxide. Consequently, the Committee decided to introduce a method based on inductively coupled plasma–atomic emission spectrophotometry (ICP-AES) in the specifications monograph of magnesium dihydrogen diphosphate.

The Committee may consider replacing corresponding methods for other diphosphate additives at a future meeting.

2.5.2 *Inclusion of titanium dioxide in the evaluation of dietary exposure to aluminium from food additives*

The Committee noted that the current specifications monograph for titanium dioxide has a provision for aluminium oxide, up to a maximum level of 2%. Consequently, the Committee may consider inclusion of titanium dioxide in evaluations of the dietary exposure to aluminium.

2.5.3 *Food additives containing aluminium and/or silicon*

The Committee, while reviewing the specifications of food additives containing aluminium and silicon, considered it relevant to update the test methods for the determination of aluminium oxide and silicon dioxide. Some of the test methods for the food additives listed below use potentially corrosive or hazardous reagents that are not always permitted in current laboratory practices because of safety concerns. The Committee also noted that the specifications of some additives were rather old or tentative and that it requires additional information to revise the specifications. Consequently, the Committee recommends placing these additives on the agenda for re-evaluation.

Food additive	Specifications	Remarks on current assay
Calcium aluminium silicate (INS No. 556)	28th, 1984	Assay by gravimetry, includes hydrofluoric acid and perchloric acid
Aluminium silicate (INS No. 559)	57th , 2001	No assay
Calcium silicate (INS No. 552)	17th, 1973	No assay
Silicon dioxide (INS No. 551)	17th, 1973	Assay by gravimetry and includes hydrofluoric acid
Sodium aluminosilicate (INS No. 554)	17th, 1973	No assay, identification test includes hydrofluoric acid
Potassium aluminium silicate (INS No. 555)	74th , 2011	Tentative specifications; assay method is based on alkali fusion followed by ICP-AES determination
Potassium aluminium silicate–based pearlescent pigments	74th , 2011	Tentative specifications; assay method is based on alkali fusion followed by ICP-AES determination

2.5.4 *Test methods for modified starches*

In addition to revising the specific test for degree of substitution of starch sodium octenylsuccinate (INS No. 1450) in the specifications monograph of

modified starches, the Committee considered that it would be necessary to align the description of the test to be consistent with the end-product specifications at a future meeting. In addition, the Committee considered that it would also be necessary to revise the specifications for all the modified starches, including test methods.

2.6 Flavour specifications

2.6.1 *Improvements to the submission of specifications data for flavouring agents*

The Committee at its current meeting made recommendations to improve the quality of data submitted for flavouring agents. These include submission of raw data (e.g. spectra, molecular structure, composition of isomers, physical and chemical properties, and method for determination of minimum assay) used to establish the specifications for each flavouring agent at submission. In addition, tabulated summary data (e.g. spreadsheet) for all the flavouring agents should be provided. It is strongly recommended that for each flavouring agent, the following spectra, with detailed experimental conditions, be provided: nuclear magnetic resonance spectrometry, Fourier-transform infrared spectroscopy and mass spectrometry. Spectra should be of such quality that they can be used for identification purposes. Data provided should be consistent with the product in commerce. The data should be provided in a timely manner that permits the Committee to perform a thorough review. All data should receive a thorough quality control review by the sponsor before submission to the Committee.

2.6.2 *Improvements to the presentation of specifications data for flavouring agents*

The Committee recommends that the chemical structures for the flavouring agents be included as part of the specifications presented online. In addition, an annotation of the method used to determine the minimum assay value of the flavouring agent should be included. The Committee also noted that it would be more useful to separate the current specification for "Physical Form/Odour" into two separate entries. It was also recommended that a separate entry for melting point be included in the specifications for flavouring agents.

2.7 Evaluation of flavour modifiers

A number of the flavouring agents submitted to the present meeting (Nos 2077, 2080–2082, 2119, 2121, 2123, 2158–2162 and 2170–2172) modify the flavour of other dietary components. At the present meeting, the Committee has adopted the term *flavour modifier* for all agents that alter or mask the flavours of flavouring agents or other dietary components.

The Committee noted that the chemical structures of some flavour modifiers (e.g. Nos 2081, 2082, 2161, 2162 and 2170–2172) have characteristics that have not been found in previously evaluated flavouring agents. The flavour modifiers evaluated at the present meeting had low estimated dietary exposures and could be evaluated using the Procedure for the Safety Evaluation of Flavouring Agents. The Committee agreed that flavour modifiers would be identified in evaluations of flavouring agents. The Committee emphasized that the safety evaluations undertaken on flavouring agents and flavour modifiers relate to the use levels submitted to the Committee for evaluation.

3. Specific food additives (other than flavouring agents)

The Committee evaluated four food additives for the first time and re-evaluated one other. Four food additives were considered for revision of specifications only. Information on the safety evaluations and specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are summarized in Annex 3.

3.1 Safety evaluations

3.1.1 *Magnesium dihydrogen diphosphate*

Explanation

At the present meeting, the Committee evaluated magnesium dihydrogen diphosphate for use as an acidifier, stabilizer and raising agent. It is proposed for use as an alternative to sodium-based acidifiers and raising agents, primarily in self-raising flour, noodles (oriental style), batters and processed cereals.

Magnesium dihydrogen diphosphate has not been evaluated previously by the Committee. Phosphates, diphosphates and polyphosphates were evaluated by the Committee at its sixth, seventh, eighth, ninth, thirteenth, fourteenth, seventeenth, twenty-sixth and fifty-seventh meetings (Annex 1, references *6–8*, *11*, *19*, *22*, *32*, *59* and *154*). A maximum tolerable daily intake (MTDI) of 70 mg/kg bw was established at the twenty-sixth meeting on the basis of the lowest dietary concentration of phosphorus (1% in the diet) that caused nephrocalcinosis in rats. It was considered inappropriate to establish an ADI, because phosphorus (primarily as phosphate) is an essential nutrient and an unavoidable constituent of food. The MTDI is expressed as phosphorus and applies to the sum of phosphates naturally present in food and the phosphates derived from use of these food additives. At its seventy-first meeting, the Committee evaluated ferrous ammonium phosphate and concluded that consideration of the toxicity of phosphate did not indicate a need to revise the Committee's previous evaluation of this ion (Annex 1, reference *191*).

The MTDI was considered to cover a number of phosphate salts, according to the principle established by the Committee at its ninth, twenty-third and twenty-ninth meetings (Annex 1, references *11*, *50* and *70*) that the ADI (or MTDI) established for ionizable salts should be based on previously accepted recommendations for the constituent cations and anions. Magnesium-based salts previously discussed by the Committee and covered by the MTDI for phosphates included magnesium phosphate (monobasic, dibasic and tribasic) and monomagnesium phosphate. However, certain specific phosphate salts were not included, because specifications were lacking and because information was not available to indicate whether they were being used as food-grade materials.

The Committee has previously evaluated other magnesium salts, allocating ADIs "not limited"[1] or "not specified" to magnesium carbonate, magnesium hydroxide, magnesium chloride, magnesium DL-lactate, magnesium hydrogen carbonate, magnesium gluconate, magnesium di-L-glutamate and magnesium sulfate (Annex 1, references *11*, *50*, *70*, *77*, *137* and *187*). At its twenty-ninth meeting (Annex 1, reference *70*), the Committee highlighted that the use of magnesium salts as food additives was acceptable, provided that the following were taken into consideration:

- The minimum laxative effective dose is approximately 1000 mg of magnesium moiety from a magnesium salt (observed only when the magnesium salt is administered as a single dose).
- Infants are particularly sensitive to the sedative effects of magnesium salts.
- Individuals with chronic renal impairment retain 15–30% of administered magnesium.

At its present meeting, the Committee was asked to conduct a safety assessment and set specifications for magnesium dihydrogen diphosphate by the Forty-third Session of CCFA (*3*). The Committee received a submission that included tests for acute toxicity, skin and eye irritation and genotoxicity of magnesium dihydrogen diphosphate and considered other information available in the literature of relevance to the magnesium and phosphate ions.

Chemical and technical considerations

Magnesium dihydrogen diphosphate (chemical formula: $MgH_2P_2O_7$; Chemical Abstracts Service registry number: 20768-12-1) is the acidic magnesium salt of diphosphoric acid. It is manufactured by adding an aqueous

[1] At its eighteenth meeting (Annex 1, reference *35*), the Committee replaced the term ADI "not limited" with ADI "not specified".

dispersion of magnesium hydroxide slowly to phosphoric acid until a magnesium to phosphorus ratio of about 1:2 is reached. The temperature is held under 60 °C during the reaction. About 0.1% hydrogen peroxide is added to the reaction mixture, and the slurry is then dried and milled.

Toxicological data

Magnesium dihydrogen diphosphate ionizes into its component ions: magnesium, hydrogen and diphosphate. Therefore, the safety assessment should be based on previously accepted recommendations for the constituent cations and anions. Magnesium and phosphorus (primarily as phosphate) are essential minerals that are naturally present in the human body and in food.

The Committee received data showing that magnesium dihydrogen diphosphate does not exert acute toxicity, skin or eye irritation or genotoxicity.

At previous meetings, the Committee noted that toxicity can arise from an imbalance of calcium, magnesium and phosphate. Excessive dietary phosphorus causes hypocalcaemia, which can result in bone loss and calcification of soft tissues. The MTDI of 70 mg/kg bw was derived from studies demonstrating nephrocalcinosis in rats at dietary concentrations of 1% phosphorus. Nephrocalcinosis has been defined as calcified deposits, mainly in the form of calcium phosphate, in tubules located predominantly at the corticomedullary junction of the kidney. The exact approach taken in deriving the MTDI from this end-point is unclear. In addition, the Committee noted that there is evidence that rats are particularly sensitive to mineralization in the kidneys resulting from an imbalance of calcium and phosphate in the diet. Therefore, the relevance of mineralization in the rat kidney for safety assessment is unclear. The available toxicological information on phosphate salts did not indicate that the MTDI is insufficiently health protective.

Assessment of dietary exposure

For the evaluation of magnesium dihydrogen diphosphate as a new food additive intended to be used as an alternative to sodium-based acidifiers and raising agents, the Committee evaluated an anticipated dietary exposure based on individual food consumption data from the European Union with the maximum proposed use levels of magnesium dihydrogen diphosphate (0.1% up to 0.7% by weight in solid food, as phosphorus) in GSFA food categories such as flours, pasta, noodles and similar products, puffed products, bread and rolls and fine bakery wares.

Based on this conservative scenario, assuming that 100% of food products would be manufactured and consumed at the maximum proposed use levels, the Committee concluded that anticipated average dietary exposures to magnesium dihydrogen diphosphate would be up to approximately 20 mg

of phosphorus per kilogram of body weight per day for an adult and up to 70 mg of phosphorus per kilogram of body weight per day for a child. The 95th percentiles of exposure are estimated to be up to 40 mg of phosphorus per kilogram of body weight per day for an adult and up to 115 mg of phosphorus per kilogram of body weight per day for a child. The main food groups contributing to these overall dietary exposures within all population groups were bread and rolls (7–86%), fine bakery wares (6–58%) and flours and starches (5–98%).

The dietary exposure to magnesium estimated from the anticipated use of magnesium dihydrogen diphosphate would be 39% of the estimated exposure to phosphorus, based on the contribution to molecular weight. This corresponds to an average dietary exposure of up to approximately 8 mg of magnesium per kilogram of body weight per day for an adult and up to 27 mg of magnesium per kilogram of body weight per day for a child. The 95th percentiles of exposure are estimated to be up to 16 mg of magnesium per kilogram of body weight per day for an adult and up to 45 mg of magnesium per kilogram of body weight per day for a child.

Evaluation

Although an ADI "not specified" has been established for a number of magnesium salts used as food additives, the estimated chronic dietary exposures to magnesium (960 mg/day for a 60 kg adult at the 95th percentile) from the proposed uses of magnesium dihydrogen diphosphate are up to twice the background exposures from food previously noted by the Committee (180–480 mg/day) and in the region of the minimum laxative effective dose of approximately 1000 mg of magnesium when taken as a single dose. The estimates of dietary exposure to phosphorus from the proposed uses of magnesium dihydrogen diphosphate are in the region of, or slightly exceed, the MTDI of 70 mg/kg bw for phosphate salts, expressed as phosphorus, from this source alone. Thus, the MTDI is further exceeded when other sources of phosphate in the diet are taken into account. The Committee therefore concluded that the proposed use levels and food categories result in an estimated dietary exposure to magnesium dihydrogen diphosphate that is a potential concern.

The Committee emphasized that in evaluating individual phosphate-containing food additives, there is a need for assessment of total dietary exposure to phosphorus.

Recommendations

The Committee noted that an ADI "not specified" has been allocated individually to a number of magnesium-containing food additives and recommended

that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed. The information submitted to the Committee and in the scientific literature did not indicate that the MTDI of 70 mg/kg bw for phosphate salts, expressed as phosphorus, is insufficiently health protective. On the contrary, because the basis for its derivation might not be relevant to humans, it could be overly conservative. Therefore, there is a need to review the toxicological basis of the MTDI for phosphate salts expressed as phosphorus.

A toxicological monograph was prepared.

New specifications and a Chemical and Technical Assessment for magnesium dihydrogen diphosphate were prepared.

3.1.2 *Mineral oil (medium and low viscosity) classes II and III*

Explanation

Mineral oils (medium and low viscosity) comprise a subgroup of the family of mineral oils and waxes. They are manufactured from crude mineral oils in various refining steps, such as distillation, extraction and crystallization, and are subsequently purified by acid treatment (oleum method) and/or hydrotreatment (catalytic hydrogenation). Mineral oils (medium and low viscosity) are mixtures of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling points greater than 200 °C. Mineral oils (medium and low viscosity) are subdivided into classes I, II and III based on criteria related to viscosity, average relative molecular mass and carbon number at 5% distillation point. The specifications of classes II and III are shown in Table 1.

Food-grade mineral oils have been evaluated by the Committee at its fourteenth, seventeenth, twentieth, thirty-seventh, forty-fourth and fifty-ninth meetings (Annex 1, references *23*, *33*, *42*, *94*, *117* and *161*). At the forty-fourth meeting, the Committee reviewed the results of 90-day studies in F344 rats on a range of mineral oils and waxes, including materials in classes II and III. The materials showed evidence of accumulation and effects including focal histiocytosis, increased weights of liver, lymph nodes, spleen and kidneys, granulomas or microgranulomas of the liver, haematological changes typical of a mild chronic inflammatory reaction and biochemical changes indicative of mild hepatic damage. The Committee considered that, although the types of effects seen were essentially foreign body reactions, it was possible that a prolonged inflammatory response of this type could result in functional changes in the immune system and that this aspect required further investigation. A temporary group ADI of 0–0.01 mg/kg bw was allocated to mineral oils falling within the specifications for the N10(A), N15(H), P15(H), N70(A) and N70(H) oils. The Committee required information about the

Table 1
Characteristics of mineral oil (medium and low viscosity) classes II and III

Class	Name	Viscosity at 100 °C (mm^2/s)	Average relative molecular mass	Carbon number at 5% distillation point
II	—	7.0–8.5	400–480	≥22
	N70(H)	7.7	420	23
III	—	3.0–7.0	300–400	≥17
	P15(H)	3.5	350	17
	N15(H)	3.5	330	17

Source: Annex 1, reference *161*

compositional factors in mineral oils that influence their absorption and toxicity. It also required a study of at least 1 year's duration on one of these materials in F344 rats, which should include an assessment of immune function at appropriate time periods and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached. A recovery period of 1 year should also be included, in order to determine whether the granulomatous hepatic lesions observed in rats in the 90-day studies are fully reversible.

At its fifty-ninth meeting, the Committee reviewed a number of studies conducted with low- and medium-viscosity mineral oils, including a 2-year study of the carcinogenicity of a medium-viscosity liquid petroleum (class I medium- and low-viscosity mineral oil); studies of pharmacokinetics and studies of humoral immune function after administration of P15(H) mineral oil (class III medium- and low-viscosity mineral oil) to F344 and Sprague-Dawley rats; and a 90-day study of histopathological responses and compositional analysis related to absorbed hydrocarbons with N15(H), N70(H) and P70(H) oils (classes III, II and I medium- and low-viscosity mineral oils, respectively). Compositional analysis was not provided on hydrocarbons in liver following administration of class II or III mineral oil. Interpretation of the results of the study of humoral immune function in F344 rats in response to dietary administration of P15(H) was complicated by a treatment-related increase in spleen weight. No data were provided to determine whether this increase was associated with a change in the proportion of spleen cell types, which prevented interpretation of the results. The results of the long-term toxicity and carcinogenicity studies with P70(H) and P100(H) oils in F344 rats, in which no effects were seen at the highest doses, provided the basis for establishing an ADI of 0–10 mg/kg bw for class I medium- and low-viscosity mineral oil. However, because the materials tested were not associated with induction of liver granulomas, the studies did not allow the Committee to determine the long-term consequences or reversibility of the liver granulomas seen in F344 rats in response to classes II and III medium- and low-viscosity mineral

oil. Several studies of low melting point paraffin wax were also reviewed by the Committee, as they were considered to provide information relevant for the evaluation of low- and medium-viscosity mineral oil relating to the difference in response to mineral hydrocarbons in F344 and Sprague-Dawley rats. The results of the studies on the effects of P15(H) oil and low melting point wax in F344 and Sprague-Dawley strains indicated that the more extensive response of F344 rats, and particularly of females, was associated with greatly enhanced retention of mineral hydrocarbons in the tissues, which was considered to be probably due to a reduced ability to metabolize absorbed hydrocarbons. The Committee concluded that additional studies were needed in order to determine whether the F344 rat is an appropriate model of human response to dietary exposure to food-grade mineral hydrocarbons. In particular, elucidation of the metabolic differences between F344 rats and other strains and species, including humans, would be useful. No data were available that would permit allocation of a full ADI for medium- and low-viscosity mineral oil in classes II and III. Also at its fifty-ninth meeting, the Committee noted that the new information reviewed indicated that the observed effects of these mineral oils, on which the temporary ADI was based, may be strain and sex specific. The Committee therefore extended the temporary group ADI of 0–0.01 mg/kg bw for classes II and III medium- and low-viscosity mineral oil until 2006, pending information on the relevance to humans of the response of F344 and Sprague-Dawley rats to these materials. In order for the data to be applicable to as wide a range of mineral oils as possible, the Committee suggested that commercial mineral oils of the lowest viscosity be used in such studies (Annex 1, reference *161*).

At its sixty-ninth meeting (Annex 1, reference *190*) and again at its seventy-first meeting (Annex 1, reference *196*), the Committee was informed that relevant studies were being undertaken and agreed to maintain the temporary group ADI for mineral oil (medium and low viscosity) classes II and III, noting that it would be withdrawn at the end of 2011 if the data were not submitted by that time.

At the present meeting, the Committee reviewed the toxicological basis for the temporary group ADI and was provided with reports of toxicokinetic studies, conducted in rats and humans, of P15(H), a white oil meeting the criteria for a class III low-viscosity mineral oil, intended to address its previous questions.

Toxicological data

In studies previously reviewed by the Committee, mineral oil (medium and low viscosity) was observed to accumulate in a dose-related fashion in the liver and mesenteric lymph nodes of F344 rats following (sub)chronic

exposure. Accumulation of classes II and III mineral oil led to histopathological changes mainly in the liver and mesenteric lymph nodes. In the liver, the histopathological changes were classified as granulomas or microgranulomas, consisting of focal aggregations of macrophages surrounded by inflammatory cells and occasionally necrotic cells and fibrosis. These changes represent an adverse consequence of histiocytosis. In the mesenteric lymph nodes, histiocytosis consisting of macrophage accumulation was reported, which was not accompanied by signs of inflammation or necrotic and fibrotic changes.

Mesenteric lymph node histiocytosis occurred at lower doses than hepatic microgranuloma and was the basis for the temporary group ADI established by the Committee at its forty-fourth meeting. However, in a later evaluation of calcium lignosulfonate at its sixty-ninth meeting, the Committee discussed the finding of histiocytosis in the mesenteric lymph nodes of rats treated with high molecular weight, poorly absorbed materials, such as petroleum-derived mineral oils and waxes and copovidone (a copolymer of vinylpyrrolidone and vinyl acetate), and with polypentosan sulfate. Histiocytosis appeared to be related to an attempt by the histiocytes of the mesenteric lymph nodes to degrade the small amount of absorbed test material. Long-term studies in rats given polypentosan sulfate and copovidone indicated that the histiocytosis does not progress to any pathological lesion; thus, the Committee concluded that the histiocytosis in the mesenteric lymph nodes does not represent an adverse effect (Annex 1, reference *190*). Therefore, the Committee concluded that the microgranulomas in the liver are the critical effect of mineral oil (medium and low viscosity) classes II and III.

In the study of Smith et al. (*4*), the original unpublished report of which was reviewed by the Committee at its forty-fourth meeting (Annex 1, reference *117*), P15(H) was fed to male and female F344 rats at dietary concentrations of 0, 20, 200, 2000 and 20 000 mg/kg (equal to average doses of 0, 2.0, 19, 190 and 1951 mg/kg bw per day in the females) for 90 days. An additional high-dose group was maintained for a 28-day recovery period after the end of the dosing period. In the females (which demonstrated more severe effects than the males), there was a dose-related increase in liver weight up to about 120% of control at the highest dose, which was statistically significant at all doses except 2.0 mg/kg bw per day. The combined scores for incidence and severity of granulomas in the liver of the female rats were 0, 5, 10 and 30 in the 2.0, 19, 190 and 1951 mg/kg bw per day dose groups, respectively, and 80 in the 1951 mg/kg bw per day recovery dose group. The scores were 0 and 3 in the concurrent control and recovery control groups, respectively. The increase was statistically significantly different from control only in the recovery group. This increased response following the recovery period

indicates that damage can continue to develop from the mineral oil constituents that have accumulated in the liver even after exposure has ceased. The data from this study show no effects at 2.0 mg/kg bw per day; the Committee considered that the minimal changes at 19 mg/kg bw per day represent the lower end of the dose–response relationship.

The new toxicokinetic studies submitted to the Committee were conducted using a single dose of P15(H). This mineral oil, which is consistent with the criteria for a class III low-viscosity mineral oil, meets the requirement of the Committee for commercial mineral oil of the lowest viscosity to be studied, in order for the data to be applicable to as wide a range of mineral oils as possible. P15(H) was administered to groups of fasted female F344 and Sprague-Dawley rats by gavage at a single dose of 0, 200 or 1500 mg/kg bw. F344 rats were also given 20 mg/kg bw. The concentrations of mineral oil hydrocarbons in blood and liver were measured by two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS) analysis. The area under the concentration–time curve values in blood were about 4 times higher in F344 rats than in Sprague-Dawley rats. The maximum concentrations in blood (C_{max} values) were about 3 times higher in the F344 rat strain than in the Sprague-Dawley rat strain, but with no difference between the 200 and 1500 mg/kg bw dose groups. The highest reported concentrations in the liver (at 24 hours) were about 2-fold higher in F344 rats than in Sprague-Dawley rats (*5*, *6*).

Studies reviewed previously indicated that the more extensive response of F344 rats, particularly females, compared with Sprague-Dawley rats to the effects of mineral oil was associated with greatly enhanced retention of mineral hydrocarbons in the tissues. The new rat toxicokinetic study submitted to the Committee at the present meeting confirms the greater bioavailability and possibly slower elimination of P15(H) mineral oil in F344 rats compared with Sprague-Dawley rats.

In the human toxicokinetic study submitted to the Committee at the present meeting, nine fasted female volunteers received a single oral dose of P15(H) at 1 mg/kg bw in a study compliant with current guidelines for good clinical practice. The concentration of mineral hydrocarbons in plasma, analysed using GC×GC-MS, was below the detection limit (limits of detection determined for each individual, ranging from 0.095 to 0.294 mg/l, mean 0.163 mg/l) at all time points (*6*, *7*).

Although mineral oils have been shown to be bioaccumulative, Boogaard et al. (*6*) proposed that the single-dose toxicokinetic data could be used in establishing an ADI for low- and medium-viscosity mineral oil, under the assumption that the C_{max} could be used as a functional surrogate marker

for hepatic concentrations of mineral oil, and comparing blood levels in humans with blood levels in rats at the no-observed-adverse-effect level (NOAEL). The studies were conducted with administration of olive oil (control) and P15(H) doses of 20, 200 or 1500 mg/kg bw by gavage to F344 rats, which Boogaard et al. (*6*) considered had been shown to be a clear NOAEL, a lowest-observed-adverse-effect level (LOAEL) and a clear effect dose, respectively. Sprague-Dawley rats were given single doses of olive oil or of P15(H) at 200 or 1500 mg/kg bw by gavage, dose levels that had not caused liver microgranulomas following 90 days of dietary dosing in this strain. The C_{max} values for mineral hydrocarbons at the reported NOAEL doses in both strains of rat were approximately 6 mg/l in the blood and approximately 30 mg/kg in the liver. In the F344 rats, the C_{max} value in blood (at 3–6 hours post-dosing) was approximately 15 mg/l following both the 200 and 1500 mg/kg bw doses, whereas the maximum reported levels in the liver (at 24 hours) were 56 and 89 mg/kg, respectively. Boogaard et al. (*6*) noted that the margin of exposure based on blood concentration after an acute dose of low-viscosity white oil at 1 mg/kg bw in humans was at least 37 in comparison with the blood concentration of 6 mg/l associated with no effect in rats.

Evaluation

The Committee concluded that the newly submitted data did not adequately address its previous requests for information on the relevance to humans of the response of F344 and Sprague-Dawley rats to mineral oil (medium and low viscosity) classes II and III. The studies were conducted with a single administration, and it was not possible to predict the concentration in the target organ (liver) at steady state, or the potential for accumulation, in humans. Information requested at the forty-fourth meeting on compositional factors of mineral oil that influence absorption and toxicity had not been provided for materials meeting the criteria of mineral oil (medium and low viscosity) classes II and III.

The Committee noted that hydrocarbon deposits with carbon numbers consistent with mineral oils, including those of classes II and III, and associated lesions have been reported in human tissues (Annex 1, reference *102*), demonstrating the potential relevance to humans of the effects in the F344 rat. Because all blood levels were below the limit of detection in the single-dose human toxicokinetic study, it was not possible to reach conclusions on the rate of elimination of mineral oils in humans or on the concentration in the liver at steady state following prolonged exposure. Therefore, the new data did not provide information that would allow an ADI to be established based on internal exposure.

Similarly, it was not possible to establish an ADI based on external dose in the absence of information on the relative accumulation potential of classes II and III mineral oil in humans compared with rats.

The Committee noted that the temporary group ADI for mineral oil (medium and low viscosity) classes II and III had been established in 1995 and extended on a number of occasions. As data supporting establishment of a full ADI had not been made available, the previously established temporary group ADI was withdrawn.

The existing specifications for mineral oil (medium and low viscosity) classes II and III were withdrawn pending a safety assessment.

A toxicological monograph addendum was not prepared.

3.1.3 *3-Phytase from* Aspergillus niger *expressed in* Aspergillus niger

Explanation

At the request of the World Food Programme and the Global Alliance for Improved Nutrition, the Committee evaluated the safety of the 3-phytase enzyme preparation (3-phytase: *myo*-inositol hexakisphosphate 3-phosphohydrolase; Enzyme Commission number 3.1.3.8), which it had not evaluated previously. 3-Phytase catalyses the sequential hydrolysis of phosphate monoesters from phytate (phytic acid), also known as *myo*-inositol (1,2,3,4,5,6) hexakisphosphate or *myo*-inositol hexakisphosphate. Hydrolysis of phytate by 3-phytase generates a series of lower (pentakis-, tetrakis-, etc.) *myo*-inositol phosphates and inorganic phosphates. The catalytic activity of 3-phytase is relatively specific; it does not have any significant levels of secondary enzyme activities. In this report, the expression "3-phytase" refers to the 3-phytase enzyme and its amino acid sequence, and the expression "3-phytase enzyme preparation" refers to the preparation formulated for commercial use. The 3-phytase enzyme preparation is used as a food additive, in the processing of phytate-rich food, such as cereal grains and legumes, and as a dietary supplement, for co-consumption with phytate-rich foods.

Genetic modification

The enzyme 3-phytase is produced from a genetically modified *Aspergillus niger* strain containing multiple copies of 3-phytase gene from *A. niger*. *Aspergillus niger* is a filamentous fungus that commonly occurs in the environment and is non-pathogenic. It has a long history of use as a source of citric acid and enzymes used in food processing, including enzymes from genetically engineered strains of the organism.

Prior to the introduction of the 3-phytase gene, the *A. niger* host strain ISO-500 was genetically modified by deletion of the genes encoding glucoamylase

activity. The modified host strain was then transformed with an amplifiable deoxyribonucleic acid (DNA) cassette containing the phytase gene from *A. niger* and the *Aspergillus nidulans* acetamidase (*amdS*) gene, which was the selectable marker. The recombinant production strain is genetically stable and does not contain any antibiotic resistance markers or any other heterologous DNA. Batch analysis demonstrated that the 3-phytase enzyme preparations from *A. niger* were free of aflatoxin B1, T2 toxin, ochratoxin A, zearalenone and sterigmatocystin.

Chemical and technical considerations

3-Phytase is produced by submerged, fed-batch, aerobic, pure culture fermentation of the genetically modified *A. niger* production strain. The enzyme is secreted into the fermentation broth and is subsequently purified and concentrated by ultrafiltration. The enzyme concentrate is formulated with glycerol (liquid form) or with maltodextrin (powder form) to achieve the desired phytase activity and stability. The 3-phytase enzyme preparation contains food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 1, reference *184*). Phytase activity is measured in phytase units, or FTU. One FTU is defined as the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute from sodium phytate at a concentration of 5.1 mmol/l at 37 °C and pH 5.5. The mean activity of 3-phytase calculated from three different batches of the ultrafiltrate concentrate was 106 FTU per milligram of total organic solids (TOS). TOS consists of the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism and the manufacturing process. The 3-phytase enzyme preparation is typically used at a range of 5–70 g/kg of food product, depending on the intended application and on the phytate content. 3-Phytase is expected to be inactivated during processing or cooking.

Assessment of potential allergenicity

3-Phytase was evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO and WHO (*8*). The amino acid sequence of 3-phytase was compared with the amino acid sequences of known allergens. A similarity search using the Allermatch database did not produce a match with any sequence showing greater than 35% identity over any sliding window of 80 amino acids. However, two stretches of six contiguous amino acids in the *A. niger* 3-phytase sequence were found to be identical to a sequence of six contiguous amino acids in each of two allergenic proteins from the WHO–International Union of Immunological Societies (WHO-IUIS) list. One match is to Zea m 14 protein in maize, and the other match

is to Der f 18 protein in house dust mite. A comparison using the Structural Database of Allergenic Proteins produced one match with Asp n 25 protein in *A. niger* that has greater than 35% identity over several windows of 80 amino acids. Further search and bioinformatics analysis using the National Center for Biotechnology Information protein database revealed that the sequence of six contiguous amino acids of *A. niger* 3-phytase that is present in Zea m 14 is not present in other similar allergenic food proteins, but is present in many non-allergenic proteins. Similarly, the sequence of six contiguous amino acids of *A. niger* 3-phytase that is present in Der f 18 is not present in chitinases from other sources (e.g. latex, banana, papaya) that are associated with allergenicity, but is present in many other proteins, including other phytases from *A. niger* as well as phytases from other species of *Aspergillus*. The Asp n 25 protein is one of the phytases from *A. niger*. Thus, some degree of sequence similarity is expected. Therefore, the Committee considered that oral intake of 3-phytase is not anticipated to pose a risk of allergenicity.

Toxicological data

Toxicological studies were performed with the 3-phytase enzyme preparation representative of commercial material with an activity of 83.8 FTU per milligram of TOS. In a 13-week study of general toxicity in rats, no treatment-related, toxicologically relevant effects were seen when the 3-phytase enzyme preparation was administered daily by gavage at doses up to 833 mg of TOS per kilogram of body weight. The NOAEL was identified as the highest dose tested (i.e. 833 mg of TOS per kilogram of body weight per day). The 3-phytase enzyme preparation was not mutagenic in a bacterial reverse mutation assay in vitro and was not clastogenic in an assay for chromosomal aberrations in human lymphocytes in vitro.

Assessment of dietary exposure

3-Phytase is expected to be inactivated in processed food. An estimate of the theoretical maximum dietary exposure to the *A. niger* 3-phytase enzyme preparation was made by the Committee using the conservative budget method approach. Based on the level of TOS of 28% in the enzyme preparation and its maximum proposed use levels in a variety of phytate-rich food applications, such as ready-to-use foods, vitamin and mineral supplements, fortified blended foods, fortified flour, breakfast cereals and beverages, the Committee estimated theoretical maximum daily exposures of 1.2 mg of TOS per kilogram of body weight in adults and 3.5 mg of TOS per kilogram of body weight in children. These estimates are conservative, as they are made assuming that 100% of food products would be manufactured using the enzyme preparation and that 100% of the enzyme preparation would remain in the final food.

Evaluation

Comparing the conservative exposure estimate with the NOAEL from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 250. The Committee allocated an ADI "not specified" for the 3-phytase enzyme preparation from *A. niger* expressed in *A. niger* used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.1.4 *Serine protease (chymotrypsin) from* Nocardiopsis prasina *expressed in* Bacillus licheniformis

Explanation

At the request of CCFA at its Forty-third Session (*3*), the Committee evaluated an enzyme preparation containing a serine protease with chymotrypsin specificity (chymotrypsin: Enzyme Commission number 3.4.21.1), which it had not evaluated previously. Serine protease (chymotrypsin) catalyses the hydrolysis of peptide bonds in a protein, preferably at the carboxyl end of Tyr (Tyr-X), Phe (Phe-X) and Trp (Trp-X), where X is not proline. It also catalyses the hydrolysis of peptide bonds at the carboxyl end of other amino acids, primarily Met and Leu, albeit at a slower rate. In this report, the expression "serine protease (chymotrypsin)" refers to the serine protease (chymotrypsin) enzyme and its amino acid sequence, and the expression "serine protease (chymotrypsin) enzyme preparation" refers to the serine protease (chymotrypsin) enzyme preparation as formulated for commercial use. The serine protease (chymotrypsin) enzyme preparation is used as a food additive to produce partially or extensively hydrolysed proteins of vegetable and animal origin. Such protein hydrolysates may be used for various applications as ingredients in food and/or beverages.

Genetic modification

Serine protease (chymotrypsin) is produced from a genetically modified strain of *Bacillus licheniformis* containing the serine protease (chymotrypsin) gene from *Nocardiopsis prasina*. *Bacillus licheniformis* is a Gram-positive bacterium that is widely distributed in nature. It has a long history of use in the production of enzymes used in food processing, including enzymes from genetically engineered strains of the organism.

Prior to the introduction of the serine protease (chymotrypsin) gene, the *B. licheniformis* host strain was genetically modified through deletion of genes responsible for sporulation and two endoproteases. The modified host strain was then transformed with an amplifiable DNA cassette containing the

serine protease (chymotrypsin) gene from *N. prasina*. A strain containing multiple copies of serine protease (chymotrypsin) gene was selected. The recombinant production strain was free of any markers, including antibiotic resistance genes. The final production strain is genetically stable and does not contain antibiotic resistance genes or other heterologous DNA.

Chemical and technical considerations

Serine protease (chymotrypsin) is produced by submerged, fed-batch, pure culture fermentation of the genetically modified *B. licheniformis* production strain. The enzyme is secreted into the fermentation broth and is subsequently purified and concentrated. The enzyme concentrate is formulated with sodium benzoate, potassium sorbate, glycerol and sorbitol to achieve the desired activity and stability. The serine protease (chymotrypsin) enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 1, reference *184*). Serine protease (chymotrypsin) activity is measured in protease units (PROT). One PROT is defined as the amount of enzyme that releases 1 μmol of *p*-nitroaniline per minute from substrate (Suc-Ala-Ala-Pro-Phe-pNA) at a concentration of 1 mmol/l at pH 9.0 and at 37 °C. The mean protease activity of three unstandardized batches of enzyme concentrate was 476.3 PROT per milligram of enzyme concentrate. TOS consists of the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism and the manufacturing process.

The serine protease (chymotrypsin) enzyme preparation is typically used up to a level of 20 g/kg of protein in the product. Serine protease (chymotrypsin) enzyme preparation is expected to be inactivated during processing.

Assessment of potential allergenicity

Serine protease (chymotrypsin) was evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO and WHO (*8*). The amino acid sequence of serine protease (chymotrypsin) was compared with the amino acid sequences of known allergens. No matches of six contiguous amino acids were found between the *N. prasina* serine protease (chymotrypsin) and any allergenic proteins in the Structural Database of Allergenic Proteins. Also, no matches of 35% amino acid identity were found between the *N. prasina* serine protease (chymotrypsin) and any allergenic proteins using a sliding window of 80 amino acids. However, a 35% amino acid identity was found with Pla a 2 (*Platanus acerifolia*; London plane tree, which is not listed as a food allergen in the WHO-IUIS list) if the window length was extended beyond 80 amino acids and gaps were introduced. Nevertheless, multiple gap openings and gap extensions needed to obtain this

identity suggest that the identity is most likely not biologically meaningful. Therefore, the Committee considered that oral intake of serine protease (chymotrypsin) is not anticipated to pose any risk of allergenicity.

Toxicological data

Toxicological studies were performed with the serine protease (chymotrypsin) enzyme preparation, which was produced according to the procedure used for commercial production and had an activity of 574.7 PROT per milligram of TOS. In a 13-week study of general toxicity in rats, no toxicologically relevant treatment-related effects were seen when the serine protease (chymotrypsin) enzyme preparation was administered daily by gavage at doses up to 500 mg of TOS per kilogram of body weight. The NOAEL was identified as the highest dose tested (i.e. 500 mg of TOS per kilogram of body weight per day). The serine protease (chymotrypsin) enzyme preparation was not mutagenic in a bacterial reverse mutation assay in vitro and was not clastogenic in an assay for chromosomal aberrations in human lymphocytes in vitro.

Assessment of dietary exposure

The serine protease (chymotrypsin) enzyme preparation is expected to be inactivated in processed food. An estimate of the theoretical maximum dietary exposure to serine protease (chymotrypsin) was made by the Committee using the conservative budget method approach. Based on the level of TOS of 7.7% in the enzyme preparation and its uses in a variety of applications as ingredients in food and/or beverages at the maximum proposed use levels per kilogram of processed protein, the Committee estimated theoretical maximum dietary exposures of 2.9 mg of TOS per kilogram of body weight per day for adults and 8.7 mg of TOS per kilogram of body weight per day for children.

The Committee noted that the above exposure estimates were too conservative, because they were made assuming that 100% of food products would be manufactured using the enzyme preparation and that 100% of the enzyme preparation would remain in the final food products. Therefore, the Committee concluded that a more refined estimate was necessary. Assuming that the serine protease (chymotrypsin) enzyme preparation was used to hydrolyse the entire daily human protein requirement of 1 g/kg bw (*9*) and using a maximum proposed use level of 1540 mg of TOS per kilogram of protein hydrolysate gives a dietary exposure estimate of 1.5 mg of TOS per kilogram of body weight per day. The Committee considered this estimate to be more relevant for the purpose of the safety assessment of the enzyme preparation, as it is based on human physiological protein requirements.

Evaluation

Comparing the exposure estimate with the NOAEL from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 350. The Committee allocated an ADI "not specified" for the serine protease (chymotrypsin) enzyme preparation from *N. prasina* expressed in the production strain *B. licheniformis*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.1.5 *Serine protease (trypsin) from* Fusarium oxysporum *expressed in* Fusarium venenatum

Explanation

At the request of CCFA at its Forty-third Session (*3*), the Committee evaluated an enzyme preparation containing a serine protease with trypsin specificity (trypsin: Enzyme Commission number 3.4.21.4), which it had not evaluated previously. Serine protease (trypsin) catalyses the hydrolysis of peptide bonds in a protein, primarily at the carboxyl side of lysine (Lys-X) or arginine (Arg-X), where X is not proline. In this report, the expression "serine protease (trypsin)" refers to the serine protease (trypsin) enzyme and its amino acid sequence, and the expression "serine protease (trypsin) enzyme preparation" refers to the serine protease (trypsin) enzyme preparation as formulated for commercial use. The serine protease (trypsin) enzyme preparation is used as a food additive in the manufacture of partially or extensively hydrolysed proteins for applications in food and beverages, for protein fortification and for emulsification or flavour enhancement.

Genetic modification

Serine protease (trypsin) is produced from a genetically modified strain of *Fusarium venenatum* containing the serine protease (trypsin) gene from *F. oxysporum*. *Fusarium venenatum* is a fungus belonging to the class of hyphomycetales. It is a saprophyte found in the soil and is not considered to be a human pathogen. Although the *Fusarium* species are known for their ability to produce mycotoxins, this *F. venenatum* strain is genetically modified to be non-toxigenic.

Prior to the introduction of the serine protease (trypsin) gene, the *F. venenatum* host strain was rendered incapable of producing trichothecenes and other related toxins by the deletion of the *tri5* gene encoding trichodiene synthase and replacing it with the acetamidase (*amdS*) gene from *A. nidulans*. The modified host strain was then transformed with an amplifiable

plasmid DNA fragment harbouring the serine protease (trypsin) gene from *F. oxysporum*. The individual transformed colonies were spore-purified, and a high-yielding transformant was selected for enzyme production. The final production strain is genetically stable and does not contain antibiotic resistance genes or other heterologous DNA. Batch analysis demonstrated that the serine protease (trypsin) enzyme preparations from *F. venenatum* were free of aflatoxin B1, T2 toxin, ochratoxin A, zearalenone, sterigmatocystin and diacetoxyscirpenol.

Chemical and technical considerations

Serine protease (trypsin) is manufactured by submerged, fed-batch, pure culture fermentation of a genetically modified *F. venenatum* production strain. The enzyme is secreted into the fermentation broth and is subsequently purified and concentrated. The enzyme concentrate is formulated with sodium benzoate, potassium sorbate, glycerol and water to achieve the desired activity and stability. The serine protease (trypsin) enzyme preparation contains commonly used food-grade materials and conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 1, reference *184*).

Serine protease (trypsin) activity is measured in Kilo Microbial Trypsin Units (KMTU). One KMTU is defined as the amount of enzyme that releases 1 μmol of *p*-nitroaniline per minute from substrate (Ac-Arg-pNA) at a concentration of 1 mmol/l at pH 8.0 and at 37 °C. The mean protease activity of two unstandardized batches of enzyme concentrate was 78 KMTU per gram of enzyme concentrate. TOS consists of the enzyme of interest and residues of organic materials, such as proteins, peptides and carbohydrates, derived from the production organism and the manufacturing process. The serine protease (trypsin) enzyme preparation is typically used up to a level of 12 g/kg of protein in the product. Serine protease (trypsin) enzyme preparation is expected to be inactivated during processing.

Assessment of potential allergenicity

Serine protease (trypsin) was evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO and WHO (*8*). A similarity search using the Allermatch database produced multiple matches showing a 35% or greater identity in a sliding window of 80 amino acids between the *F. oxysporum* serine protease (trypsin) and several allergenic proteins (Blo t 3, Der f 3, Der f 6, Der p 3, Der p 9, Eur m 3) that are not identified as food allergens in the WHO-IUIS list. Similarly, a comparison using the Structural Database of Allergenic Proteins produced multiple matches showing 35% or greater identity in different sliding windows of 80 amino acids with the mite allergen Blo t 3. A second similarity search was performed using the

Allermatch database and the Structural Database of Allergenic Proteins to identify the six contiguous amino acid stretches of the *F. oxysporum* serine protease (trypsin) that are shared by allergenic proteins. The search produced multiple matches of six contiguous amino acids between the *F. oxysporum* serine protease (trypsin) and many allergenic proteins from the WHO-IUIS list that are not identified as food allergens. Further search and bioinformatics analysis using the National Center for Biotechnology Information protein database revealed that these sequences are widely distributed in various trypsin, trypsin-like, chymotrypsin, chymotrypsin-like and other serine proteases and in non-trypsin and non-allergenic proteins in prokaryotes as well as lower and higher eukaryotes. Many of the six contiguous amino acid sequences of the *F. oxysporum* serine protease (trypsin) that are shared by the allergenic proteins Blo t 3, Bom p 4, Der f 3, Der p 3, Der p 9, Eur m 3 and Tyr p 3 are present in human trypsins as well. Therefore, the Committee considered that oral intake of the serine protease (trypsin) is not anticipated to pose a risk of allergenicity.

Toxicological data

Toxicological studies were performed with the serine protease (trypsin) enzyme preparation using a batch that was representative of commercial material and had an activity of 1.06 KMTU per milligram of TOS. In a 13-week study of general toxicity in rats, no toxicologically relevant treatment-related effects were seen when serine protease (trypsin) enzyme preparation was administered daily by gavage at doses up to 581 mg of TOS per kilogram of body weight. The NOAEL was identified as the highest dose tested (i.e. 581 mg of TOS per kilogram of body weight per day). The serine protease (trypsin) enzyme preparation was not mutagenic in a bacterial reverse mutation assay in vitro and was not clastogenic in an assay for chromosomal aberrations in human lymphocytes in vitro.

Assessment of dietary exposure

The serine protease (trypsin) enzyme preparation is expected to be inactivated in processed food. An estimate of the theoretical maximum dietary exposure to the serine protease (trypsin) enzyme preparation was made by the Committee using the conservative budget method approach. Based on the level of TOS of 4% in the enzyme preparation and its uses in a variety of applications as ingredients in food and/or beverages at the maximum proposed use level per kilogram of processed protein, the Committee estimated theoretical maximum daily exposures of 0.9 mg of TOS per kilogram of body weight in adults and 2.7 mg of TOS per kilogram of body weight in children.

The Committee noted that the above exposure estimates were too conservative because they were made assuming that 100% of food products would be

manufactured using the enzyme preparation and that 100% of the enzyme preparation would remain in the final food products. Therefore, the Committee concluded that a more refined estimate was necessary. Assuming that the serine protease (trypsin) enzyme preparation was used to hydrolyse the entire daily human protein requirement of 1 g/kg bw per day (*9*) and using a maximum proposed use level of 480 mg of TOS per kilogram of protein hydrolysate gives a dietary exposure estimate of 0.5 mg of TOS per kilogram of body weight per day. The Committee considered this estimate to be more relevant for the purpose of the safety assessment of the enzyme preparation, as it is based on human physiological protein requirements.

Evaluation

Comparing the dietary exposure estimate with the NOAEL from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 1200. The Committee allocated an ADI "not specified" for the serine protease (trypsin) enzyme preparation from *F. oxysporum* expressed in the production strain *F. venenatum*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.2 Revision of specifications

3.2.1 *Ethyl cellulose*

Ethyl cellulose was placed on the agenda of the present meeting at the request of the Forty-third Session of CCFA (*3*) to consider the use of propyl gallate as an antioxidant in ethyl cellulose and to add a limit for propyl gallate in the specifications monograph. The Committee decided to add a general reference to the use of antioxidants in the definition section of the specifications monograph, as described under section 2.3. Considering the proposed use levels of this secondary additive and estimated exposure compared with the ADI of propyl gallate, the Committee concluded that a limit for propyl gallate was not necessary. Therefore, the Committee amended the specifications monograph for ethyl cellulose so that antioxidants permitted for use in food may be added for stabilizing purposes.

3.2.2 *Mineral oil (medium viscosity)*

During the evaluation of mineral oil (medium and low viscosity) classes II and III at the present meeting, the Committee withdrew the existing temporary ADI for these additives. Consequently, it was decided to withdraw the existing specifications for mineral oil (medium and low viscosity). However,

as the specifications monograph for mineral oil (medium and low viscosity) covered not only classes II and III but also class I, for which an ADI of 0–10 mg/kg bw was established at the fifty-ninth meeting of the Committee (Annex 1, reference *160*), it was found necessary to prepare specifications for class I only. For this purpose, the provisions for class I and the methods were extracted from the existing monograph to prepare the new specifications monograph with the title Mineral oil (medium viscosity). The provisions and methods were not reviewed.

3.2.3 *Modified starches*

The Committee at its current meeting recognized an error in the specifications monograph for modified starches. The error referred to the use of filter cake instead of filtrate in the titration step of the test method for the degree of substitution of starch sodium octenyl succinate. The specifications were revised to include the amended test method. The corrected test method will be published in FAO JECFA Monographs 13 (2012), and the online version will be amended accordingly.

3.2.4 *Titanium dioxide*

Titanium dioxide was placed on the agenda of the present meeting at the request of the Forty-third Session of CCFA (*3*) to consider alternative methods for the purity of titanium dioxide.

The Committee at its seventy-third meeting (Annex 1, reference *202*) prepared specifications for titanium dioxide and included titrimetric methods for the assay of titanium dioxide and determination of aluminium oxide, as well as a gravimetric method for the determination of silicon dioxide, in the specifications monograph. The Committee at its current meeting replaced the methods for the determination of aluminium oxide and silicon dioxide as well as the assay method with methods based on alkali fusion followed by ICP-AES determination. The specifications were revised accordingly.

4. Flavouring agents

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

Assignment to structural class

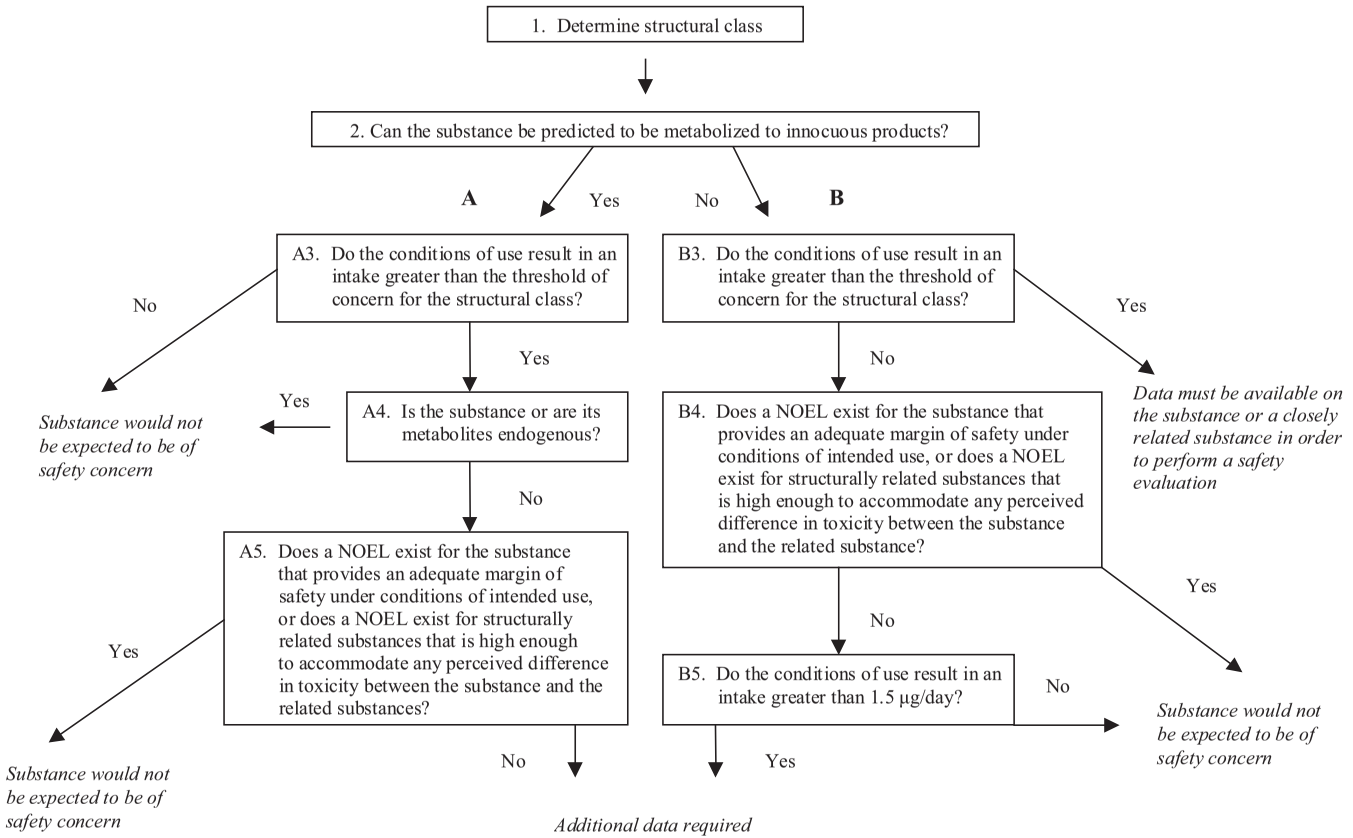
Twelve groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

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

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference *122*):

- *Innocuous metabolic products* are defined as products that are known or readily predicted to be harmless to humans at the estimated dietary exposure to the flavouring agent.
- *Endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions

Figure 1 **Procedure for the Safety Evaluation of Flavouring Agents**



are not included. The estimated dietary exposure to a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Assessment of dietary exposure

Maximized survey-derived intake (MSDI)

Estimates of the dietary exposure to flavouring agents by populations are based on annual volumes of production. These data were derived from surveys in Europe, Japan and the United States of America (USA). Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products when compiling these data. When using these production volumes to estimate dietary exposures, a correction factor of 0.8 is applied to account for under-reporting.

$$\text{MSDI (μg/day)} = \frac{\text{annual volume of production (kg)} \times 10^9 \text{ (μg/kg)}}{\text{population of consumers} \times 0.8 \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe, 13×10^6 in Japan and 31×10^6 in the USA.

Single portion exposure technique (SPET)

The SPET was developed by the Committee at its sixty-seventh meeting (Annex 1, reference *184*) to account for presumed patterns of consumer behaviour with respect to food consumption and the possible uneven distribution of dietary exposures among consumers of foods containing flavouring agents. It is based on reported use levels supplied by the industry. This single portion–derived estimate was designed to account for individuals' brand loyalty to food products and for niche products that would be expected to be consumed by only a small proportion of the population. Its use in the Procedure was endorsed at the sixty-ninth meeting of the Committee (Annex 1, reference *190*) to render the safety assessment more robust, replacing the sole use of MSDI estimates with the higher of the highest MSDI or the SPET estimate as the exposure estimate in the decision-tree. The Committee also agreed that it would not be necessary to re-evaluate flavouring agents that had already been assessed previously using the Procedure.

The SPET provides an estimate of dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day. The SPET combines an average (or usual) added use level provided by the flavour industry with a standard portion size from 75 predefined food categories as described by the Committee at its sixty-seventh meeting. The standard portion is taken to represent the mean food consumption for consumers of these

food categories. Among all the food categories with a reported use level, the calculated dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate:

$$\text{SPET (μg/day)} = \text{standard portion size of food category } i \text{ (g/day)} \times \text{use level for food category } i \text{ (μg/g)}$$

The highest result is used in the evaluation.

The use level data provided by industry for each flavouring agent evaluated at this meeting and used in the SPET calculations are available on the WHO JECFA web site at http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html.

Consideration of combined intakes from use as flavouring agents

The safety assessment of possible combined intakes of flavouring agents was based on the presence of common metabolites or a homologous series (as proposed at the sixty-eighth meeting; Annex 1, reference *187*) and using the MSDI exposure assessment (as proposed at the sixty-ninth meeting; Annex 1, reference *190*).

4.1.1 *Aliphatic and aromatic amines and amides*

Explanation

The Committee evaluated an additional seven flavouring agents belonging to the group of aliphatic and aromatic amines and amides. The additional flavouring agents comprised one aniline (No. 2043), three menthyl amides (Nos 2078–2080) and three amides with alicyclic, aromatic or alkyl side-chains, including one with a pentafluorophenyl group (Nos 2077, 2081 and 2082). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee. Four of the seven flavouring agents in this group (Nos 2077 and 2080–2082) are reported to be flavour modifiers.

The Committee evaluated 37 other members of this group of flavouring agents at its sixty-fifth meeting (Annex 1, reference *178*). For 36 of these flavouring agents, the Committee concluded that they would not give rise to safety concerns based on estimated dietary exposures. For acetamide (No. 1592), the Committee considered it inappropriate for use as a flavouring agent or for food additive purposes based on the available data indicating carcinogenicity in mice and rats. For 27 flavouring agents, the dietary exposure estimates were based on anticipated annual volumes of production, and these evaluations were conditional pending submission of use levels or poundage data,

which were provided at the sixty-ninth meeting (Annex 1, reference *190*). For the evaluation of 2-isopropyl-*N*-2,3-trimethylbutyramide (No. 1595), additional data available at the sixty-ninth meeting raised safety concerns, and the Committee concluded that the Procedure could not be applied to this flavouring agent until additional safety data became available.

At its sixty-eighth meeting, the Committee evaluated 12 additional members of this group of flavouring agents and concluded that all 12 were of no safety concern at estimated dietary exposures (Annex 1, reference *187*).

The Committee evaluated nine additional members of this group of flavouring agents at its seventy-third meeting (Annex 1, reference *202*). The Committee concluded that five of the nine flavouring agents did not raise any safety concerns at estimated dietary exposures. For one of the remaining four flavouring agents (No. 2007), the available data did not provide an adequate margin of exposure, and for the other three flavouring agents (Nos 2005, 2010 and 2011), no additional data were available. The Committee concluded that for these four flavouring agents, further data would be required to complete the safety evaluation.

One of the seven flavouring agents considered at the current meeting—namely, 2-aminoacetophenone (No. 2043)—is a natural component of food and has been detected in maize, corn chips, tuna, egg white, milk, soya milk, green tea, honey and beer.

Assessment of dietary exposure

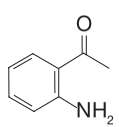
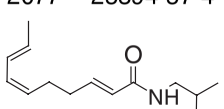
The total annual volumes of production of the seven aliphatic and aromatic amines and amides are approximately 362 kg in the USA, 0.1 kg in Europe and 0.1 kg in Japan. Approximately 83% of the total annual volume of production in the USA is accounted for by one flavouring agent in this group—namely, (2*E*,6*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077). Dietary exposures were estimated using the MSDI method and the SPET. The highest estimates were all derived using the SPET and are reported in Table 2.

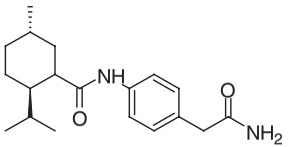
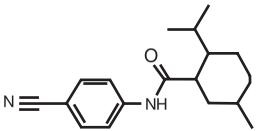
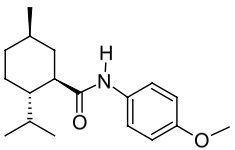
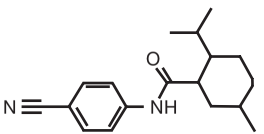
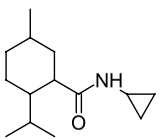
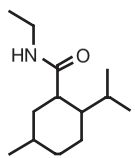
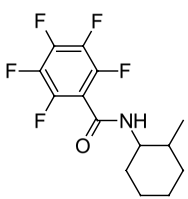
The highest estimated daily dietary exposure is for (2*E*,6*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077) (4500 μg, the SPET value obtained from non-alcoholic beverages). For the other flavouring agents, daily dietary exposures as SPET or MSDI estimates range from 0.01 to 3000 μg, with the SPET yielding the highest estimate in each case.

Absorption, distribution, metabolism and elimination

The metabolism of aliphatic and aromatic amines and amides was described in the reports of the sixty-fifth, sixty-eighth and seventy-third meetings of the Committee (Annex 1, references *178*, *187* and *202*).

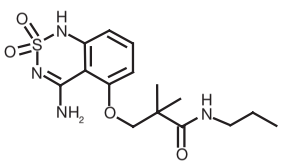
Table 2
Summary of the results of the safety evaluations of aliphatic and aromatic amines and amides used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step A4/B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class I							
2-Aminoacetophenone	2043	551-93-9 	A3: No, SPET: 10	NR	Note 1		No safety concern
Structural class III							
(2*E*,6*E*/*Z*,8*E*)-*N*-(2-Methylpropyl)-2,6,8-decatrienamide	2077	25394-57-4 	B3: Yes, SPET: 4500	The NOEL of 572 mg/kg bw per day in a 28-day study in rats is 7600 times the estimated daily dietary exposure to No. 2077 when used as a flavouring agent.	Note 2		No safety concern

(2*S*,5*R*)-*N*-[4-(2-Amino-2-oxoethyl)-phenyl]-5-methyl-2-(propan-2-yl)-cyclohexanecarboxamide 	2078	1119711-29-3	B3: Yes, SPET: 3000	The NOAEL of 300 mg/kg bw per day for the structurally related *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) in a 90-day study in rats is 6000 times the estimated daily dietary exposure to No. 2078 when used as a flavouring agent.	Note 2	*N*-*p*-Benzeneacetonitrile menthanecarboxamide (No. 2009) 	No safety concern
(1*R*,2*S*,5*R*)-*N*-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl) cyclohexanecarboxamide 	2079	68489-09-8	B3: Yes, SPET: 625	The NOAEL of 300 mg/kg bw per day for the structurally related *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) in a 90-day study in rats is 29 000 times the estimated daily dietary exposure to No. 2079 when used as a flavouring agent.	Note 2	*N*-*p*-Benzeneacetonitrile menthanecarboxamide (No. 2009) 	No safety concern
N-Cyclopropyl-5-methyl-2-isopropyl-cyclohexanecarboxamide 	2080	73435-61-7	B3: Yes, SPET: 3000	The NOEL of 8 mg/kg bw per day for the structurally related *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) in a 28-day study in rats is 160 times the SPET estimate (3000 µg/day) and 480 000 times the MSDI estimate (1 µg/day) when No. 2080 is used as a flavouring agent.	Note 2	*N*-Ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) 	No safety concern
N-(2-Methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide 	2081	1003050-32-5	B3: No, SPET: 50	B4: Yes. The NOAEL of 130 mg/kg bw per day in a 28-day study in rats is 160 000 times the estimated daily dietary exposure to No. 2081 when used as a flavouring agent.	Note 3		No safety concern

continued

Table 2 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step A4/B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
3[(4-Amino-2,2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)-oxy]-2,2-dimethyl-*N*-propylpropanamide	2082	1093200-92-0 	B3: Yes, SPET: 1250	The NOEL of 20 mg/kg bw per day in a 90-day study in rats is 960 times the estimated daily dietary exposure to No. 2082 when used as a flavouring agent.	Note 3		No safety concern

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

[a] Fifty-eight flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *178*, *187* and *202*).

[b] *Step 1*: One flavouring agent is in structural class I (No. 2043), and six flavouring agents (Nos 2077–2082) are in structural class III.

[c] *Step 2*: Flavouring agent No. 2043 is expected to be metabolized to innocuous products. The remaining six flavouring agents (Nos 2077–2082) cannot be predicted to be metabolized to innocuous products.

[d] The thresholds for human dietary exposure for structural classes I and III are 1800 and 90 µg/person per day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

[e] The margins of exposure were calculated based on the estimated dietary exposure calculated using the SPET. In cases where the resulting margin of exposure was relatively low, a comparison with the MSDI was also made.

Notes:

1. 2-Aminoacetophenone is a product of tryptophan metabolism and is expected to be transformed to anthranilic acid, which is excreted in the urine or involved in subsequent metabolic pathways.
2. Amides are expected to undergo hydrolysis and/or oxidation and enter into known pathways of metabolism.
3. Minimal metabolism of Nos 2081 and 2082 was observed in vitro; small amounts of hydroxylation products were formed for each flavouring agent.

In general, aliphatic and aromatic amines and amides are rapidly absorbed from the gastrointestinal tract and metabolized by deamination, hydrolysis or oxidation to polar metabolites that are readily eliminated in the urine. Aliphatic amides have been reported to undergo hydrolysis in mammals; however, the rate of hydrolysis is dependent on the chain length and may involve a number of different enzymes.

In relation to these additional flavouring agents, only limited information regarding metabolic pathways is available for specific substances.

Published studies indicate that 2-aminoacetophenone (No. 2043) is a minor intermediate of tryptophan metabolism that is further metabolized to anthranilic acid, which is excreted in urine or involved in subsequent metabolic pathways.

Distribution and metabolism studies were provided for *N*-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide (No. 2081). Widespread tissue distribution of parent compound and/or metabolites was observed following oral administration to rats. Minimal metabolite formation was observed following incubation with rat liver microsomes. Those metabolites that were identified were hydroxylation products.

A pharmacokinetic study on 3[(4-amino-2,2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (No. 2082) indicated rapid absorption following oral administration to rats; however, oral bioavailability was less than 10%. Minimal metabolite formation was observed following incubation with rat and human liver microsomes. Identified metabolites were hydroxylation products.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the additional flavouring agents in this group, the Committee assigned one flavouring agent (No. 2043) to structural class I. The remaining six flavouring agents (Nos 2077–2082) were assigned to structural class III (*10*).

Step 2. One of the flavouring agents (No. 2043) in this group is predicted to be metabolized to innocuous products. The evaluation of this flavouring agent therefore proceeded via the A-side of the Procedure. The remaining flavouring agents (Nos 2077–2082) in this group could not be predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step A3. The highest dietary exposure of 2-aminoacetophenone (No. 2043) is below the threshold of concern (i.e. 1800 μg/person per day for class I). This flavouring agent would not be expected to be of safety concern at current estimated dietary exposures.

Step B3. The highest dietary exposures for five flavouring agents in structural class III are above the threshold of concern (i.e. 90 µg/person per day for class III). Accordingly, for all of these flavouring agents, data are required on the flavouring agent or a closely related substance in order to perform a safety evaluation. For one of the flavouring agents in structural class III (No. 2081), the highest estimated dietary exposure is below the threshold of concern. Accordingly, evaluation of this flavouring agent proceeded to step B4.

Step B4. For *N*-(2-methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide (No. 2081), the NOAEL of 130 mg/kg bw per day in a 28-day study in rats provides a margin of exposure of 160 000 in relation to the highest estimated dietary exposure to No. 2081 (SPET = 50 µg/day) when used as a flavouring agent.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

Toxicity data on flavouring agent No. 2009, previously evaluated by the Committee (Annex 1, reference *202*), were used in support of the safety evaluation of two flavouring agents (Nos 2078 and 2079). Toxicity data on flavouring agent No. 1601, previously evaluated by the Committee (Annex 1, reference *178*), were used in support of the safety evaluation of flavouring agent No. 2080. Toxicity data on flavouring agent No. 2077 were previously evaluated by the Committee (Annex 1, reference *178*).

For (2*E*,6*E*/*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077), the no-observed-effect level (NOEL) of 572 mg/kg bw per day in a 28-day study in rats provides a margin of exposure of 7600 in relation to the highest estimated dietary exposure to No. 2077 (SPET = 4500 µg/day) when used as a flavouring agent.

For (2*S*,5*R*)-*N*-[4-(2-amino-2-oxoethyl)phenyl]-5-methyl-2-(propan-2-yl)-cyclohexanecarboxamide (No. 2078), the NOAEL of 300 mg/kg bw per day for the structurally related substance *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) in a 90-day study in rats is 6000 times the highest estimated dietary exposure to No. 2078 (SPET = 3000 µg/day) when used as a flavouring agent.

For (1*R*,2*S*,5*R*)-*N*-(4-methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide (No. 2079), the NOAEL of 300 mg/kg bw per day for the structurally related substance *N*-*p*-benzeneacetonitrile menthanecarboxamide (No. 2009) in a 90-day study in rats is 29 000 times the highest estimated dietary exposure to No. 2079 (SPET = 625 µg/day) when used as a flavouring agent.

For *N*-cyclopropyl-5-methyl-2-isopropylcyclohexanecarboxamide (No. 2080), the NOEL of 8 mg/kg bw per day for the structurally related substance

N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) in a 28-day study in rats is 160 times the SPET estimate (3000 μg/day) and 480 000 times the MSDI estimate (1 μg/day) when No. 2080 is used as a flavouring agent.

For3[(4-amino-2,2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide (No. 2082), the NOEL of 20 mg/kg bw per day in a 90-day study in rats provides a margin of exposure of 960 in relation to the highest estimated dietary exposure to No. 2082 (SPET = 1250 μg/day) when used as a flavouring agent.

The Committee concluded that the five flavouring agents with high exposure evaluated via the B-side of the decision-tree (Nos 2077–2080 and 2082) would not pose a safety concern at current estimated dietary exposures.

Table 2 summarizes the evaluations of the seven additional flavouring agents belonging to the group of aliphatic and aromatic amines and amides (Nos 2043 and 2077–2082).

Consideration of combined intakes from use as flavouring agents

The highest MSDI values for members of the current group are 33 μg/day (No. 2077), 6 μg/day (No. 2078) and 1 μg/day (No. 2080). The seven additional flavouring agents in this group have diverse structures, with various potential sites of metabolism, and are not likely to be metabolized to common products. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of the flavouring agents in this group would not saturate metabolic pathways, and the combined intakes, including those of previously evaluated members of the group, would not raise safety concerns.

Conclusion

In the previous evaluations of members of this group of flavouring agents, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. Previously evaluated data from short-term studies of toxicity were used to support the safety evaluations of four members of the current group. Data from short-term studies of toxicity on two members of the current group (Nos 2081 and 2082) were evaluated at the present meeting. The Committee concluded that none of the seven flavouring agents evaluated at the present meeting, which are additions to the group of aliphatic and aromatic amines and amides evaluated previously, raise any safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.2 *Aliphatic and aromatic ethers*

Explanation

The Committee evaluated a group of 10 aliphatic and aromatic ethers used as flavouring agents. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these agents have previously been evaluated.

The Committee previously evaluated 29 other members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*), including benzyl butyl ether (No. 1253) and dibenzyl ether (No. 1256), which were evaluated only for specifications at the twenty-fourth meeting (Annex 1, reference *53*). The Committee concluded that all 29 flavouring agents in that group were of no safety concern at estimated dietary exposures.

Seven of the 10 flavouring agents (Nos 2133–2135, 2137–2139 and 2142) in this group have been reported to occur naturally and can be found in strawberry, lychee, *Salvia* species, dill blossom, dill herb, clary sage, grape brandy, arctic bramble, black currant (buds), *Cinnamomum*, elder flower, elderberry juice, grapefruit juice, green tea (roasted), fresh tomato and white wine.

Assessment of dietary exposure

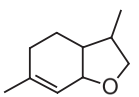
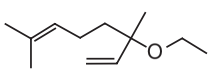
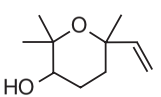
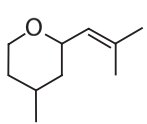
The total annual volumes of production of the 10 aliphatic and aromatic ethers are approximately 20 kg in the USA, 0.1 kg in Europe and 371 kg in Japan. Two flavouring agents in this group, butyl β-naphthyl ether (No. 2141) and linalool oxide pyranoid (No. 2135), with annual volumes of production of 202 kg and 159 kg, respectively, account for approximately 97% of the total annual volume of production in Japan. Approximately 95% of the reported volume of production in the USA is accounted for by 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran (No. 2133).

Dietary exposures were estimated using the MSDI method and the SPET, with the highest values reported in Table 3. The estimated daily dietary exposure is highest for digeranyl ether (No. 2142) (10 000 μg, the SPET value obtained from gelatines and puddings). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 5000 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

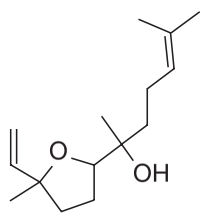
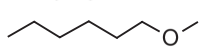
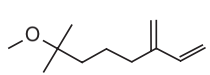
Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic and aromatic ethers has previously been described in the report of the sixty-first meeting (Annex 1, reference *166*).

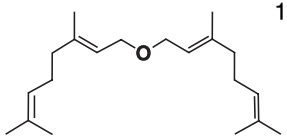
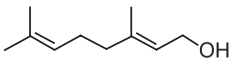
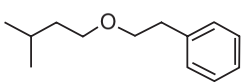
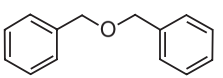
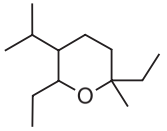
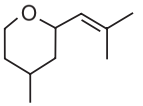
Table 3
Summary of the results of the safety evaluations of aliphatic and aromatic ethers used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II							
3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	2133	70786-44-6 	No, SPET: 180	NR	Note 1		No safety concern
Ethyl linalyl ether	2134	72845-33-1 	No, SPET: 0.1	NR	Note 2		No safety concern
Linalool oxide pyranoid	2135	14049-11-7 	Yes, SPET: 600	Yes. The NOEL of 2.5 mg/kg bw per day in a 90-day study in rats for the structurally related tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran (No. 1237) is 250 times the estimated dietary exposure to No. 2135 calculated using the SPET and 3300 times compared with the MSDI (45 µg/day) when No. 2135 is used as a flavouring agent.	Note 1	Tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran (No. 1237) 	No safety concern

continued

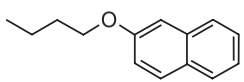
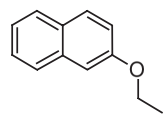
Table 3 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Nerolidol oxide	2137	1424-83-5 	Yes, SPET: 2500	No	Note 1		Additional data required to complete evaluation
Methyl hexyl ether	2138	4747-07-3 	Yes, SPET: 5000	Yes. The NOEL of 900 mg/kg bw per day in a 90-day study in rats for the structurally related methyl *tert*-butyl ether is 11 000 times the estimated dietary exposure to No. 2138 when used as a flavouring agent.	Note 2	Methyl *tert*-butyl ether 	No safety concern
Myrcenyl methyl ether	2139	24202-00-4 	No, SPET: 63	NR	Note 2		No safety concern

Digeranyl ether	2142	31147-36-1 	Yes, SPET: 10 000	Yes. The NOEL of 50 mg/kg bw per day in a 196-day study in rats for the structurally related geraniol (No. 1223) is 300 times the estimated dietary exposure to No. 2142 calculated using the SPET and 300 million times compared with the MSDI (0.01 µg/day) when used as a flavouring agent.	Note 2	Geraniol (No. 1223) 	No safety concern
Structural class III							
Isoamyl phenethyl ether	2136	56011-02-0 	Yes, SPET: 150	Yes. The NOEL of 196 mg/kg bw per day in a 90-day study in rats for the structurally related dibenzyl ether (No. 1256) is 78 000 times the estimated dietary exposure to No. 2136 when used as a flavouring agent.	Note 3	Dibenzyl ether (No. 1256) 	No safety concern
5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran	2140	1120363-98-5 	Yes,SPET: 2500	Yes. The NOEL of 2.5 mg/kg bw per day in a 90-day study in rats for the structurally related tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran (No. 1237) is 60 times the estimated dietary exposure to No. 2140 calculated using the SPET and 15 million times compared with the MSDI (0.01 µg/day) when used as a flavouring agent.	Note 1	Tetrahydro-4-methyl-2-(2-methyl-propen-1-yl)-pyran (No. 1237) 	No safety concern

continued

Table 3 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Butyl β-naphthyl ether	2141	10484-56-7 	Yes, SPET: 400	Yes. The NOEL of 5.1 mg/kg bw per day in a 90-day study in rats for the structurally related β-naphthyl ethyl ether (No. 1258) is 770 times the estimated dietary exposure to No. 2141 calculated using the SPET and 5300 times compared with the MSDI (58 µg/day) when used as a flavouring agent.	Note 3	β-Naphthyl ethyl ether (No. 1258) 	No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because dietary exposure to the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

[a] Twenty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *166*).

[b] *Step 1*: Seven flavouring agents in this group (Nos 2133–2135, 2137–2139 and 2142) are in structural class II. Three flavouring agents in this group (Nos 2136, 2140 and 2141) are in structural class III (*10*).

[c] *Step 2*: All of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human dietary exposure for structural classes II and III are 540 and 90 µg/person per day, respectively. All dietary exposures are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

[e] The margins of exposure were calculated based on the estimated dietary exposure calculated using the SPET. In cases where the resulting margin of exposure was relatively low, a comparison with the MSDI was also made.

Notes:

1. Alicyclic ethers are expected to undergo ring hydroxylation by cytochrome P450, conjugation with glucuronic acid and then excretion in the urine.
2. The straight-chain aliphatic ethers are expected to undergo *O*-dealkylation to yield the corresponding alcohol and aldehyde, which subsequently undergo complete oxidation in the fatty acid pathway and tricarboxylic acid cycle.
3. The aromatic ethers are expected to undergo ring hydroxylation, *O*-demethylation or side-chain oxidation, depending upon the position of the substituents, followed by conjugation with glucuronic acid, sulfate or glycine.

The straight-chain aliphatic ethers (Nos 2134, 2138, 2139 and 2142) are predicted to undergo *O*-dealkylation in vivo to yield the corresponding alcohol and aldehyde, which subsequently undergo complete oxidation via the fatty acid pathway and tricarboxylic acid cycle. Alternatively, the aliphatic ether may undergo ω-1 oxidation to yield polar hydroxylated metabolites. These are conjugated with glucuronic acid and excreted or are further oxidized and/or excreted. The alicyclic ethers (Nos 2133, 2135, 2137 and 2140) are predicted to largely undergo ring hydroxylation by cytochrome P450. The resulting metabolites undergo conjugation with glucuronic acid, followed by excretion in the urine. The aromatic ethers (Nos 2136 and 2141) are predicted to undergo ring hydroxylation, *O*-demethylation or side-chain oxidation (depending upon the position of the substituents). These metabolites can undergo conjugation with glucuronic acid, sulfate or glycine, the products of which are readily excreted.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 10 flavouring agents in this group of aliphatic and aromatic ethers, the Committee assigned 7 flavouring agents to structural class II (Nos 2133–2135, 2137–2139 and 2142) and 3 flavouring agents to structural class III (Nos 2136, 2140 and 2141) (*10*).

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

Step A3. The highest dietary exposures to three of the flavouring agents (Nos 2133, 2134 and 2139) in structural class II are below the threshold of concern (i.e. 540 μg/person per day for class II). According to the Procedure, these flavouring agents raise no safety concern at their current estimated dietary exposures. The estimated dietary exposures to four of the flavouring agents (Nos 2135, 2137, 2138 and 2142) are above the threshold of concern for structural class II (i.e. 540 μg/person per day for class II). The three flavouring agents in structural class III (Nos 2136, 2140 and 2141) have estimated dietary exposures above the threshold of concern (i.e. 90 μg/person per day for class III). The evaluation of the seven flavouring agents with estimated dietary exposures above the thresholds of concern proceeded to step A4.

Step A4. Because all of the flavouring agents with estimated dietary exposures above the thresholds of concern, and their metabolites, are not endogenous, the evaluation proceeded to step A5.

Step A5. For linalool oxide pyranoid (No. 2135), the NOEL of 2.5 mg/kg bw per day for the structurally related substance tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran (No. 1237) from a 90-day dietary study in rats provides a margin of exposure of 250 in relation to the dietary exposure to No. 2135 as calculated using the SPET (600 μg/person per day) or 3300 in relation to the MSDI (45 μg/day) when No. 2135 is used as a flavouring agent.

For isoamyl phenethyl ether (No. 2136), the NOEL of 196 mg/kg bw per day for the structurally related substance dibenzyl ether (No. 1256) from a 90-day dietary study in rats provides a margin of exposure of 75 000 in relation to the dietary exposure to No. 2136 calculated using the SPET (150 μg/day) when No. 2136 is used as a flavouring agent.

For nerolidol oxide (No. 2137), a NOAEL for this flavouring agent or a structurally related substance was not available. The Committee therefore concluded that additional data would be necessary to complete the safety evaluation.

For methyl hexyl ether (No. 2138), the NOEL of 900 mg/kg bw per day for the structurally related substance methyl *tert*-butyl ether from a 90-day study in rats provides a margin of exposure of 10 000 in relation to the dietary exposure to No. 2138 (SPET = 5000 μg/day) when used as a flavouring agent.

For 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran (No. 2140), the NOEL of 2.5 mg/kg bw per day for the structurally related substance tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran (No. 1237) from a 90-day dietary study in rats provides a margin of exposure of 60 in relation to the dietary exposure to No. 2140 as the SPET value (2500 μg/day) or 15 million in relation to the MSDI (0.01 μg/day) when No. 2140 is used as a flavouring agent.

For butyl β-naphthyl ether (No. 2141), the NOEL of 5.1 mg/kg bw per day for the structurally related β-naphthyl ethyl ether (No. 1258) from a 90-day study in rats provides a margin of exposure of 770 relative to the SPET value (400 μg/day) or 5000 in relation to the MSDI (58 μg/day) when No. 2141 is used as a flavouring agent.

For digeranyl ether (No. 2142), the NOEL of 50 mg/kg bw per day from a 196-day dietary study in rats for the structurally related substance, and predicted metabolite, geraniol (No. 1223; a mixture of 3,7-dimethyl-2,6-octadienol and 3,7-dimethyl-1,6-octadienol) provides a margin of exposure of 300 relative to the SPET value (10 000 μg/day) or 300 million in relation to the MSDI (0.01 μg/day) when No. 2142 is used as a flavouring agent.

The Committee therefore concluded that 9 of the 10 additional flavouring agents (Nos 2133–2136 and 2138–2142) in this group of aliphatic and aromatic ethers would not pose a safety concern at current estimated dietary exposures. Table 3 summarizes these evaluations.

Consideration of combined intakes from use as flavouring agents

Flavouring agents Nos 2142, 1257, 1258 and 1259 are ethers of 2-naphthol, which could be a common metabolite, but the combined intake of these is less than the threshold of concern for structural class III (i.e. 90 μg/person per day). Flavouring agents Nos 2136, 1252, 1253 and 1254 are benzyl or phenethyl ethers, but the combined intake of these is also less than the threshold of concern for structural class III. The other flavouring agents in this group have diverse structures, with various potential sites of metabolism, and are not likely to be metabolized to common products. The Committee concluded that combined intakes of the flavouring agents in this group would not raise safety concerns.

Consideration of secondary components

One flavouring agent in this group (No. 2135) has a minimum assay value of less than 95% (see Annex 4). The secondary component of linalool oxide pyranoid (No. 2135) is linalool (No. 356), which was previously evaluated (Annex 1, reference *137*) and considered not to present a safety concern at estimated dietary exposures.

Conclusion

In the previous evaluation of flavouring agents in this group of aliphatic and aromatic ethers, studies of metabolism and acute toxicity, short-term and long-term studies of toxicity, and studies of genotoxicity were available. The results of those studies did not raise safety concerns. The additional data from acute toxicity studies (Nos 1237, 2136 and 2140), short-term studies of toxicity (No. 1239), studies of reproductive toxicity (No. 1243) and genotoxicity studies (Nos 2133 and 2140 and several flavouring agents previously evaluated) considered at this meeting support the previous evaluation (Annex 1, reference *166*) and raised no safety concerns.

The Committee concluded that 9 of these 10 flavouring agents, which are additions to the group of aliphatic and aromatic ethers evaluated previously, would not give rise to safety concerns at current estimated dietary exposures. For one flavouring agent (No. 2137), additional data are required to complete the evaluation.

An addendum to the toxicological monograph was prepared.

4.1.3 *Aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution*

Explanation

At the present meeting, the Committee evaluated a group of 39 aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution as flavouring agents. This group contains 36 flavouring agents from the original 40 flavouring agents evaluated previously, because 4 flavouring agents (Nos 1487–1490) had been withdrawn from evaluation since the sixty-ninth meeting due to the current lack of use as flavouring agents. Three new flavouring agents—(*E*)-ethyl 3-(2-furyl)acrylate (No. 2103), di-2-furylmethane (No. 2104) and 2-methylbenzofuran (No. 2105)—that were not part of the original evaluation have been added to the current submission.

The Committee previously evaluated a group of 40 aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution at the sixty-fifth meeting (Annex 1, reference *178*). The Committee took note of the extensive positive genotoxicity data for several members of this group of flavouring agents and the paucity of in vivo genotoxicity data to allay concern and concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group.

At the sixty-ninth meeting (Annex 1, reference *190*), the Committee reviewed new in vitro and in vivo genotoxicity studies that had been submitted for the same group of 40 aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution. The Committee again concluded that the Procedure could not be applied to this group, because of unresolved toxicological concerns. Studies that would assist in the safety evaluation included investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group. In response to this, in vivo comet assays using furan and 2-pentylfuran (No. 1491) (*11*) were submitted and evaluated at the present meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

The Committee discussed the results of the in vivo comet assays (*11*). In these studies, the potential genotoxicity of 2-pentylfuran was compared

with that of furan using isolated liver cells from male B6C3F1 mice that were gavaged with 2-pentylfuran or furan. Before the conduct of the main studies, pilot comet assays were performed with a single oral furan dose of 250 mg/kg bw, and DNA damage was measured in liver cells at 3, 6 and 24 hours. The 3-hour time point was found to be optimal for measuring the genotoxic response to furan. Based on this finding, the 3-hour time point was used in three separate studies with 2-pentylfuran.

In the first study, 2-pentylfuran (508 mg/kg bw) produced an increase in per cent DNA in the comet tail in the absence of proteinase K compared with the vehicle control, indicating DNA damage. In the second study, 2-pentylfuran (762 mg/kg bw) produced no significant changes in the per cent DNA in the comet tail in either the absence or presence of proteinase K, indicating the absence of DNA damage. In the third study, both 2-pentylfuran doses of 508 and 762 mg/kg bw were used. Changes in the comet tail induced by 2-pentylfuran at 508 mg/kg bw in the first study were not observed in the third study. However, in the third study, 2-pentylfuran at 762 mg/kg bw, both without and with proteinase K, provided evidence of DNA damage. The Committee concluded that the combined results of the three comet assays did not allow conclusions to be reached on the genotoxic potential of 2-pentylfuran and its mechanism. Additionally, the Committee also questioned the selection of only a single time point for the analysis of 2-pentylfuran genotoxicity based on the results of the furan.

Conclusion

The Committee concluded that the Procedure could not be applied to this group because of the unresolved toxicological concerns. Studies that could assist in the safety evaluation include investigations of the influence of the nature and position of furan ring substitutions on metabolism and covalent binding to macromolecules, demonstration of the ring opening and reactivity of the resulting products. Depending on the findings, additional genotoxicity or other studies might be needed.

An addendum to the toxicological monograph was not prepared.

4.1.4 *Aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters*

Explanation

The Committee evaluated a group of flavouring agents consisting of five aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters. This group included *trans*-2-nonenyl acetate (No. 2163), propyl sorbate (No. 2164), *cis*-2-octenol (No. 2165), *trans*-2-tridecenol (No. 2166) and ethyl 2-hexenoate (mixture of isomers) (No. 2167).

2-Phenyl-4-methyl-2-hexenal (No. 2069) was submitted for evaluation, but the Committee considered that it did not belong to this group of flavouring agents, and the compound was therefore not further considered. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee.

The Committee previously evaluated 37 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The Committee concluded that all 37 flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee also evaluated 22 additional members of this group of flavouring agents at its sixty-ninth meeting (Annex 1, reference *190*). The Committee concluded that the 22 additional flavouring agents in that group were of no safety concern at estimated dietary exposures.

Two of the five flavouring agents (Nos 2165 and 2167) in this group have been reported to occur naturally and can be found in chicken, ginger, mushroom, raspberry, blackberry, boysenberry, apple, grapes, guava, feyoa, mangifera, passiflora and wine.

Assessment of dietary exposure

The total annual volumes of production of the five aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters are approximately 0.1 kg in the USA and 113 kg in Japan. Two flavouring agents in this group, propyl sorbate (No. 2164) with 80 kg and ethyl 2-hexenoate (mixture of isomers) (No. 2167) with 33 kg, account for approximately 99% of the total annual volume of production in Japan.

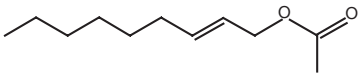
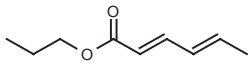
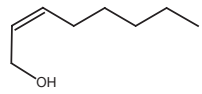
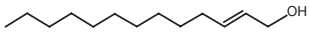
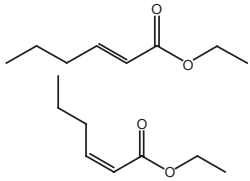
Dietary exposures were estimated using both the MSDI method and the SPET, with the highest values reported in Table 4. The estimated daily dietary exposure is highest for propyl sorbate (No. 2164) (300 μg, the SPET value obtained from non-alcoholic beverages). For the other flavouring agents, the estimated daily dietary exposures range up to 25 μg.

Absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters has previously been described in the report of the sixty-third meeting (Annex 1, reference *173*). The alkenols and alkenoic acid esters in this group are expected to be hydrolysed and completely metabolized by the fatty acid β-oxidation pathway or the tricarboxylic acid cycle.

Table 4

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

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I					
trans-2-Nonenyl acetate	2163	30418-89-4 	No, SPET: 2	Note 1	No safety concern
Propyl sorbate	2164	10297-72-0 	No, SPET: 300	Note 1	No safety concern
cis-2-Octenol	2165	26001-58-1 	No, SPET: 20	Note 2	No safety concern
trans-2-Tridecenol	2166	74962-98-4 	No, SPET: 3	Note 2	No safety concern
Ethyl 2-hexenoate (mixture of isomers)	2167	1552-67-6 	No, SPET: 25	Note 1	No safety concern

CAS, Chemical Abstracts Service

[a] Fifty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *173* and *190*).

[b] *Step 1*: All of the flavouring agents in this group (Nos 2163–2167) are in structural class I.

[c] *Step 2*: All of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class I is 1800 µg/person per day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

Notes:

1. Hydrolysed to corresponding alcohols and acids, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.
2. The alcohol group would be oxidized to the corresponding carboxylic acid and completely metabolized by the fatty acid pathway.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the five additional flavouring agents in this group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters, the Committee assigned all five to structural class I (Nos 2163–2167) (*10*).

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

Step A3. The estimated dietary exposures to the five flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, none of the five flavouring agents raise safety concern at their current estimated dietary exposures.

Table 4 summarizes the evaluations of the five aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters (Nos 2163–2167) in this group.

Consideration of combined intakes from use as flavouring agents

The highest MSDI for any member of this group is 23 μg/person per day (No. 2164), which is less than 2% of the threshold of concern, 1800 μg/person per day. Consideration of combined intakes is not deemed necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluations of the aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. None raised safety concerns. New data on acute toxicity and genotoxicity were available at the present meeting, and these supported the previous safety evaluations (Annex 1, references *173* and *190*).

The Committee concluded that these five flavouring agents, which are additions to the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.5 *Amino acids and related substances*

Explanation

The Committee evaluated six additional flavouring agents belonging to the group of amino acids and related substances. The additional flavouring agents included three L-amino acids (Nos 2118–2120), two dipeptides (Nos 2121 and 2122) and one tripeptide (No. 2123). None of these flavouring agents have previously been evaluated by the Committee. The safety of the submitted substance (3*R*)-4-[[(1*S*)-1-benzyl-2-methoxy-2-oxo-ethyl]amino]-3-[3-(3-hydroxy-4-methoxy-phenyl)propylamino]-4-oxo-butanoic acid hydrate (Advantame, No. 2124) was not assessed; the Committee decided that it would not be appropriate to evaluate this substance as a flavouring agent, because it is a low-calorie intense sweetener. Three of the flavouring agents in this group (Nos 2119, 2121 and 2123) evaluated at this meeting are reported to be flavour modifiers.

The Committee considered that the use of the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*) was inappropriate for two members of this group—namely, L-isoleucine (No. 2118) and L-threonine (No. 2119). These substances are macronutrients and normal components of protein; as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from their use as flavouring agents. For the remaining four members of the group (Nos 2120–2123), the evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents.

The Committee previously evaluated 20 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The Committee concluded that all 20 flavouring agents in that group were of no safety concern at estimated dietary exposures.

As noted by the Committee at its sixty-third meeting (Annex 1, reference *173*), amino acids may react with other food constituents upon heating. The mixtures thus formed are commonly referred to as "process flavours". The safety of process flavours has not been reviewed by the Committee at the sixty-third meeting or at the current meeting and may be considered at a future meeting. The evaluation of the flavouring agents belonging to the group of amino acids and related substances is therefore conducted on the basis that these flavouring agents are present in an unchanged form at the point of consumption.

In addition to Nos 2118 and 2119, which are normal components of protein, Nos 2120 and 2123 have been reported to occur in protein-rich foods, fish sauce, soya sauce, shrimp paste and scallops.

Assessment of dietary exposure

The total annual volume of production of the six amino acids and related substances for use as flavouring agents only is approximately 49 140 kg in the USA, with no reported volume available for Europe or Japan. Approximately 92% of the total annual volume of production in the USA is accounted for by one substance in this group—namely, L-alanyl-L-glutamine (No. 2121).

Dietary exposures were estimated using both the SPET and the MSDI method. The highest estimated dietary exposure for each flavouring agent is reported in Table 5. The estimated daily dietary exposure is highest for L-alanyl-L-glutamine (No. 2121) (280 000 μg, the SPET value obtained from milk products). For the other flavouring agents, the estimated daily dietary exposures, calculated using either the SPET or the MSDI method, range from 0.02 to 60 000 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of amino acids has previously been described in the monograph of the sixty-third meeting (Annex 1, reference *173*). Also, dipeptides and tripeptides are readily hydrolysed into constituent amino acids in the intestine during absorption.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

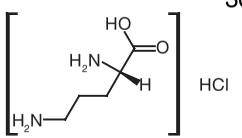
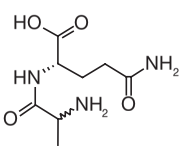
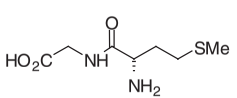
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the four flavouring agents in this group of amino acids and related substances, the Committee assigned all four flavouring agents (Nos 2120–2123) to structural class I (*10*).

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

Step A3. The highest estimated dietary exposure to one flavouring agent in structural class I (No. 2122) is below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, this flavouring agent is not of safety concern at current estimated dietary exposure. The highest estimated dietary exposures of the three remaining flavouring agents in structural class I are above the threshold of concern. Accordingly, the evaluation of these flavouring agents proceeded to step A4.

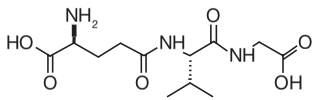
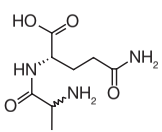
Step A4. L-Ornithine (No. 2120) is an endogenous compound, and L-alanyl-L-glutamine (No. 2121) is metabolized to the non-essential, endogenous amino acids, L-alanine and L-glutamine. According to the Procedure, these

Table 5
Summary of the results of the safety evaluations of amino acids and related substances used as flavouring agents[a,b,c]

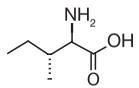
Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of exposure for the flavouring agent or a related substance?	Related structure name (No.) and structure (if applicable)	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I								
L-Ornithine (as the monochlorohydrate)	2120	3184-13-2 	Yes, SPET: 30 000	Yes	NR	NR	Note 1	No safety concern
L-Alanyl-L-glutamine	2121	39537-23-0 	Yes, SPET: 280 000	Yes	NR	NR	Note 2	No safety concern
L-Methionylglycine	2122	14486-03-4 	No, SPET: 400	NR	NR	NR	Note 2	No safety concern

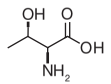
continued

Table 5 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of exposure for the flavouring agent or a related substance?	Related structure name (No.) and structure (if applicable)	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Glutamyl-valyl-glycine	2123	38837-70-6 	Yes, SPET: 4000	No	Yes. The NOAEL of 3130 mg/kg bw per day in a 91-day study in rats for the related compound L-alanyl-L-glutamine (No. 2121) is 47 000 times the estimated daily dietary exposure to No. 2123 when used as a flavouring agent.	L-Alanyl-L-glutamine (No. 2121) 	Note 2	No safety concern

Amino acids not evaluated by the Procedure

L-α-Amino acids	No.	CAS No. and structure	Conclusion
L-Isoleucine	2118	73-32-5 	This substance is a macronutrient and a normal component of protein; as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from its use as a flavouring agent.

L-Threonine	2119	72-19-5		This substance is a macronutrient and a normal component of protein; as such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from its use as a flavouring agent.

CAS, Chemical Abstracts Service; NR, not required for evaluation

[a] Twenty flavouring agents in this group were previously evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*).

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

[c] *Step 2*: All of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class I is 1800 µg/person per day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

Notes:

1. As part of the urea cycle, a carbamoyl group is transferred to ornithine from citrulline.
2. Hydrolysed to constituent amino acids.

two flavouring agents are not of safety concern at current estimated dietary exposures. Also, glutamyl-valyl-glycine (No. 2123) is metabolized to its constituent amino acids, but valine is an essential amino acid that cannot be synthesized in the human body and is therefore not an endogenous compound. Therefore, the evaluation of this flavouring agent proceeded to step A5.

Step A5. For glutamyl-valyl-glycine (No. 2123), the NOAEL of 3130 mg/kg bw per day in a 91-day study in rats for the related compound L-alanyl-L-glutamine (No. 2121) provides a margin of exposure of approximately 47 000 in relation to the highest estimated dietary exposure to glutamyl-valyl-glycine (SPET = 4000 μg/day) when used as a flavouring agent. The Committee therefore concluded that glutamyl-valyl-glycine is not of safety concern at current estimated dietary exposure.

Table 5 summarizes the evaluations of the four amino acids and related substances used as flavouring agents in this group, as well as the evaluations of the two amino acids for which the Procedure was not used.

Consideration of combined intakes from use as flavouring agents

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

Conclusion

In the previous evaluation of flavouring agents in the group of amino acids and related substances, biochemical data and studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of in vitro and in vivo genotoxicity and reproductive toxicity were available (Annex 1, reference *173*). The toxicity data for the current evaluation (biochemical data, studies of acute toxicity, short-term studies of toxicity and studies of in vitro genotoxicity) supported the previous safety evaluation.

In view of the fact that No. 2118 (L-isoleucine) and No. 2119 (L-threonine) are macronutrients and normal components of protein, the use of these substances as flavouring agents would not raise any safety concerns at current estimated dietary exposures. The Committee also concluded that the use of the other four flavouring agents in this group of amino acids and related substances would not raise any safety concerns.

An addendum to the toxicological monograph was prepared.

4.1.6 *Epoxides*

Explanation

The Committee evaluated a group of seven epoxides that includes two phenylglycidate derivatives (Nos 2143 and 2144), two terpene epoxides (Nos 2145 and 2146) and three aliphatic epoxides (Nos 2147–2149). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated.

The Committee previously evaluated nine other members of this group of flavouring agents at the sixty-fifth meeting (Annex 1, reference *178*). The Committee concluded that the nine flavouring agents in that group were of no safety concern at estimated dietary exposures.

One of the seven flavouring agents (No. 2145) has been reported to occur naturally in food and has been detected in angelica oil, black currants, cardamom, dill, ginger, orange oil and pepper.

Assessment of dietary exposure

The total annual volume of production of the seven epoxides is approximately 19 kg in Japan, with no reported volumes of production from the USA or Europe. Two flavouring agents in this group, methyl β-phenylglycidate (No. 2144) and ethyl α-ethyl-β-methyl-β-phenylglycidate (No. 2143), with annual volumes of production of 8 kg and 6 kg, respectively, account for approximately 74% of the total annual volume of production in Japan.

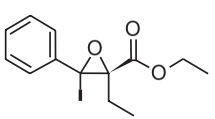
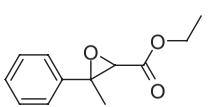
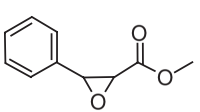
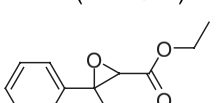
Dietary exposures were estimated using both the MSDI method and the SPET for each flavouring agent, with the highest values reported in Table 6. The estimated dietary exposures are highest for ethyl α-ethyl-β-methyl-β-phenylglycidate (No. 2143) and methyl β-phenylglycidate (No. 2144) (SPET = 60 μg/day for each flavouring agent). This SPET value was obtained from gelatines and non-alcoholic beverages for No. 2143 and from non-alcoholic beverages for No. 2144. For the other flavouring agents, the estimated daily dietary exposures range from 0.03 to 40 μg, with the SPET yielding the highest estimates.

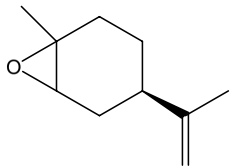
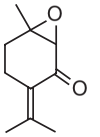
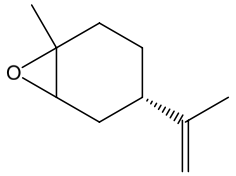
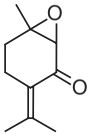
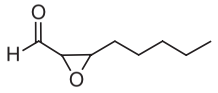
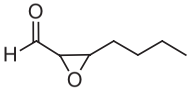
Absorption, distribution, metabolism and elimination

The hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of epoxides have previously been described in the report of the sixty-fifth meeting (Annex 1, reference *178*).

Epoxides are three-membered rings containing an oxygen atom. The inherent ring strain and polarity of the C–O bond in the epoxide ring are factors

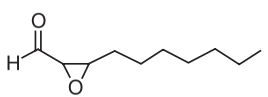
Table 6
Summary of the results of the safety evaluations of epoxides used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	*Step B5* Do the conditions of use result in an estimated dietary exposure greater than 1.5 µg/day?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III								
Ethyl α-ethyl-β-methyl-β-phenylglycidate	2143	19464-94-9 	No, SPET: 60	B4: Yes. The NOEL of 35 mg/kg bw per day for the structurally related ethyl methylphenylglycidate (No. 1577) in a 2-year study in rats is 35 000 times the estimated dietary exposure to No. 2143 when used as a flavouring agent.	NR	Note 1	Ethyl methyphenylglycidate (No. 1577) 	No safety concern
Methyl β-phenylglycidate	2144	37161-74-3 	No, SPET: 60	B4: Yes. The NOEL of 35 mg/kg bw per day for the structurally related ethyl methylphenylglycidate (No. 1577) in a 2-year study in rats is 35 000 times the estimated dietary exposure to No. 2144 when used as a flavouring agent.	NR	Note 2	Ethyl methylphenylglycidate (No. 1577) 	No safety concern

d-8-*p*-Menthene-1,2-epoxide	2145 1195-92-2 	No, SPET: 15	B4: Yes. The NOEL of 48 mg/kg bw per day for the structurally related piperitenone oxide (No. 1574) in a 28-day study in rats is 190 000 times the estimated dietary exposure to No. 2145 when used as a flavouring agent.	NR		Note 3	Piperitenone oxide (No. 1574) 	No safety concern
l-8-*p*-Menthene-1,2-epoxide	2146 203719-53-3 	No, SPET: 40	B4: Yes. The NOEL of 48 mg/kg bw per day for the structurally related piperitenone oxide (No. 1574) in a 28-day study in rats is 72 000 times the estimated dietary exposure to No. 2146 when used as a flavouring agent.	NR		Note 3	Piperitenone oxide (No. 1574) 	No safety concern
2,3-Epoxyoctanal	2147 42134-50-9 	No, SPET: 30	B4: No	Yes		Note 3		Additional data required to complete evaluation
2,3-Epoxyheptanal	2148 58936-30-4 	No, SPET: 30	B4: No	Yes		Note 3		Additional data required to complete evaluation

continued

Table 6 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	*Step B5* Do the conditions of use result in an estimated dietary exposure greater than 1.5 µg/day?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
2,3-Epoxydecanal	2149	102369-06-2 	No, SPET: 30	B4: No	Yes	Note 3		Additional data required to complete evaluation

CAS, Chemical Abstracts Service

[a] Nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *178*).

[b] *Step 1*: The seven flavouring agents in this group (Nos 2143–2149) are in structural class III.

[c] *Step 2*: The seven epoxides (Nos 2143–2149) are not expected to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class III is 90 µg/person per day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.

[e] The margins of exposure were calculated based on the estimated dietary exposures calculated using the SPET.

Notes:

1. The ester group is hydrolysed by carboxyl esterases, followed by loss of carbon dioxide and rearrangement to 2-phenyl-3-pentanone.
2. The ester group is hydrolysed by carboxyl esterases, followed by loss of carbon dioxide and rearrangement to phenylacetaldehyde.
3. The epoxide is hydrolysed via epoxide hydrolase to form a vicinal *trans*-diol, which forms a glucuronic acid conjugate and is eliminated in the urine, and/or the epoxide is directly conjugated with glutathione via glutathione *S*-transferase and is eliminated in the urine.

that promote cleavage of the three-membered ring in the presence of suitable nucleophiles. Epoxide hydrolases, enzymes with wide tissue distribution, catalyse epoxide ring cleavage by water to yield *trans*-diols. The diols are then excreted primarily in the urine unchanged or as the glucuronic acid or sulfate conjugates. Alternatively, epoxides may be conjugated with glutathione mediated by glutathione *S*-transferases to yield the corresponding mercapturic acid conjugates, which are also excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the seven flavouring agents in this group of epoxides, the Committee assigned all seven (Nos 2143–2149) to structural class III (*10*).

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

Step B3. The highest dietary exposures to the seven flavouring agents in this group are below the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of all seven flavouring agents in the group proceeded to step B4.

Step B4. For ethyl α-ethyl-β-methyl-β-phenylglycidate (No. 2143), the NOEL of 35 mg/kg bw per day for the structurally related flavouring agent ethyl methylphenylglycidate (No. 1577) in a 2-year study in rats provides a margin of exposure of 35 000 in relation to the dietary exposure to No. 2143 (SPET = 60 μg/day) when used as a flavouring agent.

For methyl β-phenylglycidate (No. 2144), the NOEL of 35 mg/kg bw per day for the structurally related flavouring agent ethyl methylphenylglycidate (No. 1577) in a 2-year study in rats provides a margin of exposure of 35 000 in relation to the dietary exposure to No. 2144 (SPET = 60 μg/day) when used as a flavouring agent.

For d-8-*p*-menthene-1,2-epoxide (No. 2145), the NOEL of 48 mg/kg bw per day for the structurally related flavouring agent piperitenone oxide (No. 1574) in a 28-day study in rats provides a margin of exposure of 190 000 in relation to the dietary exposure to No. 2145 (SPET = 15 μg/day) when used as a flavouring agent.

For l-8-*p*-menthene-1,2-epoxide (No. 2146), the NOEL of 48 mg/kg bw per day for the structurally related flavouring agent piperitenone oxide (No. 1574) in a 28-day study in rats provides a margin of exposure of 72 000 in relation to the dietary exposure to No. 2146 (SPET = 40 μg/day) when used as a flavouring agent.

For 2,3-epoxyoctanal (No. 2147), 2,3-epoxyheptanal (No. 2148) and 2,3-epoxydecanal (No. 2149), no toxicological data are available on the flavouring agents or structurally related substances with which to calculate margins of exposure. Therefore, the evaluation of these flavouring agents proceeded to step B5.

Step B5. The conditions of use for 2,3-epoxyoctanal (No. 2147), 2,3-epoxyheptanal (No. 2148) and 2,3-epoxydecanal (No. 2149) result in dietary exposures greater than 1.5 μg/day. Therefore, the Committee concluded that additional data would be necessary to complete the evaluation of these flavouring agents.

Table 6 summarizes the evaluations of the seven additional flavouring agents in this group of epoxides (Nos 2143–2149).

Consideration of combined intakes from use as flavouring agents

A number of the flavouring agents in this group (Nos 1576–1578, 2143 and 2144) are alkyl phenylglycidates or closely related compounds. If these were to be consumed at the same time, the combined intake would exceed the class III threshold, primarily due to Nos 1576 and 1577; their combined intake was considered in detail at the sixty-fifth meeting of the Committee (Annex 1, reference *178*). The other flavouring agents in this group have diverse structures, with various potential sites of metabolism, and are not likely to be metabolized to common products. The Committee concluded that the combined intake of these flavouring agents was not a safety concern.

Consideration of secondary components

Three flavouring agents in this group (Nos 2144, 2148 and 2149) have minimum assay values of less than 95% (see Annex 4). The secondary component of methyl β-phenylglycidate (No. 2144) is ethyl β-phenylglycidate (No. 1576); the secondary component of 2,3-epoxyheptanal (No. 2148) is *trans*-2-heptenal (No. 1360); and the secondary component of 2,3-epoxydecanal (No. 2149) is *trans*-2-decenal (No. 1349). These secondary components are considered not to present a safety concern at current estimated dietary exposures.

Conclusion

In the previous evaluation of flavouring agents in this group of epoxides, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. The results of a short-term study of the toxicity of No. 1574 and in vitro genotoxicity studies on Nos 1574 and 2145 considered at this meeting support the previous safety evaluation (Annex 1, reference *178*).

The Committee concluded that four flavouring agents (Nos 2143–2146), which are additions to the group of epoxides evaluated previously, would not give rise to safety concerns at the current estimated dietary exposures.

The Committee concluded that additional toxicity data on the flavouring agents or structurally related substances would be necessary to complete the evaluations of Nos 2147–2149.

An addendum to the toxicological monograph was prepared.

4.1.7 *Furfuryl alcohol and related substances*

Explanation

At the present meeting, the Committee considered four additional flavouring agents (Nos 2099–2102) belonging to the group of furfuryl alcohol and related substances: one furfuryl alcohol (No. 2099), one furfural acetal (No. 2100) and two furfuryl esters (Nos 2101 and 2102). Three flavouring agents (Nos 2103–2105) were originally assigned to the current group, but it was considered appropriate for these three to be evaluated in the group of aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution (see section 4.1.3).

Furfural (No. 450) was evaluated previously by the Committee at its thirty-ninth and fifty-first meetings (Annex 1, references *101* and *137*). An ADI was not established at either meeting because of concern about the findings of tumours in male mice given furfural by gavage and the fact that no NOEL was identified for hepatotoxicity in male rats. In the mouse study, the combined incidence of adenomas and carcinomas was increased in males at the highest dose tested. In order to address the concern regarding the formation of liver tumours in mice, the Committee at its fifty-first meeting requested the results of studies of DNA binding or adduct formation in vivo to clarify whether furfural interacts with DNA in the liver of mice (Annex 1, reference *137*). While no specific studies of DNA binding were submitted, the results of an assay for unscheduled DNA synthesis in mice in vivo was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference *149*). This study, in which single doses of up to 350 mg/kg bw were given, was considered to be particularly relevant, as it addressed potential DNA repair in the cells in which tumours arose—namely, hepatocytes. The negative results obtained in this assay were considered by the Committee to provide evidence that the liver tumours observed in the long-term study in mice were unlikely to have occurred through a genotoxic mechanism. The Committee concluded that the concerns raised previously with respect to the liver tumours in mice were adequately addressed by this study and that a study of DNA binding was unnecessary.

For furfuryl alcohol (No. 451), carcinogenicity studies in mice and rats using the inhalation route were considered by the Committee at its fifty-fifth meeting (Annex 1, reference *150*). Under the conditions of these 2-year inhalation studies, male mice exposed to the highest tested concentration had a significantly increased incidence of renal tubular degeneration and a significantly increased combined incidence of renal tubular adenomas and carcinomas. The Committee considered that, while these studies are of limited value for assessing the potential toxicity of oral exposure to furfuryl alcohol, the findings may provide some indication of potential long-term toxicity.

The Committee evaluated 15 members of this group of flavouring agents at its fifty-fifth meeting and concluded that all 15 were of no safety concern at estimated dietary exposures (Annex 1, reference *149*).

A group ADI of 0–0.5 mg/kg bw was established by the Committee at its fifty-fifth meeting for 12 members of the group—namely, furfural (No. 450), furfuryl alcohol (No. 451), furfuryl acetate (No. 739), furfuryl propionate (No. 740), furfuryl pentanoate (No. 741), furfuryl octanoate (No. 742), furfuryl 3-methylbutanoate (No. 743), methyl 2-furoate (No. 746), propyl 2-furoate (No. 747), amyl 2-furoate (No. 748), hexyl 2-furoate (No. 749) and octyl 2-furoate (No. 750). Furfural, furfuryl alcohol and these 10 derivatives are metabolized to the same metabolite, 2-furoic acid (Annex 1, reference *149*). The ADI was established on the basis of a NOEL of 53 mg/kg bw per day in a 13-week rat study with furfural and using a safety factor of 100.

At the current meeting, the Committee included new in vitro and in vivo genotoxicity studies on several members of this group in its re-evaluation of the group of furfuryl alcohol and related substances. Genotoxicity data were available for one of the four additional flavouring agents in this group (5-methylfurfuryl alcohol; No. 2099). Additional genotoxicity studies were also available on two flavouring agents previously evaluated by the Committee—namely, furfuryl alcohol (No. 451) and furfuryl acetate (No. 739). In addition, new published genotoxicity data on furfural (No. 450) were available; however, these data were evaluated by the Committee at the fifty-fifth meeting (Annex 1, reference *149*) as separate unpublished in vitro and in vivo studies.

Metabolism

All four members of the current group of flavouring agents would be expected to be metabolized to furfuryl alcohol or a structural analogue that would undergo further oxidation to the corresponding 2-furoic acid.

Genotoxicity

Positive genotoxicity findings were observed in several in vitro and in vivo studies with three flavouring agents in this group. In reverse mutation assays

with *Salmonella typhimurium* TA100-derived strains engineered to express various mouse and human sulfotransferases, furfuryl alcohol (No. 451) and 5-methylfurfuryl alcohol (No. 2099) exhibited mutagenic activity. Furfuryl acetate (No. 739) was tested in the conventional TA100 strain only and exhibited mutagenic activity. These studies suggested that furfuryl alcohol is converted by intracellular sulfate conjugation to 2-sulfo-oxymethylfuran, an electrophile reacting with DNA. Subsequent experiments resulted in the detection of nucleoside adducts of 2′-deoxyadenosine and 2′-deoxyguanosine in porcine liver DNA incubated with freshly prepared 2-sulfo-oxymethylfuran. These adducts were also observed in the DNA of liver, lung and kidney of mice administered furfuryl alcohol (No. 451) at a dose of 390 mg/kg bw per day via drinking-water for 28 days. Another study indicated that furfuryl alcohol (No. 451) can induce sister chromatid exchange in human lymphocytes in vitro and in vivo.

Conclusion

New in vitro and in vivo studies raise concerns regarding the potential genotoxicity of furfuryl alcohol and derivatives that can be metabolized to furfuryl alcohol (e.g. furfuryl esters). The Committee concluded that this group of flavouring agents could not be evaluated according to the Procedure because of the unresolved concerns regarding genotoxicity. In addition, the group ADI previously established by the Committee will need to be reconsidered at a future meeting.

An addendum to the toxicological monograph was prepared.

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

Explanation

The Committee evaluated nine flavouring agents (Nos 2177–2185) in the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). Five of the nine flavouring agents are esters (Nos 2179, 2180 and 2182–2184). The remaining four flavouring agents are linear unsaturated alcohols (Nos 2177 and 2178), an unsaturated acid (No. 2181) and an aldehyde (No. 2185). None of these flavouring agents have previously been evaluated.

The Committee evaluated 42 other members of this group of flavouring agents at the fifty-first meeting (Annex 1, reference *137*). Forty-one of the 42 flavouring agents in that group were concluded to be of no safety concern at estimated dietary exposures. The evaluation of one flavouring agent, ethyl

2-methyl-3,4-pentadienoate (No. 353), was deferred pending review of a 90-day rat feeding study. It was evaluated at the sixty-eighth meeting of the Committee (Annex 1, reference *187*) and concluded to be of no safety concern at the estimated dietary exposure.

The Committee evaluated 20 other members of this group of flavouring agents at the sixty-first meeting (Annex 1, reference *166*). All of the flavouring agents in that group were concluded to be of no safety concern at estimated dietary exposures.

The nine flavouring agents (Nos 2177–2185) in this group are natural components of foods. They have been detected in cucumber, maize, melon, peas, potato, pulasam, apple, banana, guava, raspberry, blackberry, boysenberry, tomato, wine, pork, grape, starfruit, tea, pepino fruit and prickly pear.

Assessment of dietary exposure

The total annual volumes of production of the nine linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters are approximately 56 kg in Europe, 18 kg in the USA and 6 kg in Japan. Approximately 98% of the total annual volume of production in Europe is accounted for by one flavouring agent in this group, *trans*-3-hexenyl acetate (No. 2180). Two flavouring agents, *trans*-3-nonen-1-ol (No. 2178) and *cis*,*cis*-3,6-nonadienyl acetate (No. 2179), with annual volumes of production of 10 and 7 kg, respectively, account for 94% of the volume in the USA. In Japan, all of the volume of production is accounted for by *cis*-3-nonen-1-ol (No. 2177) and *trans*-3-hexenyl acetate (No. 2180), with 1 kg and 5 kg, respectively.

Dietary exposures were estimated for each flavouring agent using both the SPET and the MSDI method, with the highest values reported in Table 7. The estimated daily dietary exposure is highest for (*Z*)-5-octenyl acetate (No. 2184) (2400 μg, the SPET value obtained from instant tea and coffee). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 600 μg, with the SPET yielding the highest estimates.

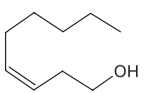
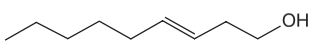
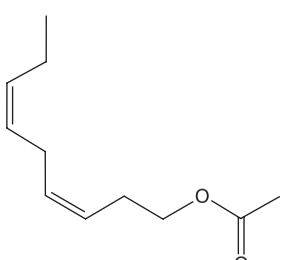
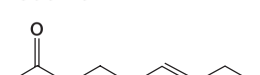
Absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters has previously been described in the reports of the fifty-first and sixty-first meetings (Annex 1, references *137* and *166*).

The aliphatic esters in this group are predicted to be hydrolysed to the corresponding unsaturated aliphatic alcohol and carboxylic acid in the gastrointestinal

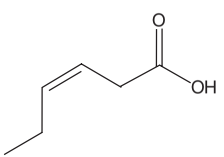
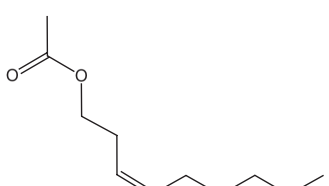
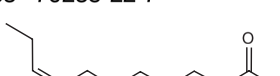
Table 7

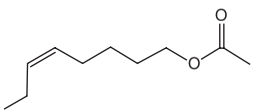
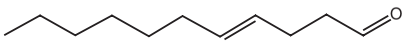
Summary of the results of the safety evaluations of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class I								
cis-3-Nonen-1-ol	2177	10340-23-5 	No, SPET: 30	NR	NR	Note 1		No safety concern
trans-3-Nonen-1-ol	2178	10339-61-4 	No, SPET: 80	NR	NR	Note 1		No safety concern
cis,*cis*-3,6-Nonadienyl acetate	2179	83334-93-4 	No, SPET: 2	NR	NR	Note 2		No safety concern
trans-3-Hexenyl acetate	2180	3681-82-1 	No, SPET: 600	NR	NR	Note 2		No safety concern

continued

Table 7 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
cis-3-Hexenoic acid	2181	1775-43-5 	No, SPET: 600	NR	NR	Note 3		No safety concern
cis-3-Nonenyl acetate	2182	13049-88-2 	No, SPET: 2	NR	NR	Note 2		No safety concern
cis-6-Nonenyl acetate	2183	76238-22-7 	No, SPET: 2	NR	NR	Note 2		No safety concern

(*Z*)-5-Octenyl acetate	2184	71978-00-2 	Yes, SPET: 2400	No	Yes. The NOEL of 120 mg/kg bw per day in a 98-day study in rats for the structurally related *cis*-3-hexenol (No. 315) is 3000 times the estimated dietary exposure to No. 2184 when used as a flavouring agent.	Note 2	*cis*-3-Hexenol (No. 315) 	No safety concern
(*E*)-4-Undecenal	2185	68820-35-9 	No, SPET: 15		NR	Note 4		No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because dietary exposure of the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

[a] Sixty-two flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *137* and *166*).
[b] *Step 1*: All nine additional flavouring agents in this group (Nos 2177–2185) are in structural class I (*10*).
[c] *Step 2*: All of the flavouring agents in this group can be predicted to be metabolized to innocuous products.
[d] The threshold for human dietary exposure for structural class I is 1800 µg/person per day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The highest estimated dietary exposures were calculated by the SPET in all cases.
[e] The margins of exposure were calculated based on the estimated dietary exposures calculated using the SPET.

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

tract. Once formed, the linear and branched-chain unsaturated primary alcohols will be rapidly absorbed and oxidized to their corresponding aldehydes and acids. Aliphatic aldehydes and acids are readily absorbed. Once absorbed, the aldehydes are oxidized to their corresponding unsaturated carboxylic acids.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned all nine flavouring agents (Nos 2177–2185) to structural class I (*10*).

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

Step A3. The estimated dietary exposures to eight of the flavouring agents in this group are below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, the safety of these eight flavouring agents raises no concern at their current estimated dietary exposures. The highest estimated dietary exposure for one flavouring agent (No. 2184) in this group is above the threshold of concern (i.e. 1800 μg/person per day for class I). Accordingly, the evaluation of this flavouring agent proceeded to step A4.

Step A4. The flavouring agent No. 2184 and its metabolites are not endogenous, and therefore its evaluation proceeded to step A5.

Step A5. For (*Z*)-5-octenyl acetate (No. 2184), the NOEL of 120 mg/kg bw per day for the structurally related *cis*-3-hexenol (No. 315) obtained in a 98-day study in rats provides a margin of exposure of 3000 in relation to the estimated dietary exposure to No. 2184 (SPET = 2400 μg/day) when used as a flavouring agent.

The Committee concluded that none of the nine additional flavouring agents (Nos 2177–2185) belonging to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters would pose a safety concern at current estimated dietary exposures.

Table 7 summarizes the evaluations of these additional flavouring agents.

Consideration of combined intakes from use as flavouring agents

The nine additional flavouring agents in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters have very low MSDIs (0.01–1 μg/day). Consideration of combined intake is not deemed necessary, because these additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluations of flavouring agents in this group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available (Annex 1, references *137* and *166*). The results of a subchronic toxicity study on No. 345 and genotoxicity studies on Nos 336 and 349 considered at this meeting support the previous safety evaluations.

The Committee concluded that the nine flavouring agents evaluated at this meeting, which are additions to the group of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters evaluated previously, would not present safety concerns at the current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.9 *Miscellaneous nitrogen-containing substances*

Explanation

The Committee evaluated two additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances. The additional flavouring agents were imidazolidines (Nos 2161 and 2162) that were initially submitted as additional flavouring agents to the group of pyridine, pyrrole and quinoline derivatives. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). Neither of these flavouring agents has previously been evaluated by the Committee. Both flavouring agents in this group that were evaluated at this meeting are reported to be flavour modifiers.

The Committee previously evaluated 16 other members of this group of flavouring agents at its sixty-fifth meeting (Annex 1, reference *178*). The Committee concluded that the use of these 16 flavouring agents would not present a safety concern at estimated dietary exposures. For 10 flavouring agents, the evaluation was conditional, because the estimated exposures were based on anticipated annual volumes of production. At its sixty-ninth meeting (Annex 1, reference *190*), the actual volumes of production for these flavouring agents were provided, and the Committee confirmed that these 10 flavouring agents were of no safety concern based on estimated dietary exposures. The Committee also evaluated 14 other members of this group of flavouring agents at its sixty-ninth meeting and concluded that all 14 flavouring agents in that group were of no safety concern at estimated dietary exposures.

Assessment of dietary exposure

The total annual volume of production of the two miscellaneous nitrogen-containing substances is 2.2 kg in the USA, with no reported data from Europe or Japan. 3-(1-((3,5-Dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-imidazolidine-2,4-dione (No. 2161), with 2 kg, accounts for approximately 90% of the total annual volume of production.

Dietary exposure estimates were made using the MSDI method and the SPET, with the highest values (calculated using the SPET) reported in Table 8. The highest estimated daily dietary exposure is 4000 μg for each flavouring agent (the SPET value obtained from gelatines and puddings and from reconstituted vegetables).

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of miscellaneous nitrogen-containing substances has previously been described in the monographs of the sixty-fifth and sixty-ninth meetings (Annex 1, references *179* and *191*).

The imidazolidines under evaluation are rapidly absorbed, metabolized and excreted. They undergo hydroxylation of the benzyl ring as well as of the dimethylisoxazole group and conjugation with sulfates or glucuronides.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the two flavouring agents in this group of miscellaneous nitrogen-containing substances, the Committee assigned both flavouring agents to structural class III (*10*).

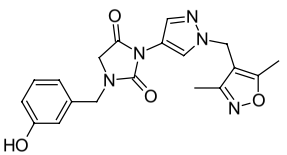
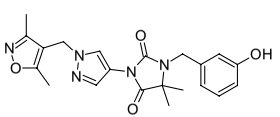
Step 2. The two flavouring agents in this group cannot be predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step B3. For both flavouring agents, the highest estimated dietary exposure is 4000 μg/day (calculated using the SPET) and above the threshold of concern (i.e. 90 μg/person per day for class III). Therefore, additional data are necessary for the evaluation of these flavouring agents.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

In accordance with the Procedure, additional data were evaluated for 3-(1-((3,5-dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-imidazolidine-2,4-dione (No. 2161) and 3-(1-((3,5-dimethylisoxazol-4-yl)-methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-5,5-dimethylimidazolidine-2,4-dione

Table 8
Summary of the results of the safety evaluations of miscellaneous nitrogen-containing substances used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does extimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class III						
3-(1-((3,5-Dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-imidazolidine-2,4-dione	2161	1119831-25-2 	Yes, SPET: 4000	Yes. The NOAEL of 100 mg/kg bw per day from a 91-day study in rats is 1500 times the estimated daily dietary exposure to No. 2161 when used as a flavouring agent.	Note 1	No safety concern
3-(1-((3,5-Dimethyl-isoxazol-4-yl)-methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-5,5-dimethylimidazolidine-2,4-dione	2162	1217341-48-4 	Yes, SPET: 4000	Yes. The NOAEL of 100 mg/kg bw per day from a 28-day study in rats is 1500 times the estimated daily dietary exposure to No. 2162 when used as a flavouring agent.	Note 1	No safety concern

CAS, Chemical Abstracts Service

[a] Thirty flavouring agents belonging to the group of miscellaneous nitrogen-containing substances were previously evaluated by the Committee at its sixty-fifth and sixty-ninth meetings (Annex 1, references *178* and *190*).

[b] *Step 1*: Both flavouring agents in this group are in structural class III.

[c] *Step 2*: Neither of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The highest estimated dietary exposures were calculated by the SPET in both cases.

[e] The margins of exposure were calculated based on the estimated dietary exposures calculated using the SPET.

Note:
1. Ring hydroxylation and conjugation and elimination in the urine.

(No. 2162), as their estimated dietary exposures exceeded the threshold of concern for structural class III (90 μg/person per day).

For No. 2161, data on kinetics, oral 28- and 91-day studies of toxicity in rats and in vitro and in vivo studies of genotoxicity are available. For No. 2162, data on kinetics, an oral 28-day study of toxicity in rats and in vitro studies of genotoxicity are available.

Nos 2161 and 2162 undergo hydroxylation of the benzyl ring as well as of the dimethylisoxazole group and conjugation with sulfate or glucuronic acid. No. 2162 is rapidly excreted. Twenty-four hours after oral administration of 10–100 mg/kg bw and 4–8 hours after intravenous administration of 1 mg/kg bw, blood levels of No. 2162 were below the limit of quantification.

No. 2161 was negative in a bacterial reverse mutation test with and without S9, an in vitro chromosomal aberration test in human lymphocytes and an in vivo micronucleus test in mice. No. 2162 was negative in two bacterial reverse mutation assays with and without S9.

The NOAEL of 100 mg/kg bw per day in a 91-day study in rats provides a margin of exposure of 1500 for No. 2161 (SPET = 4000 μg/day) when used as a flavouring agent. The NOAEL of 100 mg/kg bw per day in a 28-day study in rats provides a margin of exposure of 1500 for No. 2162 (SPET = 4000 μg/day) when used as a flavouring agent. The Committee therefore concluded that these two flavouring agents are not of safety concern at the current estimated dietary exposures.

Table 8 summarizes the evaluations of the two miscellaneous nitrogen-containing substances (Nos 2161 and 2162) in this group.

Consideration of combined intakes from use as flavouring agents

Both additional flavouring agents in this group of miscellaneous nitrogen-containing substances have MSDI values less than 20% of the threshold of concern for structural class III. Consideration of combined intakes is therefore not deemed necessary.

Conclusion

In the previous evaluation of flavouring agents in the group of miscellaneous nitrogen-containing substances, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity and studies of genotoxicity were available (Annex 1, references *179* and *191*). The majority of these data were on alkyl isothiocyanates. The additional toxicity data available for this evaluation on the two imidazolidines do not raise safety concerns.

The Committee concluded that the two flavouring agents evaluated at this meeting (Nos 2161 and 2162), which are additions to the group of miscellaneous nitrogen-containing substances evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.10 *Phenol and phenol derivatives*

Explanation

The Committee evaluated three additional flavouring agents belonging to the group of phenol and phenol derivatives that was evaluated previously. The additional flavouring agents included a flavone (No. 2170), a dihydrochalcone (No. 2171) and a flavanone (No. 2172). The safety of the two submitted substances rebaudioside C (No. 2168) and rebaudioside A (No. 2169) was not assessed; the Committee decided that it would not be appropriate to evaluate these substances as flavouring agents, as they had already been evaluated as food additives (sweeteners). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee. All three flavouring agents evaluated at the current meeting are reported to be flavour modifiers.

The Committee previously evaluated 48 other members of this group of flavouring agents at its fifty-fifth meeting (Annex 1, reference *149*). The Committee concluded that all 48 flavouring agents in that group were of no safety concern at estimated dietary exposures.

The Committee also evaluated 13 other members of this group of flavouring agents at its seventy-third meeting (Annex 1, reference *202*). The Committee concluded that all 13 flavouring agents in that group were of no safety concern at estimated dietary exposures.

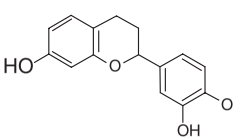
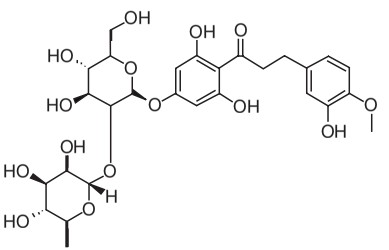
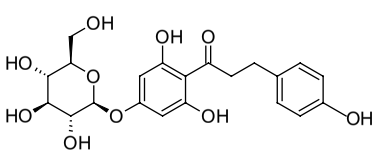
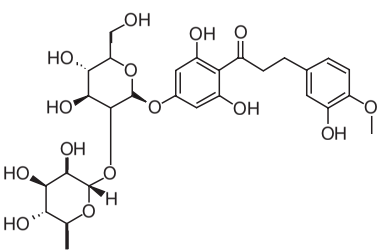
One of the three flavouring agents in this group (No. 2172) is a natural component of food and has been detected in grapefruit.

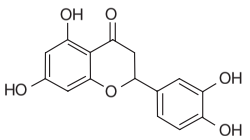
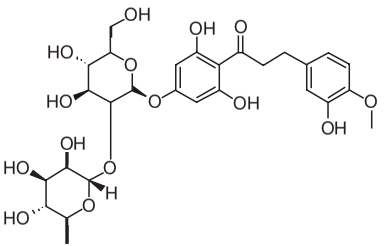
Assessment of dietary exposure

The total annual volume of production of the three flavouring agents belonging to the group of phenol and phenol derivatives is 14.2 kg in the USA, with no reported data from Europe or Japan. Approximately 99% of the total annual volume of production in the USA is accounted for by trilobatin (No. 2171).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 9. The estimated daily

Table 9
Summary of the results of the safety evaluations of phenol and phenol derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate NOAEL for flavouring agent or related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III								
3′,7-Dihydroxy-4′-methoxyflavan	2170	76426-35-2 	Yes, SPET: 15 000	No	Yes. The NOAEL of 760 mg/kg bw per day for structurally related neohesperidin dihydrochalcone in a 90-day study in rats is 3000 times the estimated dietary exposure to No. 2170 when used as a flavouring agent.	Note 1	Neohesperidin dihydrochalcone 	No safety concern
Trilobatin 	2171	4192-90-9	Yes, SPET: 50 000	No	Yes. The NOAEL of 760 mg/kg bw per day for structurally related neohesperidin dihydrochalcone in a 90-day study in rats is 910 times the estimated dietary exposure to No. 2171 when used as a flavouring agent.	Notes 1 and 2	Neohesperidin dihydrochalcone 	No safety concern

(±)-Eriodictyol 	2172	4049-38-1	Yes, SPET: 6000	No	Yes. The NOAEL of 760 mg/kg bw per day for structurally related neohesperidin dihydrochalcone in a 90-day study in rats is 7600 times the estimated dietary exposure to No. 2172 when used as a flavouring agent.	Note 1	Neohesperidin dihydrochalcone 	No safety concern

CAS, Chemical Abstracts Service

[a] In total, 61 flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *149* and *202*).
[b] *Step 1*: All three flavouring agents in this group are in structural class III.
[c] *Step 2*: All three flavouring agents in this group can be predicted to be metabolized to innocuous products.
[d] The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in each case.
[e] The margins of exposure were calculated based on the estimated dietary exposure calculated by the SPET.

Notes:
1. Aglycones are methylated and form sulfates or glucuronic acid conjugates prior to elimination.
2. Glycosides are expected to undergo hydrolysis to the aglycone.

dietary exposure is highest for trilobatin (No. 2171) (50 000 μg, the SPET value obtained from milk products). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 15 000 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of phenol and phenol derivatives has previously been described in the monographs of the fifty-fifth and seventy-third meetings (Annex 1, references *150* and *203*). Additional information on the absorption, distribution, metabolism and elimination of polyphenols was available for this meeting.

Glycoside conjugates of polyphenols are hydrolysed on the brush border of small intestine epithelial cells or within the epithelial cells. Polyphenols are rapidly but incompletely absorbed after oral administration. Metabolism occurs in the gastrointestinal tract and after absorption. Polyphenols are metabolized through hydrolysis, sulfation, glucuronidation and/or methylation. Urinary excretion is rapid to relatively slow, and biliary excretion also occurs. Metabolites not absorbed in the small intestine may undergo further metabolism in the large intestine. The microflora cleave conjugated moieties, with the resultant aglycones undergoing ring fission, leading to phenolic acid and cinnamic acid derivatives. These metabolites can be absorbed and ultimately excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the three flavouring agents in this group of phenol and phenol derivatives, the Committee assigned all three flavouring agents (Nos 2170–2172) to structural class III (*10*).

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

Step A3. The highest estimated dietary exposures to all three flavouring agents are above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of all three flavouring agents proceeded to step A4.

Step A4. None of the three flavouring agents or their metabolites are endogenous substances. Accordingly, the evaluation of all three flavouring agents proceeded to step A5.

Step A5. The NOAEL of 760[2] mg/kg bw per day for the structurally related substance neohesperidin dihydrochalcone from a 90-day study in rats provides adequate margins of exposure of 3000, 910 and 7600 for 3′,7-dihydroxy-4′-methoxyflavan (No. 2170; SPET = 15 000 µg/day), trilobatin (No. 2171; SPET = 50 000 µg/day) and (±)-eriodictyol (No. 2172; SPET = 6000 µg/day), respectively, when used as flavouring agents. The Committee therefore concluded that these flavouring agents would not pose a safety concern at currently estimated dietary exposures.

Table 9 summarizes the evaluations of the three flavouring agents belonging to the group of phenol and phenol derivatives (Nos 2070–2072).

Consideration of combined intakes from use as flavouring agents

The three additional flavouring agents in this group of phenol and phenol derivatives have low MSDIs (0.01–2 µg/day). The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluation of flavouring agents in the group of phenol and phenol derivatives, studies of acute toxicity, short-term and long-term studies of toxicity (18 days to 2 years), and studies of carcinogenicity, genotoxicity, and reproductive and developmental toxicity were available (Annex 1, references *150* and *203*).

For the present evaluation, additional biochemical data and in vitro studies of genotoxicity were available for two flavouring agents in this group (Nos 2170 and 2172), three flavouring agents previously evaluated in this group (Nos 706–708) and one related substance (neohesperidin dihydrochalcone). For neohesperidin dihydrochalcone, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity and studies of reproductive and developmental toxicity were also available. The studies available for the present evaluation support the previous safety evaluations.

The Committee concluded that these three flavouring agents, which are additions to the group of phenol and phenol derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

---

[2] Previously rounded to 750 mg/kg bw per day (Annex 1, reference *202*).

4.1.11 *Pyrazine derivatives*

Explanation

The Committee evaluated a group of eight pyrazine derivatives used as flavouring agents. This group includes five alkyl-substituted pyrazine derivatives (Nos 2125–2128 and 2130), two alkoxy-substituted pyrazine derivatives (Nos 2129 and 2131) and one sulfide-substituted pyrazine derivative (No. 2132). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these agents have been evaluated previously.

The Committee previously evaluated 41 other members of this group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *154*). All 41 substances in that group were concluded to be of no safety concern based on estimated dietary exposures.

Seven of the eight pyrazine derivatives (Nos 2125–2130 and 2132) in this group have been reported to occur naturally and can be found in chicken, cocoa, oats, malt, peanut, sesame seed, barley, beef, beer, coconut, coffee, pork, potato, shrimp, wild rice, popcorn, hazelnut, *Capsicum* species and beans.

Assessment of dietary exposure

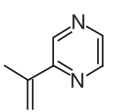
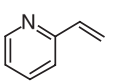
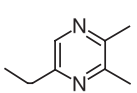
The total annual volumes of production of the eight pyrazine derivatives are approximately 1 kg in each of Europe, the USA and Japan. More than 90% of the total annual volumes of production is accounted for by the mixture of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine and 2,7-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (No. 2128) in Europe, 2-methyl-5-vinylpyrazine (No. 2127) in the USA and 2-ethyl-3-methylthiopyrazine (No. 2132) in Japan.

Dietary exposures were estimated using the MSDI method and the SPET, with the highest values reported in Table 10. The estimated daily dietary exposure is highest for the mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine (No. 2130) (5000 μg, the SPET value obtained from seasonings and flavours). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 3000 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

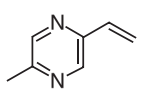
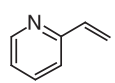
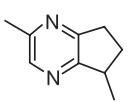
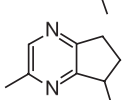
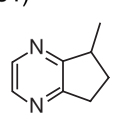
Information on the absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of pyrazine derivatives has been described in the report of the fifty-seventh meeting (Annex 1, reference *154*).

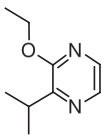
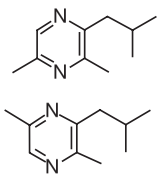
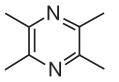
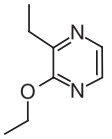
Table 10
Summary of the results of the safety evaluations of pyrazine derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II								
Isopropenylpyrazine	2125	38713-41-6 	B3: Yes, SPET: 3000	NR	Yes. The NOAEL of 14 mg/kg bw per day in a 92-day study in rats for the structurally related 2-vinylpyridine is 280 times the estimated dietary exposure to No. 2125 relative to the SPET value and 84 million times relative to the MSDI (0.01 µg/day) when used as a flavouring agent.	Note 1	2-Vinylpyridine 	No safety concern
5-Ethyl-2,3-dimethylpyrazine	2126	15707-34-3 	A3: No, SPET: 400	A4: NR	A5: NR	Note 1		No safety concern

continued

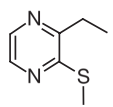
Table 10 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
2-Methyl-5-vinylpyrazine	2127	13925-08-1 	B3: Yes, SPET: 2000	NR	Yes. The NOAEL of 14 mg/kg bw per day in a 92-day study in rats for the structurally related 2-vinylpyridine is 420 times the estimated dietary exposure to No. 2127 relative to the SPET value and 8.4 million times relative to the MSDI (0.1 µg/day) when used as a flavouring agent.	Note 1	2-Vinylpyridine 	No safety concern
Mixture of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine and 2,7-dimethyl-6,7-dihydro-5H-cyclopentapyrazine	2128	38917-61-2; 38917-62-3  	A3: Yes, SPET: 3000	A4: No	A5: Yes. The NOAEL of 50 mg/kg bw per day in a 90-day study in rats for the structurally related 5-methyl-6,7-dihydro-5H-cyclopentapyrazine (No. 781) is 1000 times the estimated dietary exposure to No. 2128 when used as a flavouring agent.	Note 1	5-Methyl-6,7-dihydro-5H-cyclopentapyrazine (No. 781) 	No safety concern

2-Ethoxy-3-isopropylpyrazine	2129	72797-16-1 	A3: No, SPET: 1	A4: NR	A5: NR	Note 2		No safety concern
Structural class III								
Mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine	2130	38888-81-2; 70303-42-3 	A3: Yes, SPET: 5000	A4: No	A5: Yes. The NOEL of 44 mg/kg bw per day in a 90-day study in rats for the structurally related 2,3,5,6-tetramethylpyrazine (No. 780) is 530 times the estimated dietary exposure to No. 2130 relative to the SPET value and 260 million times relative to the MSDI (0.01 µg/day) when used as a flavouring agent.	Note 1	2,3,5,6-Tetramethylpyrazine (No. 780) 	No safety concern
2-Ethoxy-3-ethylpyrazine	2131	35243-43-7 	A3: No, SPET: 2	A4: NR	A5: NR	Note 2		No safety concern

continued

Table 10 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
2-Ethyl-3-methylthiopyrazine	2132	72987-62-3 	A3: No, SPET: 5	A4: NR	A5: NR	Note 3		No safety concern

CAS, Chemical Abstracts Service; NR, not required for evaluation because dietary exposure to the flavouring agent was determined to be of no safety concern at step A3 of the Procedure

[a] Forty-one flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *154*).

[b] *Step 1*: Five flavouring agents in this group (Nos 2125–2129) are in structural class II. Three flavouring agents in this group (Nos 2130–2132) are in structural class III (*10*).

[c] *Step 2*: Six of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human dietary exposures for structural classes II and III are 540 and 90 µg/day, respectively. All dietary exposures are expressed in µg/day. The dietary exposure values listed represent the highest estimated dietary exposures calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposures in all cases.

[e] The margins of exposure were calculated based on the estimated dietary exposures calculated using the SPET.

Notes:

1. The biotransformation of substituted pyrazines is expected to occur primarily via oxidation of the side-chain. Alkyl-ring substituents (>C1) are expected to undergo oxidation to the corresponding secondary alcohol, which may be further oxidized to the corresponding ketone for excretion unchanged or conjugated in the urine. An alternative pathway for substituted pyrazines and primary pathway for pyrazine involves hydroxylation of the pyrazine ring. Methyl-substituted pyrazines are oxidized to yield the corresponding pyrazine-2-carboxylic acid derivatives. Products of oxidative metabolism may be excreted unchanged or conjugated with glycine, glucuronic acid or sulfate prior to excretion.
2. Pyrazine or pyrazine derivatives with a ring-activating alkoxy side-chain primarily undergo ring hydroxylation. Additionally, the methoxy groups will undergo demethylation, and the resulting hydroxyl groups will undergo conjugation with glucuronic acid.
3. The presence of sulfur in the side-chain permits rapid oxidation. Alkyl and aromatic sulfides are oxidized to sulfoxides and then to sulfones, which are excreted in the urine.

The pyrazine derivatives in this group are predicted to be absorbed rapidly from the gastrointestinal tract and excreted. The biotransformation of alkyl-substituted pyrazine derivatives (Nos 2125–2128 and 2130) is predicted to occur primarily by oxidation of the side-chains to yield the corresponding secondary alcohols. The resulting alcohols are predicted to undergo conjugation with glucuronic acid. Additional products of oxidative metabolism can be excreted unchanged or conjugated with glycine, glucuronic acid or sulfate before excretion. Hydroxylation of the pyrazine ring may also occur.

Additionally, the alkoxy side-chains are predicted to undergo *O*-dealkylation reactions, followed by excretion as glucuronic acid conjugates in the urine. The presence of sulfur in the side-chain (No. 2132) permits rapid oxidation to sulfoxides and then to sulfones, which are metabolically stable and predicted to be excreted in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these eight flavouring agents, the Committee assigned five flavouring agents (Nos 2125–2129) to structural class II and three flavouring agents (Nos 2130–2132) to structural class III (*10*).

Step 2. Six flavouring agents (Nos 2126 and 2128–2132) are predicted to be metabolized to innocuous products. Therefore, the safety evaluation for these flavouring agents proceeded via the A-side of the Procedure. The remaining two flavouring agents (Nos 2125 and 2127) are not predicted to be metabolized to innocuous products. The safety evaluation for these two flavouring agents proceeded down the B-side of the Procedure.

Step A3. Estimated dietary exposures to two of the three flavouring agents in this group in structural class II (Nos 2126 and 2129) are below the threshold of concern (i.e. 540 μg/person per day for class II). These flavouring agents would not be expected to be of concern at current estimated dietary exposures. The estimated dietary exposure to the other flavouring agent in this group (No. 2128) in structural class II is above the threshold of concern, and therefore its safety evaluation proceeded to step A4. The estimated daily dietary exposures to two of the three flavouring agents in structural class III (Nos 2131 and 2132) are below the threshold of concern (i.e. 90 μg/person per day for class III). These flavouring agents would not be expected to be of concern at current estimated dietary exposures. The estimated dietary exposure to the remaining flavouring agent (No. 2130) in structural class III is above the threshold of concern, and the safety evaluation of this flavouring agent proceeded to step A4.

Step A4. The two flavouring agents (Nos 2128 and 2130) and their respective metabolites considered at this step are not endogenous, and the safety evaluations for these flavouring agents therefore proceeded to step A5.

Step A5. For the mixture of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine and 2,7-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (No. 2128), the NOAEL of 50 mg/kg bw per day for the structurally related 5-methyl-6,7-dihydro-5H-cyclopentapyrazine (No. 781) from a 90-day dietary study in rats provides a margin of exposure of 1000 in relation to the current estimated dietary exposure to No. 2128 (SPET = 3000 μg/day) when used as a flavouring agent.

For the mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine (No. 2130), the NOEL of 44 mg/kg bw per day for the structurally related 2,3,5,6-tetramethylpyrazine (No. 780) from a 90-day dietary study in rats provides a margin of exposure of 530 in relation to the current estimated dietary exposure to No. 2130 calculated using the SPET (5000 μg/day) or 260 million in relation to the MSDI (0.01 μg/day), when used as a flavouring agent.

Step B3. The estimated dietary exposures to two flavouring agents (Nos 2125 and 2127) in structural class II are above the threshold of concern (540 μg/person per day for class II). Accordingly, for these flavouring agents, data are required on the flavouring agent or a closely related substance in order to perform a safety evaluation.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

For isopropenylpyrazine (No. 2125), the NOAEL of 14 mg/kg bw per day (based on a dose of 20 mg/kg bw per day administered 5 days/week) for the structurally related 2-vinylpyridine from a 92-day oral toxicity study in rats provides a margin of exposure of 280 in relation to the current estimated dietary exposure to No. 2125 calculated using the SPET (3000 μg/day) or 84 million compared with the MSDI (0.01 μg/day) when used as a flavouring agent.

For 2-methyl-5-vinylpyrazine (No. 2127), the NOAEL of 14 mg/kg bw per day (based on a dose of 20 mg/kg bw per day administered 5 days/week) for the structurally related 2-vinylpyridine from a 92-day oral toxicity study in rats provides a margin of exposure of 420 in relation to the current estimated dietary exposure to No. 2127 calculated using the SPET (2000 μg/day) or 8.4 million compared with the MSDI (0.1 μg/day) when used as a flavouring agent.

The Committee therefore concluded that none of the eight additional flavouring agents (Nos 2125–2132) in this group of pyrazine derivatives would pose a safety concern at current estimated dietary exposures.

Table 10 summarizes the evaluations of all eight flavouring agents in this group.

Consideration of combined intakes from use as flavouring agents

The highest MSDI for any of these eight pyrazine derivatives is 0.2 μg/day. Consideration of combined intakes is not deemed necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluation of the flavouring agents in this group of pyrazine derivatives, biochemical data, metabolism and acute toxicity studies, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available (Annex 1, reference *154*). The additional toxicity data on subchronic toxicity (No. 784 and the structurally related 2-vinylpyridine) and on genotoxicity (several previously evaluated pyrazine derivatives and 2-vinylpyridine) considered at this meeting support the previous evaluation.

The Committee concluded that these eight flavouring agents, which are additions to the group of pyrazine derivatives evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.12 *Pyridine, pyrrole and quinoline derivatives*

Explanation

The Committee evaluated 11 additional flavouring agents belonging to the group of pyridine, pyrrole and quinoline derivatives. The additional flavouring agents included two pyrroles (Nos 2150 and 2152), eight alkylated pyridines (Nos 2151, 2153–2156 and 2158–2160) and one quinoline (No. 2157). The Committee decided to evaluate two imidazolidines that were originally submitted in this group (Nos 2161 and 2162) as additional flavouring agents belonging to the group of miscellaneous nitrogen-containing substances (see section 4.1.9). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee. Three of the flavouring agents in this group (Nos 2158–2160) that were evaluated at this meeting are reported to be flavour modifiers.

The Committee previously evaluated 22 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The Committee concluded that all 22 flavouring agents in that group were of no safety concern at estimated dietary exposures.

Three of the 11 flavouring agents evaluated at the current meeting are natural components of food (Nos 2150–2152) and have been detected in black and green teas, coffee, cocoa, mate, skim milk powder, beer, wine, cognac, onion, popcorn, raisin, clam, cheese, egg, oats, pork, shoyu, wheaten bread, liquorice, honey, peanut, potato, okra, soya bean and tamarind.

Assessment of dietary exposure

The total annual volumes of production of the 11 pyridine, pyrrole and quinoline derivatives are approximately 0.4 kg in the USA, 0.2 kg in Europe and 64 kg in Japan. Approximately 98% of the total annual volume of production in Japan is accounted for by one flavouring agent in this group—namely, 2-methoxypyridine (No. 2156).

Dietary exposures were estimated using the MSDI method and the SPET, with the highest values reported in Table 11. The estimated dietary exposure is highest for 2,4-dimethylpyridine (No. 2151) (4000 μg/day, the SPET value obtained from soups). For the other flavouring agents, the estimated daily dietary exposures, calculated using either the MSDI method or the SPET, range from 0.01 to 1500 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of pyridine, pyrrole and quinoline derivatives has previously been described in the monograph of the sixty-third meeting (Annex 1, reference *174*).

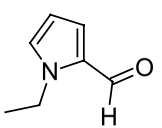
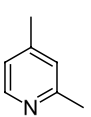
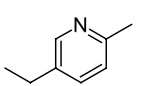
New structural elements not evaluated previously are aldehyde-substituted pyrroles (Nos 2150 and 2152). The aldehyde group of aldehyde-substituted pyrroles can be expected to be oxidized to the corresponding carboxylic acid, as was shown for pyrrole-2-carboxaldehyde.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 11 flavouring agents in this group of pyridine, pyrrole and quinoline derivatives, the Committee assigned 3 flavouring agents to structural class II (Nos 2150–2152) and 8 flavouring agents to structural class III (Nos 2153–2160) (*10*).

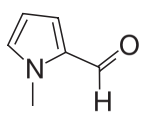
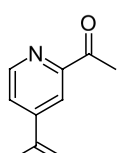
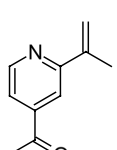
Step 2. Four of the flavouring agents (Nos 2151 and 2053–2155) in this group can be predicted to be metabolized to innocuous products. The evaluation of these flavouring agents therefore proceeded via the A-side of the Procedure. The remaining flavouring agents (Nos 2050, 2052 and 2156–2160) in this group cannot be predicted to be metabolized to innocuous products.

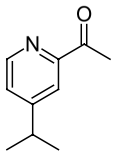
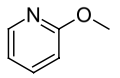
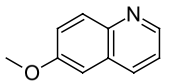
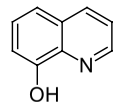
Table 11
Summary of the results of the safety evaluations of pyridine, pyrrole and quinoline derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5/B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step A3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	*Step B5* Do the conditions of use result in a dietary exposure greater than 1.5 µg/day?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II									
1-Ethyl-2-pyrrolecarboxaldehyde	2150	2167-14-8 	B3: No, SPET: 510		B4: No	Yes	Notes 1–3		Additional data required to complete evaluation
2,4-Dimethylpyridine	2151	108-47-4 	A3: Yes, SPET: 4000	No	A5: Yes. The NOAEL of 30 mg/kg bw per day for the related substance 5-ethyl-2-methylpyridine (No. 1318) based on an abstract describing a 28-day study in rats is 450 (based on the SPET) and 180 million (based on the MSDI) times the estimated daily dietary exposure to No. 2151 when used as a flavouring agent.		Notes 3 and 4	5-Ethyl-2-methylpyridine (No. 1318) 	No safety concern (temporary)

continued

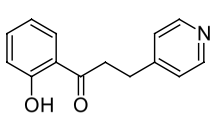
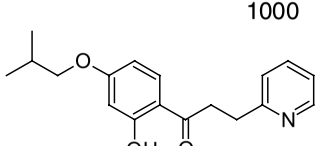
Table 11 (*continued*)

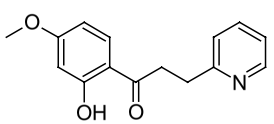
Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5/B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step A3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	*Step B5* Do the conditions of use result in a dietary exposure greater than 1.5 µg/day?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
1-Methyl-1H-pyrrole-2-carboxaldehyde	2152	1192-58-1 	B3: No, SPET: 300	NR	B4: No	Yes	Notes 1–3		Additional data required to complete evaluation
Structural class III									
2-Acetyl-4-isopropenylpyridine	2153	142896-11-5 	A3: No, SPET: 3	NR	NR	NR	Notes 4–6		No safety concern
4-Acetyl-2-isopropenylpyridine	2154	142896-12-6 	A3: No, SPET: 3	NR	NR	NR	Notes 4–6		No safety concern

2-Acetyl-4-isopropylpyridine	2155	142896-09-1 	A3: No, SPET: 3	NR	NR	NR	Notes 3–5		No safety concern
2-Methoxypyridine	2156	1628-89-3 	B3: No, SPET: 40	NR	B4: No	Yes	Notes 3, 4 and 7		Additional data required to complete evaluation
6-Methoxyquinoline	2157	5263-87-6 	B3: No, SPET: 25	NR	B4. Yes. The NOAEL of 140 mg/kg bw per day for the structurally related substance 8-hydroxyquinoline in a 2-year study in rats and mice is 340 000 times the estimated daily dietary exposure to No. 2157 when used as a flavouring agent.		Notes 4, 7 and 8	8-Hydroxyquinoline 	No safety concern

continued

Table 11 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5/B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step A3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	*Step B5* Do the conditions of use result in a dietary exposure greater than 1.5 µg/day?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
1-(2-Hydroxyphenyl)-3-(pyridin-4-yl)-propan-1-one	2158	1186004-10-3 	B3: Yes, SPET: 1500	NR	Additional data: Genotoxic potential in vitro was demonstrated for No. 2158.	NR	Note 7		Additional data required to complete evaluation
1-(2-Hydroxy-4-isobutoxyphenyl)-3-(pyridin-2-yl)-propan-1-one	2159	1190230-47-7 	B3: Yes, SPET: 1000	NR	Additional data: Genotoxic potential in vitro was demonstrated for the structurally related flavouring agent No. 2158.	NR	Note 7		Additional data required to complete evaluation

1-(2-Hydroxy-4-methoxyphenyl)-3-(pyridin-2-yl)-propan-1-one 	2160	1190229-37-8	B3: Yes, SPET: 1000	NR	Additional data: Genotoxic potential in vitro was demonstrated for the structurally related flavouring agent No. 2158.	NR	Note 7	Additional data required to complete evaluation

CAS, Chemical Abstracts Service; NR, not required for evaluation

[a] Twenty-two flavouring agents belonging to the group of pyridine, pyrrole and quinoline derivatives were previously evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*).

[b] *Step 1*: Three flavouring agents in this group (Nos 2150–2152) are in structural class II. The other eight flavouring agents in this group (Nos 2153–2160) are in structural class III.

[c] *Step 2*: Four of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human dietary exposure for structural classes II and III are 540 and 90 µg/person per day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure values listed represent the highest estimated dietary exposures calculated using either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposures in all cases.

[e] The margins of exposure were calculated based on the estimated dietary exposures calculated using the SPET.

Notes:

1. The pyrrole ring undergoes hydroxylation and is excreted in the urine as the corresponding glucuronic acid conjugate.
2. The aldehyde group can be expected to be oxidized to the corresponding carboxylic acid. Alkyl side-chain oxidation may also occur.
3. Alkyl side-chain oxidation followed by glucuronic acid conjugation and excretion or oxidation to the corresponding carboxylic acid.
4. The pyridine ring system undergoes hydroxylation and is excreted in the urine as the corresponding glucuronic acid conjugate.
5. The acetyl group is reduced and conjugated with glucuronic acid.
6. Alkenyl side-chain oxidation followed by glucuronic acid conjugation and excretion or oxidation to the corresponding carboxylic acid.
7. *N*-oxidation and *O*-dealkylation followed by glucuronic acid or sulfate conjugation.
8. Forms a reactive epoxide metabolite that is detoxified through glutathione conjugation.

Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step A3. The highest estimated dietary exposure to one flavouring agent in structural class II (No. 2151) is above the relevant threshold of concern (i.e. 540 μg/person per day for class II). Accordingly, the evaluation of this flavouring agent proceeded to step A4.

The highest estimated dietary exposures to all three flavouring agents in structural class III (Nos 2153–2155) are below the threshold of concern (i.e. 90 μg/person per day for class III). The Committee therefore concluded that these flavouring agents are not of safety concern at current estimated dietary exposures.

Step A4. Neither the flavouring agent 2,4-dimethylpyridine (No. 2151) nor its metabolites are endogenous substances. Accordingly, the evaluation of this flavouring agent proceeded to step A5.

Step A5. The NOAEL for the structurally related substance 5-ethyl-2-methylpyridine (No. 1318) of 30 mg/kg bw per day based on an abstract describing a 28-day study in rats provides a margin of exposure of 450 in relation to the highest estimated dietary exposure to 2,4-dimethylpyridine (No. 2151; SPET = 4000 μg/day) when used as a flavouring agent. The Committee noted that the margin of exposure for No. 2151 based on the MSDI of 0.01 μg/day is 180 million and concluded that the margins of exposure of 450 (based on the SPET) and 180 million (based on the MSDI) are adequate.

The Committee therefore concluded that this flavouring agent is not of safety concern at current estimated dietary exposures. However, because the full study was not available, the safety evaluation for No. 2151 was considered temporary.

Step B3. The highest estimated dietary exposures to the two flavouring agents in structural class II (Nos 2150 and 2152) and two flavouring agents in structural class III (Nos 2156 and 2157) are below the relevant threshold of concern (i.e. 540 μg/person per day for class II and 90 μg/person per day for class III). Accordingly, the evaluation of these four flavouring agents proceeded to step B4.

The highest estimated dietary exposures to the three remaining flavouring agents in structural class III (Nos 2158–2160) are above the relevant threshold of concern. Therefore, additional data are necessary for the evaluation of these flavouring agents.

Step B4. The NOAEL of 140 mg/kg bw per day for the structurally related substance 8-hydroxyquinoline in a 2-year study in rats and mice provides a margin of exposure of 340 000 for 6-methoxyquinoline (No. 2157; SPET = 25 μg/day) when used as a flavouring agent.

For 1-ethyl-2-pyrrolecarboxaldehyde (No. 2150), 1-methyl-1H-pyrrole-2-carboxaldehyde (No. 2152) and 2-methoxypyridine (No. 2156), no toxicological data are available on the flavouring agents or adequate related substances with which to calculate a margin of exposure. Therefore, the evaluation of these flavouring agents proceeded to step B5.

Step B5. The conditions of use for 1-ethyl-2-pyrrolecarboxaldehyde (No. 2150), 1-methyl-1H-pyrrole-2-carboxaldehyde (No. 2152) and 2-methoxypyridine (No. 2156) result in dietary exposures greater than 1.5 μg/day. Therefore, the Committee determined that additional data would be necessary to complete the evaluation of these flavouring agents.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

In accordance with the Procedure, additional data were evaluated for 1-(2-hydroxyphenyl)-3-(pyridin-4-yl)propan-1-one (No. 2158), 1-(2-hydroxy-4-isobutoxyphenyl)-3-(pyridin-2-yl)propan-1-one (No. 2159) and 1-(2-hydroxy-4-methoxyphenyl)-3-(pyridin-2-yl)propan-1-one (No. 2160), as the estimated dietary exposures exceeded the threshold of concern for structural class III (90 μg/person per day).

For the three structurally related compounds Nos 2158–2160, the data available indicate potential genotoxicity.

No. 2158 was negative in two bacterial reverse mutation tests with and without metabolic activation. It was positive for clastogenicity in an in vitro chromosomal aberration assay using human lymphocytes at concentrations of 245 μg/ml or more without metabolic activation in two independent tests. No. 2159 was negative in a bacterial reverse mutation test with and without metabolic activation and a forward mutation test in mouse lymphoma L5178Y cells. For No. 2160, no genotoxicity data are available. The data available for No. 2158 demonstrate that this compound has genotoxic potential in vitro. As in vivo genotoxicity data are not available for No. 2158 and as no tests on clastogenicity are available for the structurally related compounds Nos 2159 and 2160, the Committee determined that additional data on genotoxicity would be necessary to complete the safety evaluations of Nos 2158–2160.

Table 11 summarizes the evaluations of the 11 pyridine, pyrrole and quinoline derivatives (Nos 2150–2160) in this group.

Consideration of combined intakes from use as flavouring agents

The 11 additional flavouring agents in this group of pyridine, pyrrole and quinoline derivatives all have MSDI values less than or equal to 20% of the threshold of concern for structural class III. Consideration of combined intakes is therefore not deemed necessary.

Conclusion

In the previous evaluation of flavouring agents in the group of pyridine, pyrrole and quinoline derivatives, studies of acute toxicity, short-term and long-term studies of toxicity and carcinogenicity (21–460 days) and studies of genotoxicity were available (Annex 1, reference *173*). The toxicity data available for this evaluation generally supported those from previous evaluations. However, in the previous evaluation of this group, it was concluded, on the basis of the available evidence, that the 22 pyridine, pyrrole and quinoline derivatives evaluated would not demonstrate genotoxic potential. On the basis of the data available for the current evaluation, this conclusion cannot be extended to Nos 2158–2160. The Committee concluded that, due to concerns with genotoxicity, additional data on genotoxicity would be necessary to complete the evaluation of Nos 2158–2160 at current estimated dietary exposures.

The Committee concluded that additional toxicity data on the flavouring agents or adequate related substances would be necessary to complete the evaluation of Nos 2150, 2152 and 2156 at current estimated dietary exposures.

The Committee concluded that five flavouring agents (Nos 2151, 2153–2155 and 2157), which are additions to the group of pyridine, pyrrole and quinoline derivatives, would not give rise to safety concerns at current estimated dietary exposures. However, for No. 2151, the safety evaluation was temporary, pending the submission of the full report of the critical study for the next JECFA meeting at which flavouring agents are evaluated.

An addendum to the toxicological monograph was prepared.

4.1.13 *Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids*

Explanation

The Committee evaluated four additional flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids that was evaluated previously. All four are aliphatic branched-chain aldehydes (Nos 2173–2176). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have been evaluated previously by the Committee.

The Committee previously evaluated 25 other members of this group of flavouring agents at its forty-ninth meeting (Annex 1, reference *131*). The Committee concluded that all 25 flavouring agents in that group were of no safety concern at estimated dietary exposures. The Committee had evaluated 2 of these 25 flavouring agents before the forty-ninth meeting. Isobutyl alcohol

(2-methyl-2-propanol) was considered at the twenty-third meeting (Annex 1, reference *50*), when an evaluation of this compound was not possible owing to a paucity of toxicological data. 2-Ethyl-1-hexanol was evaluated at the forty-first meeting (Annex 1, reference *107*), when it was allocated an ADI of 0–0.5 mg/kg bw.

Three of the four flavouring agents evaluated at the current meeting (Nos 2173–2175) are natural components of food and have been detected in foods such as herbs and spices (*Zanthoxylum alatum*, *Codonopsis pilosula*), citrus fruit (yuzu) and meat (chicken, beef and pork) (*12–15*).

Assessment of dietary exposure

The total annual volumes of production of the four saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and carboxylic acids are approximately 0.3 kg in the USA, 0.1 kg in Europe and 0.2 kg in Japan (*16–19*).

Dietary exposures were estimated using both the SPET and the MSDI method, with the highest values reported in Table 12. Two flavouring agents have the highest estimated daily dietary exposure: 3-methylhexanal (No. 2173) and 3,7-dimethyloctanal (No. 2176) (150 μg, the SPET value obtained from non-alcoholic beverages [No. 2173] or instant coffee and tea [No. 2176]). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 30 μg, with the SPET yielding the highest estimates.

Annual volumes of production of this group of flavouring agents as well as the daily dietary exposures calculated using both the MSDI method and the SPET are summarized in Table 13.

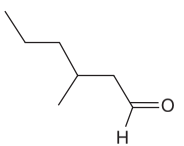
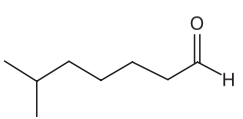
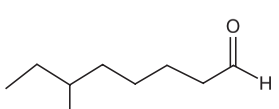
Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids has previously been described in the monograph of the forty-ninth meeting (Annex 1, reference *132*). No relevant additional data have been reported since that meeting.

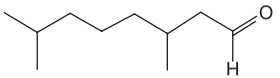
Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the four flavouring agents in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, the Committee assigned all four flavouring agents (Nos 2173–2176) to structural class I (*10*).

Table 12
Summary of the results of the safety evaluations of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does estimated dietary exposure exceed the threshold of concern?	Comments on predicted metabolism	Conclusion based on current estimated dietary exposure
Structural class I					
3-Methylhexanal	2173	19269-28-4 	No, SPET: 150	Note 1	No safety concern
6-Methylheptanal	2174	63885-09-6 	No, SPET: 30	Note 1	No safety concern
6-Methyloctanal	2175	30689-75-9 	No, SPET: 30	Note 1	No safety concern

3,7-Dimethyloctanal	2176	5988-91-0	No, SPET: 150	Note 1	No safety concern



CAS, Chemical Abstracts Service

[a] Twenty-five flavouring agents in this group were previously evaluated by the Committee at its forty-ninth meeting (Annex 1, reference *131*), 2 of which had also been evaluated before, one at the twenty-third meeting and one at the forty-first meeting (Annex 1, references *50* and *107*).

[b] *Step 1*: The four flavouring agents in this group are in structural class I.

[c] *Step 2*: All of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class I is 1800 µg/person per day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The highest estimates were all derived using the SPET.

Note:

1. Expected to undergo oxidation to form the corresponding carboxylic acid followed by metabolism in the fatty acid pathway or tricarboxylic acid cycle.

Table 13

Annual volumes of production and daily dietary exposures for saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Natural occurrence in foods
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
3-Methylhexanal (2173)				150	2.5	+
Europe	ND	ND	ND			
USA	0.2	0.02	0.0004			
Japan	ND	ND	ND			
6-Methylheptanal (2174)				30	0.5	+
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
6-Methyloctanal (2175)				30	0.5	+
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
3,7-Dimethyloctanal (2176)				150	2.5	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.1	0.03	0.0005			
Total						
Europe	0.1					
USA	0.3					
Japan	0.2					

ND, no data reported; +, reported to occur naturally in foods (*12–15*), but no quantitative data; –, not reported to occur naturally in foods

[a] From references *16–19*. Values greater than 0 kg but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1×10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 31×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*16–19*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (highest usual use level) (*19*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

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

Step A3. The highest estimated dietary exposures to all four flavouring agents are below the threshold of concern (i.e. 1800 μg/person per day for class I). The safety of these four flavouring agents raises no concern at current estimated dietary exposures.

Table 12 summarizes the evaluations of the four saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids (Nos 2173–2176) in this group.

Additional toxicological studies

Additional studies of in vitro genotoxicity have been reported for five flavouring agents previously evaluated in this group (Nos 255, 268, 269, 272 and 275). The results of these studies are summarized in Table 14 and described below.

No evidence of mutagenicity was observed in Ames assays when 2-methylbutyric acid (No. 255), 3,5,5-trimethylhexanal (No. 269), 3,7-dimethyl-1-octanol (No. 272) or 2-methylundecanal (No. 275) were incubated with *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102, TA1535, TA1537 and/or TA1538 with or without metabolic activation at concentrations up to 5000 μg/plate (*20–23*).

No evidence of clastogenic potential was observed for 3,5,5-trimethyl-1-hexanol (No. 268) in a chromosomal aberration assay in Chinese hamster CHL/IU cells (*24*).

The results support the conclusion of the previous evaluation that the weight of evidence indicates that members of the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids are not genotoxic.

Consideration of combined intakes from use as flavouring agents

The four additional flavouring agents in this group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids have very low MSDIs (0.01–0.03 μg/day). Consideration of combined intakes is not deemed necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Conclusion

In the previous evaluation of flavouring agents in the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids, studies of acute toxicity, short-term and long-term toxicity (7 days to 2 years),

Table 14
Studies of genotoxicity in vitro with saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids used as flavouring agents

No.	Flavouring agent	End-point	Test system	Concentration	Results	Reference
255	2-Methylbutyric acid	Reverse mutation	*Salmonella typhimurium* TA97a, TA98, TA100, TA102, TA1535	50–5000 µg/plate, ±S9[a]	Negative[b]	*21*
268	3,5,5-Trimethyl-1-hexanol	Chromosomal aberration	Chinese hamster CHL/IU cells	0–5000 µg/ml, ±S9[c]	Negative[d]	*24*
269	3,5,5-Trimethylhexanal	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.000 82–0.82 µg/plate (0.001–1 nl/plate), ±S9[e]	Negative	*21*
272	3,7-Dimethyl-1-octanol	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1.5–500 µg/plate, –S9[a] 1.5–1500 µg/plate, +S9[g]	Negative[f]	*22*
275	2-Methylundecanal	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5–5000 µg/plate, ±S9[a]	Negative[h]	*23*

S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Two independent experiments.
[b] Toxicity was observed in strain TA97a, with metabolic activation, at the highest dose tested.
[c] Cells were analysed either 18 hours after 6 hours of treatment (with and without metabolic activation) or immediately after 24 or 48 hours of treatment (without metabolic activation).
[d] No numerical data were provided.
[e] Calculated using the density of 3,3,5-trimethylhexanal = 0.82 g/ml.
[f] Toxicity was observed from 150 µg/plate upwards in strain TA1537 and at the highest dose tested in strains TA98, TA100, TA102 and TA1535 without metabolic activation. With metabolic activation, toxicity was observed in all strains from 500 µg/plate upwards.
[g] Two independent experiments. Dose levels ranged from 1.5 to 500 µg/plate in the first experiment and from 1.5 to 1500 µg/plate in the second experiment.
[h] Toxicity was observed in strains TA100, TA102, TA1535 and TA1537 from 500 µg/plate upwards, with and without metabolic activation, and in strain TA98 at the highest dose tested, with and without metabolic activation.

genotoxicity, and reproductive and developmental toxicity as well as special studies on peroxisome proliferation and immunotoxicity were available (Annex 1, reference *132*). None raised safety concerns. The genotoxicity data available for the present evaluation supported the previous evaluations.

The Committee concluded that these four flavouring agents, which are additions to the group of saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was not prepared.

4.1.14 *Simple aliphatic and aromatic sulfides and thiols*

Explanation

The Committee evaluated seven additional flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols. The additional flavouring agents included one acyclic sulfide with an oxidized side-chain (No. 2086), one cyclic sulfide (No. 2089), one simple thiol (No. 2083), two thiols with oxidized side-chains (Nos 2084 and 2085), one dithiol (No. 2087) and one disulfide with an oxidized side-chain (No. 2088). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee.

The Committee previously evaluated 137 other members of this group of flavouring agents at its fifty-third meeting (Annex 1, reference *143*). The group was divided into 12 subgroups on the basis of the position of the sulfur atom, in order to facilitate the assessment of the relevant data on metabolism and toxicity. The Committee concluded that all 137 flavouring agents in that group were unlikely to pose a safety concern at the estimated dietary exposures.

The Committee evaluated 12 additional members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*), another 51 at its sixty-eighth meeting (Annex 1, reference *187*) and a further 36 at its seventy-third meeting (Annex 1, reference *203*). The Committee concluded that 93 additional members of this group were unlikely to pose a safety concern at their estimated dietary exposures. For the six remaining flavouring agents (Nos 1914, 1931, 1939, 1941, 1943 and 1944), the Committee at the seventy-third meeting concluded that the evaluation could not be completed because additional data were required.

Three of the seven flavouring agents evaluated at the current meeting are natural components of food (Nos 2083, 2086 and 2088) and have been detected in guava fruit, tomato and roasted sesame oil (*13*, *25*, *26*).

Assessment of dietary exposure

The total annual volumes of production of the seven simple aliphatic and aromatic sulfides and thiols are approximately 0.7 kg in the USA and 0.2 kg in Europe, with no data available from Japan. Each of the seven flavouring agents has a reported annual volume of production of 0.1 kg in either the USA or Europe, or both.

Dietary exposure estimates were made using the MSDI method and the SPET, with the highest estimates reported in Table 15. The estimated dietary exposure is highest for 1-methyldithio-2-propanone (No. 2088) (100 μg/day, the SPET value obtained from soups and processed vegetables). For the other flavouring agents, the estimated dietary exposures range from 0.01 to 40 μg/day, with the SPET yielding the highest estimates.

Annual volumes of production of this group of flavouring agents as well as the daily dietary exposures calculated using both the MSDI method and the SPET are summarized in Table 16.

Absorption, distribution, metabolism and elimination

Information on the absorption, distribution, metabolism and elimination of the flavouring agents belonging to the group of simple aliphatic and aromatic sulfides and thiols has previously been described in the monographs of the fifty-third, sixty-first and sixty-eighth meetings (Annex 1, references *144*, *167* and *188*). No relevant additional data have been reported since these meetings.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

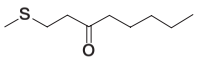
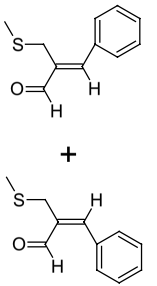
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the seven flavouring agents in this group of simple aliphatic and aromatic sulfides and thiols, the Committee assigned four flavouring agents to structural class I (Nos 2083–2086) and three flavouring agents to structural class III (Nos 2087–2089) (*10*).

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

Step B3. The highest dietary exposures of the four flavouring agents in structural class I (Nos 2083–2086) are below the relevant threshold of concern (i.e. 1800 μg/person per day). The highest dietary exposures of two flavouring agents in structural class III (Nos 2087 and 2089) are below the relevant threshold of concern (i.e. 90 μg/person per day). Accordingly, the evaluation of these six flavouring agents proceeded to step B4.

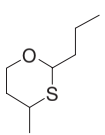
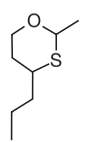
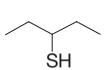
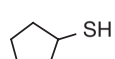
Table 15

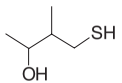
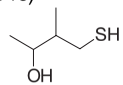
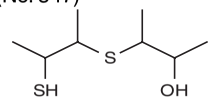
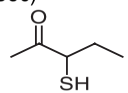
Summary of the results of the safety evaluations of simple aliphatic and aromatic sulfides and thiols used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[a] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Subgroup ii: Acyclic sulfides with oxidized side-chains							
Structural class I							
1-(Methylthio)-3-octanone	2086	61837-77-2 	No, SPET: 3	B4. Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) (*27*) from an oral 90-day study in rats is 28 000 times the estimated daily dietary exposure to No. 2086 when used as a flavouring agent.	Notes 1 and 2	2-(Methylthiomethyl)-3-phenylpropenal (No. 505) 	No safety concern

continued

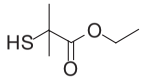
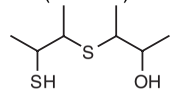
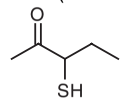
Table 15 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Subgroup iii: Cyclic sulfides							
Structural class III							
4-Methyl-2-propyl-1,3-oxathiane	2089	1064678-08-5 	No, SPET: 15	B4. Yes. The NOEL of 0.44 mg/kg bw per day for the related substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) (*28*) is 1800 times the estimated daily exposure to No. 2089 when used as a flavouring agent.	Note 2	2-Methyl-4-propyl-1,3-oxathiane (No. 464) 	No safety concern
Subgroup iv: Simple thiols							
Structural class I							
3-Pentanethiol	2083	616-31-9 	No, SPET: 0.01	B4. Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) from an oral 90-day study in rats (*29*) is 3 million times the estimated daily dietary exposure to No. 2083 when used as a flavouring agent.	Notes 3 and 5	Cyclopentanethiol (No. 516) 	No safety concern

Subgroup v: Thiols with oxidized side-chains							
Structural class I							
4-Mercapto-3-methyl-2-butanol	2084	33959-27-2 	No, SPET: 40	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, the related substances 2-mercapto-3-butanol (No. 546), α-methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from oral 90-day studies in rats (*30–32*) are 2800 times the estimated daily dietary exposure to No. 2084 when used as a flavouring agent.	Notes 3, 5 and 6	2-Mercapto-3-butanol (No. 546)  α-Methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547)  3-Mercapto-2-pentanone (No. 560) 	No safety concern

continued

Table 15 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[a] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Ethyl 2-mercapto-2-methylpropionate	2085	33441-50-8 	No, SPET: 15	B4. Yes. The NOELs of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, the related substances 2-mercapto-3-butanol (No. 546), α-methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (30–32) are 7600 times the estimated daily dietary exposure to No. 2085 when used as a flavouring agent.	Notes 3–5	2-Mercapto-3-butanol (No. 546)  α-Methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547)  3-Mercapto-2-pentanone (No. 560) 	No safety concern

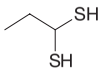
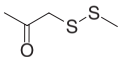
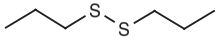
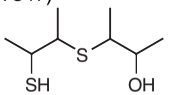
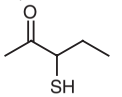
Subgroup vi: Dithiols							
Structural class III							
1,1-Propanedithiol	2087	88497-17-0 	No, SPET: 2	B4. Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2,3-butanedithiol (No. 539) from an oral 90-day study in rats (*33*) is 21 000 times the estimated daily dietary exposure to No. 2087 when used as a flavouring agent.	Notes 3 and 5	2,3-Butanedithiol (No. 539) 	No safety concern
Subgroup viii: Disulfides with oxidized side-chains							
Structural class III							
1-Methyldithio-2-propanone	2088	122861-78-3 	Yes, SPET: 100	Additional data: The NOELS of 7.3, 0.56, 1.9, 2.8 and 1.9 mg/kg bw per day for the related substances propyl disulfide (No. 566), cyclopentanethiol (No. 516), 2-mercapto-3-butanol (No. 546), α-methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from 90-day studies in rats (*29–32*, *34*) provide margins of	Notes 1, 3, 5 and 7	Propyl disulfide (No. 566)  Cyclopentanethiol (No. 516)  2-Mercapto-3-butanol (No. 546) 	No safety concern

Table 15 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance? *Follow-on from step B3*[a] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
				exposure ranging from 340 to 4400 using the SPET and greater than 3 million using the MSDI.		α-Methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547)  3-Mercapto-2-pentanone (No. 560)  	

CAS, Chemical Abstracts Service

[a] One hundred and thirty-seven flavouring agents in this group were previously evaluated by the Committee at its fifty-third meeting (Annex 1, reference *143*), 12 additional members at its sixty-first meeting (Annex 1, reference *166*), 51 additional members at its sixty-eighth meeting (Annex 1, reference *187*) and 36 additional members at its seventy-third meeting (Annex 1, reference *202*).

[b] *Step 1*: Four flavouring agents in this group are in structural class I (Nos 2083–2086), and the remaining three are in structural class III (Nos 2087–2089).

[c] *Step 2*: None of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human dietary exposure for structural classes I and III are 1800 and 90 µg/person per day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The highest estimates were all derived using the SPET.

[e] The margins of exposure were calculated based on the estimated dietary exposure calculated by the SPET. In cases where the resulting margin of exposure was relatively low, a comparison with the MSDI was also made.

Notes:

1. Detoxication of the ketone group involves reduction followed by conjugation with glucuronic acid and subsequent elimination in the urine.
2. The sulfur is expected to be oxidized to the sulfoxide and sulfone.
3. The sulfur is expected to be oxidized to sulfonic acid and/or undergo methylation followed by excretion.
4. The ester is expected to undergo hydrolysis to the corresponding carboxylic acid and alcohol.
5. Free thiols may form mixed disulfides with glutathione or cysteine.
6. The hydroxy group is expected to undergo oxidation to the carboxylic acid and/or conjugation with glucuronic acid, followed by excretion.
7. Disulfides are expected to be reduced to free thiols.

Table 16

Annual volumes of production and daily dietary exposures for simple aliphatic and aromatic sulfides and thiols used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Natural occurrence in foods
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
3-Pentanethiol (2083)				0.01	0.0002	+
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
4-Mercapto-3-methyl-2-butanol (2084)				40	1	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Ethyl 2-mercapto-2-methylpropionate (2085)				15	0.3	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
1-(Methylthio)-3-octanone (2086)				3	0.1	+
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
1,1-Propanedithiol (2087)				2	0.03	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
1-Methyldithio-2-propanone (2088)				100	2	+
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
4-Methyl-2-propyl-1,3-oxathiane (2089)				15	0.3	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			

Table 16 (*continued*)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Natural occurrence in foods
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
Total						
Europe	0.2					
USA	0.7					
Japan	ND					

ND, no data reported; +, reported to occur naturally in foods (*13*, *25*, *26*), but no quantitative data; –, not reported to occur naturally in foods

[a] From references *16–19*. Values greater than 0 kg but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1×10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 31×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the USA, Europe and the Japan surveys, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*16–19*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (highest usual use level) (*19*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

The dietary exposure of the remaining flavouring agent in structural class III (No. 2088) is 100 µg/day (calculated using the SPET). This is above the threshold of concern (i.e. 90 µg/person per day for class III), and therefore additional data are necessary for the evaluation of this flavouring agent.

Step B4. *Subgroup ii: Acyclic sulfides with oxidized side-chains.* The NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from an oral 90-day study in rats (*27*) provides a margin of exposure of 28 000 for 1-(methylthio)-3-octanone (No. 2086; SPET = 3 µg/day) when used as a flavouring agent.

Subgroup iii: Cyclic sulfides. Using the NOEL of 0.44 mg/kg bw per day for the structurally related substance 2-methyl-4-propyl-1,3-oxathiane (No. 464) from an oral 90-day study in rats (*28*), the margin of exposure is 1800 for 4-methyl-2-propyl-1,3-oxathiane (No. 2089; SPET = 15 µg/day) when used as a flavouring agent.

Subgroup iv: Simple thiols. The NOEL of 0.56 mg/kg bw per day for the structurally related substance cyclopentanethiol (No. 516) from an oral 90-day study in rats (*29*) provides a margin of exposure of greater than 3 million for 3-pentanethiol (No. 2083; SPET = 0.01 µg/day) when used as a flavouring agent.

Subgroup v: Thiols with oxidized side-chains. The NOELS of 1.9, 2.8 and 1.9 mg/kg bw per day for, respectively, the structurally related substances 2-mercapto-3-butanol (No. 546), α-methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560) from oral 90-day studies in rats (*30–32*) provide margins of exposure of at least 2800 for 4-mercapto-3-methyl-2-butanol (No. 2084; SPET = 40 μg/day) and at least 7600 for ethyl 2-mercapto-2-methylpropionate (No. 2085; SPET = 15 μg/day) when used as flavouring agents.

Subgroup vi: Dithiols. The NOEL of 0.7 mg/kg bw per day for the structurally related substance 2,3-butanedithiol (No. 539) from an oral 90-day study in rats (*33*) provides a margin of exposure of 21 000 for 1,1-propanedithiol (No. 2087; SPET = 2 μg/day) when used as a flavouring agent.

Consideration of additional data for the flavouring agent with high exposure evaluated via the B-side of the decision-tree:

In accordance with the Procedure, additional data were evaluated for 1-methyldithio-2-propanone (No. 2088), as the estimated dietary exposure exceeded the threshold of concern for structural class III (90 μg/person per day).

No toxicological data were available for 1-methyldithio-2-propanone (No. 2088). Therefore, the NOEL of 7.3 mg/kg bw per day from a 90-day feeding study in rats for the structurally related substance propyl disulfide (No. 566) (*34*) was used, resulting in a margin of exposure of 4400.

In addition, 1-methyldithio-2-propanone (No. 2088) is expected to be rapidly reduced to the thiols methyl mercaptan (No. 508) and 1-mercapto-2-propanone (No. 557). These thiols were evaluated previously using the NOELs of 0.56, 1.9, 2.8 and 1.9 mg/kg bw per day for the related substances cyclopentanethiol (No. 516), 2-mercapto-3-butanol (No. 546), α-methyl-β-hydroxypropyl α-methyl-β-mercaptopropyl sulfide (No. 547) and 3-mercapto-2-pentanone (No. 560), respectively, from 90-day studies in rats (*29–32*, *34*).

These NOELs provide margins of exposure ranging from 340 to 1700 using the SPET and greater than 3 million using the MSDI method for No. 2088.

The Committee therefore concluded that this flavouring agent is unlikely to pose a safety concern at current estimated dietary exposure.

Table 15 summarizes the evaluations of the seven simple aliphatic and aromatic sulfides and thiols (Nos 2083–2089) in this group.

Consideration of combined intakes from use as flavouring agents

The seven additional flavouring agents in this group of simple aliphatic and aromatic sulfides and thiols all have a very low MSDI (0.01 μg/day).

The Committee concluded that consideration of combined intakes is not necessary, because the additional flavouring agents would not contribute significantly to the combined intake of this flavouring group.

Consideration of secondary components

One flavouring agent in this group, 1-methyldithio-2-propanone (No. 2088), has an assay value of less than 95% (Annex 4). Two of its secondary components, 1-mercapto-2-propanone (No. 557) and 1,3-dimethyltrisulfane (No. 582), were evaluated by the Committee at its fifty-third meeting (Annex 1, reference *149*) and were considered unlikely to pose a safety concern at their estimated dietary exposures. The third secondary component, 1,1′-disulfanediyldipropan-2-one, is expected to be hydrolysed to 1-mercapto-2-propanone (No. 557) and is therefore considered not to present a safety concern at estimated dietary exposures.

Conclusion

In the previous evaluations of flavouring agents in the group of simple aliphatic and aromatic sulfides and thiols, studies of acute toxicity, short-term and long-term studies of toxicity (6 days to 2 years), and studies of genotoxicity and developmental/reproductive toxicity, as well as observations in humans, were available (Annex 1, references *144*, *167* and *188*). No relevant additional toxicity data were available for this evaluation.

The Committee concluded that the seven flavouring agents evaluated at this meeting (Nos 2083–2089), which are additions to the group of simple aliphatic and aromatic sulfides and thiols evaluated previously, would not give rise to safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was not prepared.

4.1.15 *Sulfur-containing heterocyclic compounds*

Explanation

The Committee evaluated an additional 12 flavouring agents belonging to the group of sulfur-containing heterocyclic compounds. The additional flavouring agents comprised five thiophenes (Nos 2106, 2107 and 2110–2112), four thiazoles (Nos 2108, 2109, 2113 and 2114), one thiazoline (No. 2115), one dithiazine (No. 2116) and one thiophene-pyrimidine derivative (No. 2117). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee. One of the flavouring agents in this group—namely, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride (No. 2117)—is reported to be a flavour modifier.

The Committee evaluated 47 other members of this group of flavouring agents at its fifty-ninth and sixty-eighth meetings and concluded that none would give rise to safety concerns based on estimated dietary exposures (Annex 1, references *160* and *187*).

Eight of the 12 flavouring agents (Nos 2106–2111, 2113 and 2116) in this group have been reported to occur naturally in coffee, cocoa, pig liver, sheep liver, chicken, beef, lamb, shrimp, squid, trassi, beans, soya bean, onion, shallot, leek, asparagus, oats, peanut butter, potato, coriander seed and sweet corn.

Assessment of dietary exposure

The total annual volumes of production of the 12 sulfur-containing heterocyclic compounds are approximately 1000 kg in the USA, 1 kg in Europe and 1.5 kg in Japan. Approximately 99% of the total annual volume of production in the USA is accounted for by one flavouring agent in this group—namely, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride (No. 2117).

Dietary exposures were estimated using the MSDI method and the SPET, with the highest estimates reported in Table 17. The highest estimated daily dietary exposure is for 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride (No. 2117) (4500 μg, the SPET value from sugar substitutes). For the other flavouring agents, the estimated daily dietary exposures range from 0.05 to 90 μg, with the SPET yielding the highest estimates.

Absorption, distribution, metabolism and elimination

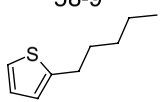
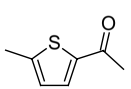
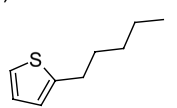
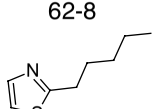
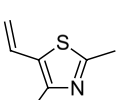
The metabolism of sulfur-containing heterocyclic compounds was described in the reports of the fifty-ninth and sixty-eighth meetings of the Committee (Annex 1, references *160* and *187*).

Thiophene derivatives are metabolized primarily by *S*-oxidation, followed by conjugation with glutathione. Thiazole and its derivatives are metabolized primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms. Dithiazine and thiazoline derivatives, being cyclic sulfides, are metabolized primarily by *S*-oxidation to yield corresponding sulfoxides and sulfones. Other routes of metabolism for sulfur-containing heterocyclic compounds, including ring oxidation and cleavage, are also possible.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

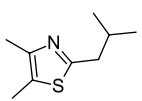
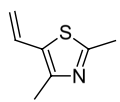
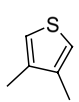
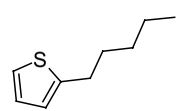
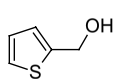
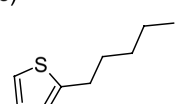
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the additional flavouring agents in this group, the Committee assigned four flavouring agents (Nos 2106–2109) to structural class II.

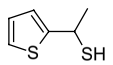
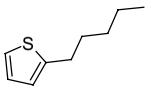
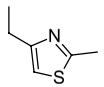
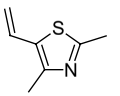
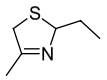
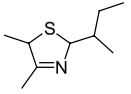
Table 17
Summary of the results of the safety evaluations of sulfur-containing heterocyclic compounds used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class II							
2-Pentylthiophene	2106	4861-58-9 	No, SPET: 40	B4: Yes. The NOEL of 3 mg/kg bw per day in a 28-day study in rats is 4500 times the estimated daily dietary exposure to No. 2106 when used as a flavouring agent.	Note 1		No safety concern
2-Acetyl-5-methylthiophene	2107	13679-74-8 	No, SPET: 0.3	B4: Yes. The NOEL of 3 mg/kg bw per day in a 28-day study in rats for the structurally related 2-pentylthiophene (No. 2106) is 600 000 times the estimated daily dietary exposure to No. 2107 when used as a flavouring agent.	Note 1	2-Pentylthiophene (No. 2106) 	No safety concern
2-Pentylthiazole	2108	37645-62-8 	No, SPET: 0.5	B4: Yes. The NOEL of 0.92 mg/kg bw per day in a 90-day study in rats for the structurally related 2,4-dimethyl-5-vinylthiazole (No. 1039) is 110 000 times the estimated daily dietary exposure to No. 2108 when used as a flavouring agent.	Note 2	2,4-Dimethyl-5-vinylthiazole (No. 1039) 	No safety concern

continued

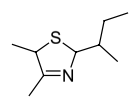
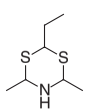
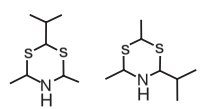
Table 17 (*continued*)

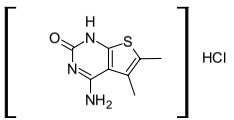
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
4,5-Dimethyl-2-isobutylthiazole	2109	53498-32-1 	No, SPET: 0.3	B4: Yes. The NOEL of 0.92 mg/kg bw per day in a 90-day study in rats for the structurally related 2,4-dimethyl-5-vinylthiazole (No. 1039) is 180 000 times the estimated daily dietary exposure to No. 2109 when used as a flavouring agent.	Note 2	2,4-Dimethyl-5-vinylthiazole (No. 1039) 	No safety concern
Structural class III							
3,4-Dimethylthiophene	2110	632-15-5 	No, SPET: 2	B4: Yes. The NOEL of 3 mg/kg bw per day in a 28-day study in rats for the structurally related 2-pentylthiophene (No. 2106) is 90 000 times the estimated daily dietary exposure to No. 2110 when used as a flavouring agent.	Note 1	2-Pentylthiophene (No. 2106) 	No safety concern
2-Thienylmethanol	2111	636-72-6 	No, SPET: 0.3	B4: Yes. The NOEL of 3 mg/kg bw per day in a 28-day study in rats for the structurally related 2-pentylthiophene (No. 2106) is 600 000 times the estimated daily dietary exposure to No. 2111 when used as a flavouring agent.	Note 1	2-Pentylthiophene (No. 2106) 	No safety concern

1-(2-Thienyl)-ethanethiol	2112	94089-02-8 	No, SPET: 0.05	B4: Yes. The NOEL of 3 mg/kg bw per day in a 28-day study in rats for the structurally related 2-pentylthiophene (No. 2106) is 3.6 million times the estimated daily dietary exposure to No. 2112 when used as a flavouring agent.	Notes 1 and 3	2-Pentylthiophene (No. 2106) 	No safety concern
5-Ethyl-2-methylthiazole	2113	19961-52-5 	No, SPET: 2	B4: Yes. The NOEL of 0.92 mg/kg bw per day in a 90-day study in rats for the structurally related 2,4-dimethyl-5-vinylthiazole (No. 1039) is 28 000 times the estimated daily dietary exposure to No. 2113 when used as a flavouring agent.	Note 2	2,4-Dimethyl-5-vinylthiazole (No. 1039) 	No safety concern
2-Ethyl-2,5-dihydro-4-methylthiazole	2114	41803-21-8 	No, SPET: 90	B4: Yes. The NOEL of 1.2 mg/kg bw per day in a 90-day study in rats for the structurally related 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) is 800 times the estimated daily dietary exposure to No. 2114 when used as a flavouring agent. The margin of exposure calculated using the MSDI of 0.01 µg/day is 7.2 million.	Note 4	2-(2-Butyl)-4,5-dimethyl-3-thiazoline (No. 1059) 	No safety concern

continued

Table 17 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
4-Methyl-3-thiazoline	2115	52558-99-3 	No, SPET: 4	B4: Yes. The NOEL of 1.2 mg/kg bw per day in a 90-day study in rats for the structurally related 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) is 18 000 times the estimated daily dietary exposure to No. 2115 when used as a flavouring agent.	Note 4	2-(2-Butyl)-4,5-dimethyl-3-thiazoline (No. 1059) 	No safety concern
2-Ethyl-4,6-dimethyldihydro-1,3,5-dithiazine	2116	54717-14-5 	No, SPET: 9	B4: Yes. The NOEL of 11 mg/kg bw per day in a 14-day study in rats for the structurally related mixture of 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1047) is 73 000 times the estimated daily dietary exposure to No. 2116 when used as a flavouring agent.	Note 4	Mixture of of 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1047)  27% 44%	No safety concern

4-Amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride 	2117	1033366-59-4	Yes, SPET: 4500	Yes. In a 90-day rat study, the NOAEL of 60 mg/kg bw per day provides margins of exposure of 800 in relation to the dietary exposure calculated using the SPET value (4500 µg/day) and 33 000 using the MSDI (110 µg/day) when No. 2117 is used as a flavouring agent.	Note 1	No safety concern

CAS, Chemical Abstracts Service

[a] Forty-seven flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *160* and *187*).

[b] *Step 1*: Four flavouring agents (Nos 2106–2109) are in structural class II, and eight flavouring agents (Nos 2110–2117) are in structural class III.

[c] *Step 2*: None of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds of human dietary exposure for structural classes II and III are 540 and 90 µg/person per day, respectively. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET or the MSDI method. The highest estimates were all derived using the SPET.

[e] The margins of exposure were calculated based on the estimated dietary exposures calculated by the SPET. In cases where the resulting margin of exposure was relatively low, a comparison with the MSDI was also made.

Notes:

1. Thiophene derivatives are metabolized primarily by *S*-oxidation, followed by conjugation with glutathione.
2. Thiazole and its derivatives are metabolized primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms.
3. The thiol group can undergo *S*-oxidation followed by conjugation with glutathione or may form mixed disulfides.
4. Dithiazine and thiazoline derivatives, being cyclic sulfides, are metabolized primarily by *S*-oxidation to yield the corresponding sulfoxides and sulfones.

The remaining eight flavouring agents (Nos 2110–2117) were assigned to structural class III (*10*).

Step 2. None of the flavouring agents in this group are predicted to be metabolized to innocuous products. Therefore, the evaluation of these flavouring agents proceeded via the B-side of the Procedure.

Step B3. The highest dietary exposure for one flavouring agent (No. 2117) is above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, for this flavouring agent, data are required on the flavouring agent or a closely related substance in order to perform a safety evaluation. For the remaining 11 flavouring agents, the dietary exposures are below the thresholds of concern (i.e. 540 μg/person per day for class II and 90 μg/person per day for class III). Accordingly, evaluation of these flavouring agents proceeded to step B4.

Step B4. For 2-pentylthiophene (No. 2106), available data give a NOEL of 3 mg/kg bw per day from a 28-day study in rats. This provides a margin of exposure of 4500 in relation to the dietary exposure to No. 2106 (SPET = 40 μg/day) when used as a flavouring agent.

For 2-acetyl-5-methylthiophene (No. 2107), 3,4-dimethylthiophene (No. 2110), 2-thienylmethanol (No. 2111) and 1-(2-thienyl)ethanethiol (No. 2112), the NOEL of 3 mg/kg bw per day for the structurally related 2-pentylthiophene (No. 2106) provides respective margins of exposure of 600 000, 90 000, 600 000 and 3.6 million in relation to the dietary exposures to No. 2107 (SPET = 0.3 μg/day), No. 2110 (SPET = 2 μg/day), No. 2111 (SPET = 0.3 μg/day) and No. 2112 (SPET = 0.05 μg/day) when used as flavouring agents.

For 2-pentylthiazole (No. 2108), 4,5-dimethyl-2-isobutylthiazole (No. 2109) and 5-ethyl-2-methylthiazole (No. 2113), available data on the structurally related 2,4-dimethyl-5-vinylthiazole (No. 1039) give a NOEL of 0.92 mg/kg bw per day from a 90-day study in rats. This provides respective margins of exposure of 110 000, 180 000 and 28 000 in relation to the dietary exposures to No. 2108 (SPET = 0.5 μg/day), No. 2109 (SPET = 0.3 μg/day) and No. 2113 (SPET = 2 μg/day) when used as flavouring agents.

For 2-ethyl-2,5-dihydro-4-methylthiazoline (No. 2114) and 4-methyl-3-thiazoline (No. 2115), available data on the structurally related 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) give a NOEL of 1.2 mg/kg bw per day from a 90-day study in rats. This provides respective margins of exposure of 800 and 18 000 in relation to the dietary exposures to No. 2114 (SPET = 90 μg/day) and No. 2115 (SPET = 4 μg/day) when used as flavouring agents. For No. 2114, the margin of exposure calculated using the MSDI of 0.01 μg/day is 7.2 million.

For 2-ethyl-4,6-dimethyldihydro-1,3,5-dithiazine (No. 2116)[3], available data on the structurally related 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture of isomers; No. 1047) give a NOEL of 11 mg/kg bw per day from a 14-day study in rats. This provides a margin of exposure of 73 000 in relation to the dietary exposure to No. 2116 (SPET = 9 μg/day) when used as a flavouring agent.

Consideration of the flavouring agent with high exposure evaluated via the B-side of the decision-tree:

Short-term toxicity data are available on 4-amino-5,6-dimethylthieno[2,3-d]-pyrimidin-2(1H)-one hydrochloride (No. 2117). In a 90-day rat study, the NOAEL of 60 mg/kg bw per day provides margins of exposure of approximately 800 in relation to the dietary exposure calculated using the SPET (4500 μg/day) and 33 000 in relation to the MSDI (110 μg/day) when No. 2117 is used as a flavouring agent. Therefore, No. 2117 is not considered to pose a safety concern at current estimated dietary exposure.

The Committee therefore concluded that none of the 12 additional flavouring agents (Nos 2106–2117) belonging to the group of sulfur-containing heterocyclic compounds would pose a safety concern at current estimated dietary exposures. Table 17 summarizes the evaluations of these additional flavouring agents.

Consideration of combined intakes from use as flavouring agents

The highest MSDI for members of the current group of flavouring agents is 110 μg/day (No. 2117). No. 2117 does not share a close structural relationship with any other members of the current group or with those members evaluated previously. The MSDI values of the remaining members of the current group are negligible (≤0.2 μg/day). Combined intakes are therefore not a safety concern.

Consideration of secondary components

Two flavouring agents in this group (Nos 2114 and 2116) have minimum assay values of less than 95% (Annex 4). The secondary components of 2-ethyl-2,5-dihydro-4-methylthiazole (No. 2114) are 2-ethyl-4-methyl-4,5-dihydrothiazole-4-ol (2–3%), 3,4-dimethylthiophene (2–3%) and 2-ethyl-4-methylthiazole (2–3%). 3,4-Dimethylthiophene (No. 2110) is a member of the current group. 2-Ethyl-4-methylthiazole (No. 1044) was evaluated at the fifty-ninth meeting (Annex 1, reference *160*) and concluded to be of no safety concern

[3] The Committee noted that No. 2116 was originally submitted as a mixture of two isomers, but that only one of the two isomers is in commercial use.

at estimated dietary exposures when used as a flavouring agent. 2-Ethyl-4-methyl-4,5-dihydrothiazole-4-ol is anticipated to undergo further oxidative metabolism and/or conjugate formation with subsequent elimination in urine. It does not present a safety concern at current estimated dietary exposures.

The secondary components of 2-ethyl-4,6-dimethyldihydro-1,3,5-dithiazine (No. 2116)—namely, 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine (No. 1049) and 3,5-diethyl-1,2,4-trithiolane (No. 1686)—were evaluated at the fifty-ninth and sixty-eighth meetings of the Committee, respectively (Annex 1, references *160* and *187*), and concluded to be of no safety concern at estimated dietary exposures.

Conclusion

In the previous evaluations of members of this group, studies of acute toxicity, short-term studies of toxicity, and studies of genotoxicity and reproductive and developmental toxicity were available. The toxicity data available for this evaluation supported the previous evaluations.

The Committee concluded that none of the 12 flavouring agents evaluated at the present meeting, which are additions to the group of sulfur-containing heterocyclic compounds evaluated previously, raise any safety concerns at current estimated dietary exposures.

An addendum to the toxicological monograph was prepared.

4.1.16 *Sulfur-substituted furan derivatives*

Explanation

The Committee evaluated a group of nine flavouring agents belonging to the group of sulfur-substituted furan derivatives. The additional flavouring agents included one furfuryl mercaptan (No. 2090), two furyl disulfides (Nos 2091 and 2092), one tetrahydrofurfuryl thiol (No. 2093), two furfuryl thioethers (Nos 2094 and 2096), one furyl thioether (No. 2095), one dihydrofuryl thiol (No. 2097) and one tetrahydrofuryl thioester (No. 2098). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (see Figure 1) (Annex 1, reference *131*). None of these flavouring agents have previously been evaluated by the Committee.

The Committee evaluated 33 other members of this group of flavouring agents at the fifty-ninth meeting (Annex 1, reference *160*). It was concluded that none of the 33 flavouring agents would give rise to safety concerns based on estimated dietary exposures.

One of the nine flavouring agents, 5-methylfurfuryl mercaptan (No. 2090), considered at the current meeting has been reported to occur naturally in food and has been detected in coffee and roasted sesame (*35*, *36*).

Assessment of dietary exposure

The total annual volumes of production of the sulfur-substituted furan derivatives are approximately 11 kg in the USA, 52 kg in Europe and 5 kg in Japan. Approximately 95% of the volume of production reported in Europe is from one substance, 2-methyl-4,5-dihydrofuran-3-thiol (No. 2097). Additionally, approximately 95% of the reported volume of production in the USA is from 3-[(2-methyl-3-furyl)thio]butanal (No. 2095), while in Japan, 99% of the reported volume of production is from 2-tetrahydrofurfuryl 2-mercaptopropionate (No. 2093).

Dietary exposures were estimated using the MSDI method and the SPET, and the highest estimates are reported in Table 18. The estimated daily dietary exposure is highest for 1-(2-furfurylthio)-propanone (No. 2096) (600 µg, the SPET value obtained from instant coffee and tea). For the other flavouring agents, the estimated daily dietary exposures range from 0.01 to 200 µg, with the SPET yielding the highest estimates except for No. 2095, for which the MSDI estimate was higher.

Annual volumes of production of this group of flavouring agents as well as the daily dietary exposures calculated using both the MSDI method and the SPET are summarized in Table 19.

Absorption, distribution, metabolism and elimination

At the fifty-ninth meeting of the Committee, no metabolism data were available for the 33 sulfur-substituted furan derivatives evaluated (Annex 1, reference *160*). Because all members of the group contain a reactive divalent sulfur atom attached to the heteroaromatic or heterocyclic ring, these flavouring agents were predicted to be metabolized via reactions of the divalent sulfur.

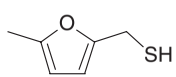
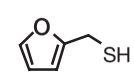
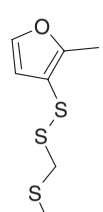
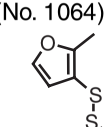
Published metabolism data were provided for the current meeting on the previously evaluated flavouring agents 2-methyl-3-furanthiol (No. 1060) and furfuryl mercaptan (No. 1072). Both flavouring agents were shown to be metabolized in vitro to their thiofuran methyl sulfide derivatives, which were subsequently *S*-oxidized to form thiofuran methyl sulfoxides (*37*).

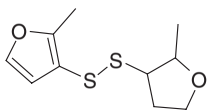
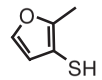
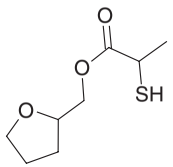
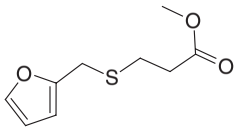
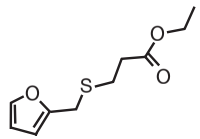
No data were provided on the nine additional sulfur-substituted furan derivatives evaluated by the Committee at its current meeting. Each of these flavouring agents contains one or more divalent sulfur atoms and is predicted to be metabolized via reactions of the divalent sulfur plus hydrolysis of ester groups and oxidation of aldehyde groups.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to these nine flavouring agents, the Committee assigned all of the flavouring agents to structural class III (*10*).

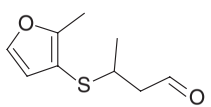
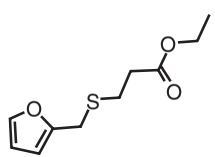
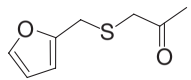
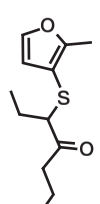
Table 18
Summary of the results of the safety evaluations of sulfur-substituted furan derivatives used as flavouring agents[a,b,c]

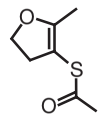
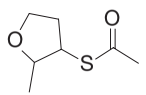
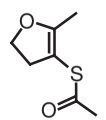
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
Structural class III							
5-Methylfurfuryl mercaptan	2090	59303-05-8 	No, SPET: 1.5	B4: Yes. The NOEL of 3 mg/kg bw per day in a 90-day study in rats for the structurally related furfuryl mercaptan (No. 1072) (*38*) is 120 000 times the estimated daily dietary exposure to No. 2090 when used as a flavouring agent.	Note 1	Furfuryl mercaptan (No. 1072) 	No safety concern
2-Methyl-3-furyl methylthiomethyl disulfide	2091	333384-99-9 	No, SPET: 0.6	B4: Yes. The NOEL of 1.2 mg/kg bw per day in a 90-day study in rats for the structurally related methyl 2-methyl-3-furyl disulfide (No. 1064) (*39*) is 120 000 times the estimated daily dietary exposure to No. 2091 when used as a flavouring agent.	Note 1	Methyl 2-methyl-3-furyl disulfide (No. 1064) 	No safety concern

2-Methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulfide	2092	252736-40-6 	No, SPET: 20	B4: Yes. The NOEL of 5 mg/kg bw per day in a 90-day study in rats for the structurally related 2-methyl-3-furanthiol (No. 1060) (*40*) is 15 000 times the estimated daily dietary exposure to No. 2092 when used as a flavouring agent.	Note 1	2-Methyl-3-furanthiol (No. 1060) 	No safety concern
2-Tetrahydrofurfuryl 2-mercaptopropionate	2093	99253-91-5 	Yes, SPET: 200	Additional data are not available.	Note 2		Additional data required to complete evaluation
Methyl 3-(furfurylthio)-propionate	2094	94278-26-9 	No, SPET: 5	B4: Yes. The NOEL of 17 mg/kg bw per day in a 90-day study in rats for the structurally related ethyl 3-(furfurylthio)propionate (No. 1088) (*41*) is 200 000 times the estimated daily dietary exposure to No. 2094 when used as a flavouring agent.	Note 1	Ethyl 3-(furfurylthio)-propionate (No. 1088) 	No safety concern

continued

Table 18 (*continued*)

Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3*[e] Are additional data available for flavouring agent with an estimated dietary exposure exceeding the threshold of concern? *Step B4*[e] Adequate margin of exposure for the flavouring agent or a related substance?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
3-[(2-Methyl-3-furyl)-thio]butanal	2095	915971-43-6 	No, MSDI: 1.1	B4: Yes. The NOEL of 17 mg/kg bw per day in a 90-day study in rats for the structurally related ethyl 3-(furfurylthio)propionate (No. 1088) (*41*) is 900 000 times the estimated daily dietary exposure to No. 2095 when used as a flavouring agent.	Note 1	Ethyl 3-(furfurylthio)-propionate (No. 1088) 	No safety concern
1-(2-Furfurylthio)-propanone	2096	58066-86-7 	Yes, SPET: 600	The NOEL of 3.8 mg/kg bw per day in a 90-day study in rats for the structurally related 3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1085) (*43*) is 380 times the SPET estimate (600 µg/day) and greater than 20 million times the MSDI estimate (0.01 µg/day) of dietary exposure to No. 2096 when used as a flavouring agent.	Note 1	3-[(2-Methyl-3-furyl)thio]-4-heptanone (No. 1085) 	No safety concern

2-Methyl-4,5-dihydrofuran-3-thiol 	2097	26486-13-5	Yes, SPET: 115	The NOEL of 8.3 mg/kg bw per day in a 1-year study in rats for the structurally related 2-methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089) (*42*) is 4300 times the estimated daily dietary exposure to No. 2097 when used as a flavouring agent.	Note 1	2-Methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089) 	No safety concern
2-Methyltetrahydrofuran-3-thiol acetate 	2098	252736-41-7	No, SPET: 5	B4: Yes. The NOEL of 8.3 mg/kg bw per day in a 1-year study in rats for the structurally related 2-methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089) (*42*) is 100 000 times the estimated daily dietary exposure to No. 2098 when used as a flavouring agent.	Note 1	2-Methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089) 	No safety concern

CAS, Chemical Abstracts Service

[a] Thirty-three flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *160*).

[b] *Step 1*: All nine flavouring agents in this group are in structural class III (*10*).

[c] *Step 2*: None of the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The threshold for human dietary exposure for structural class III is 90 µg/day. All dietary exposure values are expressed in µg/day. The dietary exposure value listed represents the highest estimated dietary exposure calculated using either the SPET (Nos 2090–2094, 2096–2098) or the MSDI method (No. 2095). See Table 19 for all values.

[e] The margins of exposure were calculated based on the highest estimated dietary exposure calculated using either the SPET or the MSDI method. In cases where the resulting margin of exposure calculated using the SPET value was relatively low, a comparison with the MSDI was also made.

Notes:

1. Metabolism is predicted to occur by biotransformation of the divalent sulfur by oxidation to unstable sulfenic acids (RSOH), which are further oxidized to the corresponding sulfinic (RSO_2H) and sulfonic acids (RSO_3H). Biotransformation by *S*-methylation to the corresponding sulfides may occur, which are then readily oxidized to sulfoxides and sulfones. Thiols may form mixed disulfides or conjugate with glucuronic acid or be directly excreted in the urine unchanged. Disulfides may be reduced to corresponding thiols.
2. 2-Tetrahydrofurfuryl 2-mercaptopropionate would be expected to be hydrolysed to tetrahydrofurfuryl alcohol, which would be expected to be oxidized to the corresponding carboxylic acid, conjugated with glucuronic acid and excreted in the urine.

Table 19
Annual volumes of production and daily dietary exposures for sulfur-substituted furan derivatives used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Natural occurrence in foods
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
5-Methylfurfuryl mercaptan (2090)				1.5	0.03	+[d]
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
2-Methyl-3-furyl methylthiomethyl disulfide (2091)				0.6	0.01	–
Europe	ND	ND	ND			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
2-Methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulfide (2092)				20	0.3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	0.2	0.1	0.001			
2-Tetrahydrofurfuryl 2-mercaptopropionate (2093)				200	3	–
Europe	ND	ND	ND			
USA	ND	ND	ND			
Japan	5	1.4	0.02			
Methyl 3-(furfurylthio)propionate (2094)				5	0.1	–
Europe	2	0.2	0.003			
USA	0.2	0.02	0.0004			
Japan	0.2	0.1	0.001			
3-[(2-Methyl-3-furyl)thio]butanal (2095)				0.6	0.01	–
Europe	ND	ND	ND			
USA	10	1.1	0.02			
Japan	ND	ND	ND			
1-(2-Furfurylthio)-propanone (2096)				600	10	–
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			

Table 19 (*continued*)

Flavouring agent (No.)	Most recent annual volume of production (kg)[a]	Dietary exposure				Natural occurrence in foods
		MSDI[b]		SPET[c]		
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	
2-Methyl-4,5-dihydrofuran-3-thiol (2097)				115	2	–
Europe	50	5	0.09			
USA	ND	ND	ND			
Japan	ND	ND	ND			
(±)-2-Methyltetrahydrofuran-3-thiol acetate (2098)				5	0.1	–
Europe	0.1	0.01	0.0002			
USA	0.1	0.01	0.0002			
Japan	ND	ND	ND			
Total						
Europe	52					
USA	11					
Japan	5.4					

ND, no data reported; +, reported to occur naturally in foods, but no quantitative data; –, not reported to occur naturally in foods

[a] From references *16–19*. Values greater than 0 kg but less than 0.1 kg were reported as 0.1 kg.

[b] MSDI (µg/person per day) calculated as follows:
(annual volume, kg) × (1×10^9 µg/kg)/(population × survey correction factor × 365 days), where population (10%, "eaters only") = 32×10^6 for Europe, 31×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys in Europe, the USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*16–19*).
MSDI (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] SPET (µg/person per day) calculated as follows:
(standard food portion, g/day) × (average use level) (*19*). The dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate.
SPET (µg/kg bw per day) calculated as follows:
(µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[d] Reported to occur naturally in food (*35*, *36*).

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

Step B3. The highest estimated dietary exposures for six of the flavouring agents (Nos 2090–2092, 2094, 2095 and 2098) are below the threshold of concern (i.e. 90 µg/person per day for class III). Accordingly, the evaluation of these six flavouring agents proceeded to step B4.

The highest estimated dietary exposures for three of the flavouring agents (Nos 2093, 2096 and 2097) are above the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, for these three flavouring agents, data are required on the flavouring agent or a closely related substance in order to perform a safety evaluation.

Step B4. For 5-methylfurfuryl mercaptan (No. 2090), available data on the structurally related furfuryl mercaptan (No. 1072) from a 90-day study in rats give a NOEL of 3 mg/kg bw per day (*38*). This provides an adequate margin of exposure of 120 000 in relation to the highest estimated dietary exposure to No. 2090 (SPET = 1.5 μg/day) when used as a flavouring agent.

For 2-methyl-3-furyl methylthiomethyl disulfide (No. 2091), available data on the structurally related methyl 2-methyl-3-furyl disulfide (No. 1064) from a 90-day study in rats give a NOEL of 1.2 mg/kg bw per day (*39*). This provides an adequate margin of exposure of 120 000 in relation to the highest estimated dietary exposure to No. 2091 (SPET = 0.6 μg/day) when used as a flavouring agent.

For 2-methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulfide (No. 2092), available data on the structurally related 2-methyl-3-furanthiol (No. 1060) from a 90-day study in rats give a NOEL of 5 mg/kg bw per day (*40*). This provides an adequate margin of exposure of 15 000 in relation to the highest estimated dietary exposure to No. 2092 (SPET = 20 μg/day) when used as a flavouring agent.

For methyl 3-(furfurylthio)propionate (No. 2094) and 3-[(2-methyl-3-furyl)thio]butanal (No. 2095), available data on the structurally related ethyl 3-(furfurylthio)propionate (No. 1088) from a 90-day study in rats give a NOEL of 17 mg/kg bw per day (*41*). This provides adequate margins of exposure of 200 000 and 900 000, respectively, in relation to the highest estimated dietary exposures to No. 2094 (SPET = 5 μg/day) and No. 2095 (MSDI = 1.1 μg/day) when used as flavouring agents.

For 2-methyltetrahydrofuran-3-thiol acetate (No. 2098), available data on the structurally related 2-methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089) from a 1-year study in rats give a NOEL of 8.3 mg/kg bw per day (*42*). This provides an adequate margin of exposure of 100 000 in relation to the highest estimated dietary exposure to No. 2098 (SPET = 5 μg/day) when used as a flavouring agent.

Consideration of flavouring agents with high exposure evaluated via the B-side of the decision-tree:

For 2-tetrahydrofurfuryl 2-mercaptopropionate (No. 2093), a NOEL for this flavouring agent or a structurally related substance was not available. Therefore, for this flavouring agent, the Committee concluded that additional data would be necessary to complete the safety evaluation.

For 1-(2-furfurylthio)-propanone (No. 2096), available data on the structurally related 3-[(2-methyl-3-furyl)thio]-4-heptanone (No. 1085) from a 90-day study in rats give a NOEL of 3.8 mg/kg bw per day (*43*). This provides a margin of exposure of 380 in relation to the SPET (600 μg/day) and greater than 20 million in relation to the MSDI (0.01 μg/day) when No. 2096 is used as a flavouring agent.

For 2-methyl-4,5-dihydrofuran-3-thiol (No. 2097), available data on the structurally related 2-methyl-3-thioacetoxy-4,5-dihydrofuran (No. 1089), which is predicted to be hydrolysed to 2-methyl-4,5-dihydrofuran-3-thiol, from a 1-year study in rats give a NOEL of 8.3 mg/kg bw per day (*42*). This provides an adequate margin of exposure of 4300 in relation to the highest estimated dietary exposure to No. 2097 (SPET = 115 μg/day) when used as a flavouring agent.

Table 18 summarizes the evaluations of the nine sulfur-substituted furan derivatives in this group (Nos 2090–2098).

Consideration of combined intakes from use as flavouring agents

The highest MSDI values for members of the current group are 5 μg/day (No. 2097), 1.4 μg/day (No. 2093) and 1.1 μg/day (No. 2095). The dietary exposures to the flavouring agents evaluated at the present meeting would not contribute significantly to the combined intakes considered at the fifty-ninth meeting.

Consideration of secondary components

One flavouring agent in this group, 2-methyl-4,5-dihydrofuran-3-thiol (No. 2097), has a minimum assay value of less than 95% (named material 55%) (Annex 4). The secondary components of 2-methyl-4,5-dihydrofuran-3-thiol (No. 2097)—namely, 2-methyl-3-furanthiol (No. 1060) (5–7%) and 2-methyl-3-tetrahydrofuranthiol (No. 1090) (35–40%)—were evaluated by the Committee at its fifty-ninth meeting and were concluded to be of no safety concern at estimated dietary exposures (Annex 1, reference *160*).

Conclusion

In the previous evaluation of sulfur-substituted furan derivatives, studies of acute toxicity and short-term studies of toxicity were available. Results from the short-term studies of toxicity were used to support the safety evaluations of eight of the nine additional members of this group considered at the present meeting.

The Committee concluded that eight of the nine flavouring agents evaluated at the present meeting, which are additions to the group of sulfur-substituted furan derivatives evaluated previously, do not raise any safety concerns at

current estimated dietary exposures. For the remaining flavouring agent (No. 2093), no appropriate toxicity data were available on the flavouring agent or a closely related substance. The Committee concluded that for this flavouring agent, additional data would be required to complete the safety evaluation.

An addendum to the toxicological monograph was not prepared.

4.2 Specifications of identity and purity of flavouring agents

4.2.1 *New specifications*

The Committee received information related to specifications for the 111 new flavouring agents from the call for data for the present meeting. Specifications were prepared for 107 flavouring agents. At the current meeting, no specifications were prepared for four substances (Nos 2069, 2124, 2168 and 2169). Specifications for the flavouring agents Nos 2153 and 2154 were made tentative pending the submission of further information (see Annex 3). The specifications prepared for 18 flavouring agents—No. 2137 (see section 4.1.2); Nos 2103–2105 (see section 4.1.3); Nos 2147–2149 (see section 4.1.6); Nos 2099–2102 (see section 4.1.7); Nos 2150, 2152, 2156 and 2158–2160 (see section 4.1.12); and No. 2093 (see section 4.1.16)—include a statement that the safety evaluations for these flavouring agents had not been completed. The evaluation for No. 2151 is temporary pending receipt of additional toxicological data (see section 4.1.12).

The Committee noted that the Flavor and Extract Manufacturers Association of the United States (FEMA) number provided for No. 2116 encompasses two substances, but only one of the substances was evaluated for safety and specifications at the current meeting. As a result, a FEMA number was not included in the specifications monograph for No. 2116.

5. Future work

Food additives (other than flavouring agents)

Food additive specifications

For the revision of the specification for paprika extract, no data were received, and, based on the commitment by the sponsor to provide data for a future meeting, this evaluation was postponed.

With respect to the introduction of a method based on ICP-AES in the specifications monograph of magnesium dihydrogen diphosphate to replace the existing method for the determination of phosphorus as phosphorus pentoxide, the Committee may consider replacing corresponding methods for other diphosphate additives at a future meeting.

The Committee noted that the current specifications monograph for titanium dioxide has a provision for aluminium oxide, up to a maximum level of 2%. Consequently, the Committee may consider inclusion of titanium dioxide in evaluations of the dietary exposure to aluminium.

Food additives containing aluminium and/or silicon

The Committee, while reviewing the specifications of food additives containing aluminium and silicon, considered it relevant to update the test methods for the determination of aluminium oxide and silicon dioxide. Some of the test methods for the food additives listed in section 2.5.3 use potentially corrosive or hazardous reagents that are not always permitted in current laboratory practices because of safety concerns. The Committee also noted that the specifications of some additives were rather old or tentative and that it requires additional information to revise the specifications. Consequently, the Committee recommends placing these additives on the agenda for re-evaluation.

Food additives containing magnesium and phosphate

The Committee noted that an ADI "not specified" has been allocated individually to a number of magnesium-containing food additives and recommended that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed.

The information submitted to the Committee for its evaluation of the food additive magnesium dihydrogen diphosphate and in the scientific literature did not indicate that the MTDI of 70 mg/kg bw for phosphate salts, expressed as phosphorus, is insufficiently health protective. On the contrary, because the basis for its derivation might not be relevant to humans, it could be overly conservative. Therefore, there is a need to review the toxicological basis of the MTDI for phosphate salts expressed as phosphorus.

Flavouring agents

Specifications for flavouring agents

The specifications for Nos 2153 (2-acetyl-4-isopropenylpyridine) and 2154 (4-acetyl-2-isopropenylpyridine) were made tentative at the current meeting because the submitted information was insufficient. The two flavouring agents are positional isomers, and the Committee concluded that the current specifications would not allow for differentiation between the two substances. Information that could be used to differentiate between the two substances (e.g. optical [specific] rotation) is requested.

Aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

The Committee concluded that the Procedure could not be applied to this group because of unresolved toxicological concerns. Studies that could assist in the safety evaluation include investigations of the influence of the nature and position of furan ring substitutions on metabolism and covalent binding to macromolecules, demonstration of the ring opening and reactivity of the resulting products. Depending on the findings, additional genotoxicity or other studies might be needed.

Furfuryl alcohol and related substances

New in vitro and in vivo studies raise concerns regarding the potential genotoxicity of furfuryl alcohol and derivatives that can be metabolized to furfuryl alcohol (e.g. furfuryl esters). The Committee concluded that this group of flavouring agents could not be evaluated according to the Procedure because of the unresolved concerns regarding genotoxicity. In addition, the group ADI previously established by the Committee will need to be reconsidered at a future meeting.

Pyridine, pyrrole and quinoline derivatives

For 2,4-dimethylpyridine (No. 2151), the safety evaluation was made temporary, pending the submission of the full report of the critical study for the next JECFA meeting at which flavouring agents are evaluated.

Additional data required to complete the evaluation according to the Procedure for the Safety Evaluation of Flavouring Agents

Additional data are required to complete the toxicological evaluations of 11 flavouring agents (Nos 2093, 2137, 2147–2150, 2152, 2156 and 2158–2160).

Acknowledgements

The Committee wishes to thank Ms M. Sheffer, Ottawa, Canada, for her assistance in the preparation of the report.

References

1. **FAO/WHO.** *Joint FAO/WHO Conference on Food Additives*. Rome, Italy, Food and Agriculture Organization of the United Nations, 1956 (FAO Nutrition Meetings Report Series, No. 11); Geneva, Switzerland, World Health Organization, 1956 (WHO Technical Report Series, No. 107).
2. **FAO/WHO.** *Principles and methods for the risk assessment of chemicals in food*. Geneva, World Health Organization, 2009 (Environmental Health Criteria, No. 240; http://www.who.int/foodsafety/chem/principles/en/index1.html).
3. **FAO/WHO.** *Report of the Forty-third Session of the Codex Committee on Food Additives, Xiamen, China, 14–18 March 2011.* Rome, Italy, Food and Agriculture Organization of the United Nations, and Geneva, Switzerland, World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Codex Committee on Food Additives, 2011 (REP11/FA).
4. **Smith JH et al.** Ninety-day feeding study in Fischer-344 rats of highly refined petroleum-derived food-grade white oils and waxes. *Toxicologic Pathology*, 1996, 24:214–230.
5. **Cnubben NHP, van Stee LLP.** Single dose pharmacokinetic study of P15 white oil in two rat strains. Unpublished report no. V8503 from TNO, Zeist, the Netherlands. Submitted to WHO by Concawe, Brussels, Belgium, 2010.
6. **Boogaard PJ et al.** Comparative toxicokinetics of low-viscosity mineral oil in Fischer 344 rats, Sprague-Dawley rats and humans—Implications for an acceptable daily intake (ADI). *Regulatory Toxicology and Pharmacology*, 2012, 63:69–77.
7. **Bakker GCM.** Absorption and kinetics of P15H oil after single oral administration of P15H oil in human volunteers. Unpublished report no. V9051 from TNO, Zeist, the Netherlands. Submitted to WHO by Concawe, Brussels, Belgium, 2011.
8. **FAO/WHO.** *Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 22–25 January 2001.* Rome, Italy, Food and Agriculture Organization of the United Nations, 2001 (http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf).

9. **WHO/FAO/UNU.** *Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation, Geneva, 9–16 April 2002.* Geneva, World Health Organization, 2007 (WHO Technical Report Series, No. 935; http://whqlibdoc.who.int/trs/WHO_TRS_935_eng.pdf).
10. **Cramer GM, Ford RA, Hall RL.** Estimation of toxic hazard—a decision tree approach. *Food and Cosmetics Toxicology*, 1978, 16:255–276.
11. **Jeffrey AM.** Comparison of furan and 2-pentylfuran genotoxicity measured by in vivo comet assays in mouse liver. Unpublished report no. NYMC-CSL 10–72 from New York Medical College, Valhalla, NY, USA. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2012.
12. **Weyerstahl P et al.** Constituents of the essential oil from the fruits of *Zanthoxylum rhetsoides* Drake from Vietnam and from the aerial parts of *Zanthoxylum alatum* Roxb. from India. *Flavour and Fragrance Journal*, 1999, 14:225–229.
13. **Nijssen LM, van Ingen-Visscher CA, Donders JJH.** Volatile Compounds in Food (VCF) database, version 13.1. Zeist, the Netherlands, TNO Triskelion, 2011 (http://www.vcf-online.nl/VcfHome.cfm, accessed November 2011).
14. **Chen Y, Wei J, Liang N.** [Volatile constituents of fresh yushen (*Codonopsis pilosula* root).] *Zhongguo Zhong Yao Za Zhi*, 1993, 18:492–493 (in Chinese).
15. **Ramarathnam N, Rubin LJ, Diosady LL.** Studies on meat flavor. 2. A quantitative investigation of the volatile carbonyls and hydrocarbons in uncured and cured beef and chicken. *Journal of Agricultural and Food Chemistry*, 1991, 39:1839–1847.
16. **European Flavor and Fragrance Association.** European inquiry on volume use. Unpublished report. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2004.
17. **Japan Flavor and Fragrance Materials Association.** Japanese inquiry on volume use. Unpublished report. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2005.
18. **Gavin CL, Williams MC, Hallagan JB.** *2005 FEMA poundage and technical effects update survey.* Washington, DC, USA, Flavor and Extract Manufacturers Association of the United States, 2008.
19. **International Organization of the Flavor Industry.** Interim inquiry on volume use and added use levels for flavoring agents to be presented at the 76th JECFA meeting. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2011.
20. **Scheerbaum D.** Methylbuttersaeure-2 nat. Reverse mutation assay (Amestest) with *Salmonella typhimurium*. Unpublished report no. USO75263 from Dr U. Noack Laboratorium für Angewandte Biologie, Sarstedt, Germany. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2000.

21. **Richold M, Jones E.** Ames metabolic activation test to assess the potential mutagenic effect of verdinal. Unpublished report no. ULR/59/791231 from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 1980.

22. **King MT.** Mutagenicity study of tetrahydrogeraniol in the *Salmonella typhimurium*/mammalian microsome reverse mutation assay (Ames-test). Unpublished report no. AM07400N from Freiburger Labor für Mutagenitätsprüfung der King & Harnasch GmbH, Kirchzarten, Germany. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 2001.

23. **King MT, Harnasch G.** Mutagenicity study of Aldehyde C12 MNA in the *Salmonella typhimurium*/mammalian microsome reverse mutation assay (Ames-test). Unpublished report no. AM08399N from Freiburger Labor für Mutagenitätsprüfung der King & Harnasch GmbH, Kirchzarten, Germany. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 1999.

24. **Kusakabe H et al.** Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on the testing results of 98 high production volume industrial chemicals. *Mutation Research*, 2002, 517:187–198.

25. **Idstein H, Schreier P.** Volatile constituents from guava (*Psidium guajava*, L.) fruit. *Journal of Agricultural and Food Chemistry*, 1985, 33:138–143.

26. **Nakamura S et al.** Identification of volatile flavor components of the oil from roasted sesame seeds. *Agricultural and Biological Chemistry*, 1989, 53:1891–1899.

27. **Cox GE, Rucci G, Babish JG.** 90-day subacute dietary toxicity study of IFF code No. 78-002-2 in Sprague-Dawley rats. Unpublished report submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, by Food and Drug Research Laboratories, Inc., 1979 [cited in Annex 1, reference *144*].

28. **British Industrial Biological Research Association.** Short-term toxicity of samples TT171, TT172, TT173, and TT174 in rats. Unpublished study submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1976 [cited in Annex 1, reference *144*].

29. **Morgareidge K, Oser BL.** 90-day feeding studies in rats with cyclopentanethiol. Unpublished study submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1970 [cited in Annex 1, reference *144*].

30. **Morgareidge K.** 90-day feeding study with 2-keto-3-pentanethiol in rats. Unpublished study submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1971 [cited in Annex 1, reference *144*].

31. **Cox GE, Bailey DE, Morgareidge K.** 90-day feeding study in rats with compound 14935 (2-mercapto-3-butanol). Unpublished report submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, by Food and Drug Research Laboratories, Inc., 1974 [cited in Annex 1, reference *144*].

32. **Morgareidge K, Bailey DE, Cox GE.** 90-day feeding study in rats with compound 14966. Unpublished study submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1974 [cited in Annex 1, reference *144*].

33. **Morgareidge K, Bailey DE, Cox GE.** 90-day feeding study on 2,3-butanedithiol in rats. Unpublished study submitted to the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1974 [cited in Annex 1, reference *144*].

34. **Posternak JM, Linder A, Vodoz CA.** Summaries of toxicological data. Toxicological tests on flavoring matters. *Food and Cosmetics Toxicology*, 1969, 7:405–407 [cited in Annex 1, reference *144*].

35. **Schieberle P.** Odour-active compounds in moderately roasted sesame. *Food Chemistry*, 1996, 55(2):145–152.

36. **Tressl R, Silwar R.** Investigation of sulfur-containing components in roasted coffee. *Journal of Agricultural and Food Chemistry*, 1981, 29(5):1078–1082.

37. **Lake BG et al.** Studies on the metabolism of the thiofurans furfuryl mercaptan and 2-methyl-3-furanthiol in rat liver. *Food and Chemical Toxicology*, 2003, 41:1761–1770.

38. **Phillips JC et al.** Short-term toxicity of furfuryl mercaptan in rats. *Food and Cosmetics Toxicology*, 1977, 15:383–387.

39. **Gallo MA, Cox GE, Babish JG.** 90-day feeding studies in rats with compound 75-31065 (methyl benzyl disulfide file). Unpublished report no. 2689e from Food and Drug Research Laboratories, Inc. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 1976.

40. **Oser BL.** 90-day feeding studies with 2-methyl-3-furanthiol in rats. Unpublished report. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1970.

41. **Bio-Research Laboratories.** Ethyl-beta-furfural-alpha-thiopropionate. Unpublished report from Bio-Research Laboratories. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 1980.

42. **Munday R, Gellatly JB.** Biological evaluation of DUS-5. Part 7. 1-year test with DUS-5 in rats. Unpublished report. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA, 1974.

43. **Gallo MA, Cox GE, Babish JG.** 90-day feeding study in rats with compound 75-15963 (3-[(2-methyl-3-furyl)-thio]-4-heptanone). Unpublished report no. 2689d from Food and Drug Research Laboratories, Inc. Submitted to WHO by the International Organization of the Flavor Industry, Brussels, Belgium, 1976.

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 Agriculture 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 Additives 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, 2000.

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, No. 896, 2000.

144. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 44, 2000.

145. *Compendium of food additive specifications, addendum 7.* FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.

146. *Evaluation of certain veterinary drug residues in food* (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.

147. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 45, 2000.

148. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/13, 2000.

149. *Evaluation of certain food additives and contaminants* (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 901, 2001.

150. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 46, 2001.

151. *Compendium of food additive specifications: addendum 8.* FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.

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, 2002.

155. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 48, 2002.

156. *Compendium of food additive specifications: addendum 9.* FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.

157. *Evaluation of certain veterinary drug residues in food* (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.

158. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 49, 2002.

159. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/14, 2002.

160. *Evaluation of certain food additives and contaminants* (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.

161. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 50, 2003.

162. *Compendium of food additive specifications: addendum 10*. FAO Food and Nutrition Paper, No. 52, Add. 10, 2002.

163. *Evaluation of certain veterinary drug residues in food* (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.

164. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 51, 2003.

165. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/15, 2003.

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. *Compendium of food additive specifications: addendum 13.* FAO Food and Nutrition Paper, No. 52, Add. 13 (with Errata), 2005.

176. *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.

177. *Safety evaluation of certain contaminants in food*. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.

178. *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.

179. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 56, 2006.

180. *Combined compendium of food additive specifications.* FAO JECFA Monographs 1, Volumes 1–4, 2005, 2006.

181. *Evaluation of certain veterinary drug residues in food* (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.

182. *Residue evaluation of certain veterinary drugs.* FAO JECFA Monographs 2, 2006.

183. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 57, 2006.

184. *Evaluation of certain food additives and contaminants* (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.

185. *Compendium of food additive specifications.* FAO JECFA Monographs 3, 2006.

186. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 58, 2007.

187. *Evaluation of certain food additives and contaminants* (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 59, 2008.

189. *Compendium of food additive specifications.* FAO JECFA Monographs 4, 2007.

190. *Evaluation of certain food additives* (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.

191. *Safety evaluation of certain food additives*. WHO Food Additives Series, No. 60, 2009.

192. *Compendium of food additive specifications.* FAO JECFA Monographs 5, 2009.

193. *Evaluation of certain veterinary drug residues in food* (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.

194. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 61, 2009.

195. *Residue evaluation of certain veterinary drugs*. FAO JECFA Monographs 6, 2009.

196. *Evaluation of certain food additives* (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.

197. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 62, 2010.

198. *Compendium of food additive specifications.* FAO JECFA Monographs 7, 2009.

199. *Evaluation of certain contaminants in food* (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.

200. *Safety evaluation of certain contaminants in food.* WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.

201. *Residue evaluation of certain veterinary drugs*. FAO JECFA Monographs 9, 2010.

202. *Evaluation of certain food additives and contaminants* (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.

203. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 64, 2011.

204. *Compendium of food additive specifications.* FAO JECFA Monographs 10, 2010.

205. *Evaluation of certain food additives and contaminants* (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.

206. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 65, 2011.

207. *Compendium of food additive specifications.* FAO JECFA Monographs 11, 2011.

208. *Evaluation of certain veterinary drug residues in food* (Seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 969, 2012.

209. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 66, 2012.

210. *Residue evaluation of certain veterinary drugs*. FAO JECFA Monographs 12, 2012.

211. *Evaluation of certain food additives* (Seventy-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 974, 2012.

212. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 67, 2012.

213. *Compendium of food additive specifications.* FAO JECFA Monographs 13, 2012.

Annex 2
Toxicological information and information on specifications

Food additives considered for specifications only

Food additive	Specifications[a]
Ethyl cellulose	R
Mineral oil (medium viscosity)	N[b]
Modified starches	R
Titanium dioxide	R

[a] N, new specifications; R, existing specifications revised.
[b] The existing specifications for mineral oil (medium and low viscosity) were withdrawn (see below).

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Magnesium dihydrogen diphosphate	N	Although an acceptable daily intake (ADI) "not specified"[b] has been established for a number of magnesium salts used as food additives, the estimated chronic dietary exposures to magnesium (960 mg/day for a 60 kg adult at the 95th percentile) from the proposed uses of magnesium dihydrogen diphosphate are up to twice the background exposures from food previously noted by the Committee (180–480 mg/day) and in the region of the minimum laxative effective dose of approximately 1000 mg of magnesium when taken as a single dose. The estimates of dietary exposure to phosphorus from the proposed uses of magnesium dihydrogen diphosphate were in the region of, or slightly exceeded, the maximum tolerable daily intake (MTDI) of 70 mg/kg body weight (bw) for phosphate salts, expressed as phosphorus, from this source alone. Thus, the MTDI is further exceeded when other sources of phosphate in the diet are taken into

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		account. **The Committee therefore concluded that the proposed use levels and food categories result in an estimated dietary exposure to magnesium dihydrogen diphosphate that is of potential concern.** **The Committee emphasized that in evaluating individual phosphate-containing food additives, there is a need for assessment of total dietary exposure to phosphorus.** **The Committee recommended that total dietary exposure to magnesium from food additives and other sources in the diet should be assessed.** The information submitted to the Committee and in the scientific literature did not indicate that the MTDI of 70 mg/kg bw for phosphate salts, expressed as phosphorus, is insufficiently health protective. On the contrary, because the basis for its derivation might not be relevant to humans, it could be overly conservative. Therefore, **the Committee recommended that the toxicological basis of the MTDI for phosphate salts expressed as phosphorus be reviewed.**
Mineral oil (medium and low viscosity) classes II and III	W	**The Committee concluded that the newly submitted data did not adequately address its previous requests for information** on the relevance to humans of the response of F344 and Sprague-Dawley rats to mineral oil (medium and low viscosity) classes II and III. The studies were conducted with a single administration, and it was not possible to predict the concentration in the target organ (liver) at steady state, or the potential for accumulation, in humans. Information requested at the forty-fourth meeting on compositional factors of mineral oils that influence absorption and toxicity had not been provided for materials meeting the criteria of mineral oil (medium and low viscosity) classes II and III. The Committee noted that hydrocarbon deposits with carbon numbers consistent with mineral oils, including those of classes II and III, and associated lesions have been reported in human tissues, demonstrating the potential relevance to humans of the effects in the F344 rat. Because all blood levels were below the limit of detection in the single-dose human toxicokinetic study, it was not possible to reach conclusions on the rate of elimination of mineral oils in humans or on the concentration in the liver at steady state following prolonged exposure. Therefore, the new data did not provide information that would allow an ADI to be established based on internal exposure.

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		Similarly, it was not possible to establish an ADI based on external dose in the absence of information on the relative accumulation potential of classes II and III mineral oils in humans compared with rats. The Committee noted that the temporary group ADI for mineral oil (medium and low viscosity) classes II and III had been established in 1995 and extended on a number of occasions. As data supporting the establishment of a full ADI had not been made available, t**he previously established temporary group ADI was withdrawn.**
3-Phytase from *Aspergillus niger* expressed in *Aspergillus niger*	N	Comparing the conservative exposure estimate with the no-observed-adverse-effect level (NOAEL) from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 250. **The Committee allocated an ADI "not specified"[b] for 3-phytase enzyme preparation from *A. niger* expressed in *A. niger*, used in the applications specified and in accordance with good manufacturing practice.**
Serine protease (chymotrypsin) from *Nocardiopsis prasina* expressed in *Bacillus licheniformis*	N	Comparing the exposure estimate with the NOAEL from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 350. **The Committee allocated an ADI "not specified"[b] for serine protease (chymotrypsin) enzyme preparation from *N. prasina* expressed in the production strain *B. licheniformis*, used in the applications specified and in accordance with good manufacturing practice.**
Serine protease (trypsin) from *Fusarium oxysporum* expressed in *Fusarium venenatum*	N	Comparing the dietary exposure estimate with the NOAEL from the 13-week study of oral toxicity in rats, the margin of exposure is approximately 1200. **The Committee allocated an ADI "not specified"[b] for serine protease (trypsin) enzyme preparation from *F. oxysporum* expressed in the production strain *F. venenatum*, used in the applications specified and in accordance with good manufacturing practice.**

[a] N, new specifications; W, existing specifications withdrawn.

[b] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to 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.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents[4]

A. Aliphatic and aromatic amines and amides

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
2-Aminoacetophenone	2043	N	No safety concern
Structural class III			
(2*E*,6*E*/*Z*,8*E*)-*N*-(2-Methylpropyl)-2,6,8-decatrienamide	2077	N	No safety concern
(2*S*,5*R*)-*N*-[4-(2-Amino-2-oxoethyl)-phenyl]-5-methyl-2-(propan-2-yl)-cyclohexanecarboxamide	2078	N	No safety concern
(1*R*,2*S*,5*R*)-*N*-(4-Methoxyphenyl)-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide	2079	N	No safety concern
N-Cyclopropyl-5-methyl-2-isopropylcyclohexanecarboxamide	2080	N	No safety concern
N-(2-Methylcyclohexyl)-2,3,4,5,6-pentafluorobenzamide	2081	N	No safety concern
3[(4-Amino-2,2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-*N*-propylpropanamide	2082	N	No safety concern

[a] N, new specifications.

B. Aliphatic and aromatic ethers

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	2133	N	No safety concern

[4] The flavouring agent **2-phenyl-2-methyl-2-hexenal (No. 2069)** was submitted for evaluation in the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters; the Committee considered that it did not belong to this group of flavouring agents, and therefore it was not further considered. The safety of the submitted substance **(3*R*)-4-[[(1*S*)-1-benzyl-2-methoxy-2-oxo-ethyl]amino]-3-[3-(3-hydroxy-4-methoxy-phenyl)propylamino]-4-oxo-butanoic acid hydrate (Advantame, No. 2124)** in the group of amino acids and related substances was not assessed; the Committee decided that it would not be appropriate to evaluate this substance as a flavouring agent, because it is a low-calorie intense sweetener. The safety of the two submitted substances **rebaudioside C (No. 2168)** and **rebaudioside A (No. 2169)** in the group of phenol and phenol derivatives was not assessed; the Committee decided that it would not be appropriate to evaluate these substances as flavouring agents, as they had already been evaluated as food additives (sweeteners).

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Ethyl linalyl ether	2134	N	No safety concern
Linalool oxide pyranoid	2135	N	No safety concern
Nerolidol oxide	2137	N	Additional data required to complete evaluation
Methyl hexyl ether	2138	N	No safety concern
Myrcenyl methyl ether	2139	N	No safety concern
Digeranyl ether	2142	N	No safety concern
Structural class III			
Isoamyl phenethyl ether	2136	N	No safety concern
5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran	2140	N	No safety concern
Butyl β-naphthyl ether	2141	N	No safety concern

[a] N, new specifications.

C. Aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

The Committee concluded that the Procedure could not be applied to this group because of unresolved toxicological concerns. Studies that could assist in the safety evaluation include investigations of the influence of the nature and position of furan ring substitutions on metabolism and covalent binding to macromolecules, demonstration of the ring opening and reactivity of the resulting products. Depending on the findings, additional genotoxicity or other studies might be needed.

Flavouring agent	No.	Specifications[a]
2-Pentylfuran	1491	M
2-Heptylfuran	1492	M
2-Decylfuran	1493	M
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	M
3-(2-Furyl)acrolein	1497	M
3-(5-Methyl-2-furyl)prop-2-enal	1499	M
2-Furyl methyl ketone	1503	M
2-Acetyl-5-methylfuran	1504	M
2-Acetyl-3,5-dimethylfuran	1505	M
2-Butyrylfuran	1507	M
(2-Furyl)-2-propanone	1508	M
2-Pentanoylfuran	1509	M

Flavouring agent	No.	Specifications[a]
1-(2-Furyl)butan-3-one	1510	M
4-(2-Furyl)-3-buten-2-one	1511	M
Ethyl 3-(2-furyl)propanoate	1513	M
Isobutyl 3-(2-furan)propionate	1514	M
Isoamyl 3-(2-furan)propionate	1515	M
Isoamyl 4-(2-furan)butyrate	1516	M
Phenethyl 2-furoate	1517	M
Furfuryl methyl ether	1520	M
Ethyl furfuryl ether	1521	M
Difurfuryl ether	1522	M
2,5-Dimethyl-3-furanthiol acetate	1523	M
Furfuryl 2-methyl-3-furyl disulfide	1524	M
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	M
O-Ethyl *S*-(2-furylmethyl)thiocarbonate	1526	M
2,3-Dimethylbenzofuran	1495	M
2,4-Difurfurylfuran	1496	M
2-Methyl-3(2-furyl)acrolein	1498	M
3-(5-Methyl-2-furyl)-butanal	1500	M
2-Furfurylidene-butyraldehyde	1501	M
2-Phenyl-3-(2-furyl)prop-2-enal	1502	M
3-Acetyl-2,5-dimethylfuran	1506	M
Pentyl 2-furyl ketone	1512	M
Propyl 2-furanacrylate	1518	M
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	M
(*E*)-Ethyl 3-(2-furyl)acrylate	2103	N
Di-2-furylmethane	2104	N
2-Methylbenzofuran	2105	N

[a] M, specifications maintained; N, new specifications.

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

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
trans-2-Nonenyl acetate	2163	N	No safety concern
Propyl sorbate	2164	N	No safety concern
cis-2-Octenol	2165	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
trans-2-Tridecenol	2166	N	No safety concern
Ethyl 2-hexenoate (mixture of isomers)	2167	N	No safety concern

[a] N, new specifications.

E. Amino acids and related substances

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
L-Ornithine (as the monochlorohydrate)	2120	N	No safety concern
L-Alanyl-L-glutamine	2121	N	No safety concern
L-Methionylglycine	2122	N	No safety concern
Glutamyl-valyl-glycine	2123	N	No safety concern

[a] N, new specifications.

The Committee considered that the use of the Procedure for the Safety Evaluation of Flavouring Agents was inappropriate for two members of this group—namely, L-isoleucine (No. 2118) and L-threonine (No. 2119). In view of the fact that these substances are macronutrients and normal components of protein, the Committee concluded that the use of these substances as flavouring agents would not raise any safety concerns at current estimated dietary exposures.

Flavouring agent	No.	Specifications[a]
L-Isoleucine	2118	N
L-Threonine	2119	N

[a] N, new specifications.

F. Epoxides

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class III			
Ethyl α-ethyl-β-methyl-β-phenylglycidate	2143	N	No safety concern
Methyl β-phenylglycidate	2144	N	No safety concern
d-8-*p*-Menthene-1,2-epoxide	2145	N	No safety concern
l-8-*p*-Menthene-1,2-epoxide	2146	N	No safety concern
2,3-Epoxyoctanal	2147	N	**Additional data required to complete evaluation**

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
2,3-Epoxyheptanal	2148	N	**Additional data required to complete evaluation**
2,3-Epoxydecanal	2149	N	**Additional data required to complete evaluation**

[a] N, new specifications.

G. Furfuryl alcohol and related substances

New in vitro and in vivo studies raise concerns regarding the potential genotoxicity of furfuryl alcohol and derivatives that can be metabolized to furfuryl alcohol (e.g. furfuryl esters). The Committee concluded that this group of flavouring agents could not be evaluated according to the Procedure because of the unresolved concerns regarding genotoxicity. In addition, the group ADI previously established by the Committee will need to be reconsidered at a future meeting.

Flavouring agent	No.	Specifications[a]
5-Methylfurfuryl alcohol	2099	N
Furfural propyleneglycol acetal	2100	N
Furfuryl formate	2101	N
Furfuryl decanoate	2102	N

[a] N, new specifications.

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

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
cis-3-Nonen-1-ol	2177	N	No safety concern
trans-3-Nonen-1-ol	2178	N	No safety concern
cis,cis-3,6-Nonadienyl acetate	2179	N	No safety concern
trans-3-Hexenyl acetate	2180	N	No safety concern
cis-3-Hexenoic acid	2181	N	No safety concern
cis-3-Nonenyl acetate	2182	N	No safety concern
cis-6-Nonenyl acetate	2183	N	No safety concern
(*Z*)-5-Octenyl acetate	2184	N	No safety concern
(*E*)-4-Undecenal	2185	N	No safety concern

[a] N, new specifications.

I. Miscellaneous nitrogen-containing compounds

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
3-(1-((3,5-Dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-imidazolidine-2,4-dione	2161	N	No safety concern
3-(1-((3,5-Dimethylisoxazol-4-yl)methyl)-1H-pyrazol-4-yl)-1-(3-hydroxybenzyl)-5,5-dimethylimidazolidine-2,4-dione	2162	N	No safety concern

[a] N, new specifications.

J. Phenol and phenol derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class III			
3′,7-Dihydroxy-4′-methoxyflavan	2170	N	No safety concern
Trilobatin	2171	N	No safety concern
(±)-Eriodictyol	2172	N	No safety concern

[a] N, new specifications.

K. Pyrazine derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
Isopropenylpyrazine	2125	N	No safety concern
5-Ethyl-2,3-dimethylpyrazine	2126	N	No safety concern
2-Methyl-5-vinylpyrazine	2127	N	No safety concern
Mixture of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine and 2,7-dimethyl-6,7-dihydro-5H-cyclopentapyrazine	2128	N	No safety concern
2-Ethoxy-3-isopropylpyrazine	2065	N	No safety concern
Structural class III			
Mixture of 3,5-dimethyl-2-isobutylpyrazine and 3,6-dimethyl-2-isobutylpyrazine	2130	N	No safety concern
2-Ethoxy-3-ethylpyrazine	2131	N	No safety concern
2-Ethyl-3-methylthiopyrazine	2132	N	No safety concern

[a] N, new specifications.

L. Pyridine, pyrrole and quinoline derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
1-Ethyl-2-pyrrolecarboxaldehyde	2150	N	**Additional data required to complete evaluation**
2,4-Dimethylpyridine	2151	N	No safety concern (temporary)[b]
1-Methyl-1H-pyrrole-2-carboxaldehyde	2152	N	**Additional data required to complete evaluation**
Structural class III			
2-Acetyl-4-isopropenylpyridine	2153	T	No safety concern
4-Acetyl-2-isopropenylpyridine	2154	T	No safety concern
2-Acetyl-4-isopropylpyridine	2155	N	No safety concern
2-Methoxypyridine	2156	N	**Additional data required to complete evaluation**
6-Methoxyquinoline	2157	N	No safety concern
1-(2-Hydroxyphenyl)-3-(pyridin-4-yl)-propan-1-one	2158	N	**Additional data required to complete evaluation**
1-(2-Hydroxy-4-isobutoxyphenyl)-3-(pyridin-2-yl)propan-1-one	2159	N	**Additional data required to complete evaluation**
1-(2-Hydroxy-4-methoxyphenyl)-3-(pyridin-2-yl)propan-1-one	2160	N	**Additional data required to complete evaluation**

[a] N, new specifications; T, tentative specifications.
[b] The evaluation for No. 2151 is temporary pending receipt of additional toxicological data.

M. Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class I			
3-Methylhexanal	2173	N	No safety concern
6-Methylheptanal	2174	N	No safety concern
6-Methyloctanal	2175	N	No safety concern
3,7-Dimethyloctanal	2176	N	No safety concern

[a] N, new specifications.

N. Simple aliphatic and aromatic sulfides and thiols

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Subgroup ii: Acyclic sulfides with oxidized side-chains			
Structural class I			
1-(Methylthio)-3-octanone	2086	N	No safety concern
Subgroup iii: Cyclic sulfides			
Structural class III			
4-Methyl-2-propyl-1,3-oxathiane	2089	N	No safety concern
Subgroup iv: Simple thiols			
Structural class I			
3-Pentanethiol	2083	N	No safety concern
Subgroup v: Thiols with oxidized side-chains			
Structural class I			
4-Mercapto-3-methyl-2-butanol	2084	N	No safety concern
Ethyl 2-mercapto-2-methylpropionate	2085	N	No safety concern
Subgroup vi: Dithiols			
Structural class III			
1,1-Propanedithiol	2087	N	No safety concern
Subgroup viii: Disulfides with oxidized side-chains			
Structural class III			
1-Methyldithio-2-propanone	2088	N	No safety concern

[a] N, new specifications.

O. Sulfur-containing heterocyclic compounds

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class II			
2-Pentylthiophene	2106	N	No safety concern
2-Acetyl-5-methylthiophene	2107	N	No safety concern
2-Pentylthiazole	2108	N	No safety concern
4,5-Dimethyl-2-isobutylthiazole	2109	N	No safety concern
Structural class III			
3,4-Dimethylthiophene	2110	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
2-Thienylmethanol	2111	N	No safety concern
1-(2-Thienyl)ethanethiol	2112	N	No safety concern
5-Ethyl-2-methylthiazole	2113	N	No safety concern
2-Ethyl-2,5-dihydro-4-methylthiazole	2114	N	No safety concern
4-Methyl-3-thiazoline	2115	N	No safety concern
2-Ethyl-4,6-dimethyldihydro-1,3,5-dithiazine	2116	N	No safety concern
4-Amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride	2117	N	No safety concern

[a] N, new specifications.

P. Sulfur-substituted furan derivatives

Flavouring agent	No.	Specifications[a]	Conclusion based on current estimated dietary exposure
Structural class III			
5-Methylfurfuryl mercaptan	2090	N	No safety concern
2-Methyl-3-furyl methylthiomethyl disulfide	2091	N	No safety concern
2-Methyl-3-furyl 2-methyl-3-tetrahydrofuryl disulfide	2092	N	No safety concern
2-Tetrahydrofurfuryl 2-mercaptopropionate	2093	N	**Additional data required to complete evaluation**
Methyl 3-(furfurylthio)propionate	2094	N	No safety concern
3-[(2-Methyl-3-furyl)thio]butanal	2095	N	No safety concern
1-(2-Furfurylthio)-propanone	2096	N	No safety concern
2-Methyl-4,5-dihydrofuran-3-thiol	2097	N	No safety concern
2-Methyltetrahydrofuran-3-thiol acetate	2098	N	No safety concern

[a] N, new specifications.

Annex 3
Further information required or desired

Paprika extract

For the revision of the specification for paprika extract, no data were received. Based on the commitment by the sponsor to provide data for a future meeting, this evaluation was postponed.

Specifications for flavouring agents

The specifications for Nos 2153 (2-acetyl-4-isopropenylpyridine) and 2154 (4-acetyl-2-isopropenylpyridine) were made tentative at the current meeting because the submitted information was insufficient. The two flavouring agents are positional isomers, and the Committee concluded that the current specifications would not allow for differentiation between the two substances. Information that could be used to differentiate between the two substances (e.g. optical [specific] rotation) is requested.

Aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution

The Committee concluded that the Procedure could not be applied to this group because of unresolved toxicological concerns. Studies that could assist in the safety evaluation include investigations of the influence of the nature and position of furan ring substitutions on metabolism and covalent binding to macromolecules, demonstration of the ring opening and reactivity of the resulting products. Depending on the findings, additional genotoxicity or other studies might be needed.

Furfuryl alcohol and related substances

New in vitro and in vivo studies raise concerns regarding the potential genotoxicity of furfuryl alcohol and derivatives that can be metabolized to furfuryl alcohol (e.g. furfuryl esters). The Committee concluded that this group of

flavouring agents could not be evaluated according to the Procedure because of the unresolved concerns regarding genotoxicity. In addition, the group ADI previously established by the Committee will need to be reconsidered at a future meeting.

Pyridine, pyrrole and quinoline derivatives

For 2,4-dimethylpyridine (No. 2151), the safety evaluation was made temporary, pending the submission of the full report of the critical study for the next JECFA meeting at which flavouring agents are evaluated.

Additional data required to complete the evaluation according to the Procedure for the Safety Evaluation of Flavouring Agents

Additional data are required to complete the toxicological evaluations of 11 flavouring agents (Nos 2093, 2137, 2147–2150, 2152, 2156 and 2158–2160).

Annex 4

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

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
Simple aliphatic and aromatic sulfides and thiols				
2088	1-Methyldithio-2-propanone	90	2–3% 1-mercapto-2-propanone; 2–3% 1,1′-disulfanediyldipropan-2-one; 1–3% 1,3-dimethyltrisulfane	1-Mercapto-2-propanone (No. 557) and 1,3-dimethyltrisulfane (No. 582) were evaluated by the Committee at its fifty-third meeting (Annex 1, reference *149*) and were concluded to be of no safety concern at estimated dietary exposures. 1,1′-Disulfanediyldipropan-2-one is expected to be hydrolysed to 1-mercapto-2-propanone (No. 557) and is therefore considered not to present a safety concern at current estimated dietary exposures to the flavouring agent.
Sulfur-substituted furan derivatives				
2097	2-Methyl-4,5-dihydrofuran-3-thiol	55	35–40% 2-methyl-3-tetrahydrofuranthiol; 5–7% 2-methyl-3-furanthiol	2-Methyl-3-tetrahydrofuranthiol (No. 1090) and 2-methyl-3-furanthiol (No. 1060) were evaluated by the Committee at the fifty-ninth meeting (Annex 1, reference *160*) and were concluded to be of no safety concern at estimated dietary exposures.
Sulfur-containing heterocyclic compounds				
2114	2-Ethyl-2,5-dihydro-4-methylthiazole	90	2–3% 2-ethyl-4-methyl-4,5-dihydrothiazole-4-ol; 2–3% 3,4-dimethylthiophene; 2–3% 2-ethyl-4-methylthiazole	2-Ethyl-4-methylthiazole (No. 1044) was evaluated by the Committee at the fifty-ninth meeting (Annex 1, reference *160*) and was concluded to be of no safety concern at estimated dietary exposures. 2-Ethyl-4-methyl-4,5-dihydrothiazole-4-ol is anticipated to undergo glucuronic acid conjugate formation and elimination in the urine. It does not present a safety concern at current estimated dietary exposures. 3,4-Dimethylthiophene (No. 2110) is a member of the current group and is expected to undergo side-chain oxidation and subsequent conjugation and elimination in the urine. It does not present a safety concern at current estimated dietary exposures to the flavouring agent.

JECFA No.	Flavouring agent	Minimum assay value	Secondary components	Comments on secondary components
2116	2-Ethyl-4,6-dimethyldihydro-1,3,5-dithiazine	90	3–5% 3,5-diethyl-1,2,4-trithiolane and 2–3% 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine	3,5-Diethyl-1,2,4-trithiolane (No. 1686) and 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine (No. 1049) were evaluated at the sixty-eighth (Annex 1, reference *187*) and the fifty-ninth (Annex 1, reference *160*) meetings, respectively, and were concluded to be of no safety concern at estimated dietary exposures.
Aliphatic and aromatic ethers				
2135	Linalool oxide pyranoid	92	3–5% linalool	Linalool (No. 356) was evaluated by the Committee at the fifty-first meeting (Annex 1, reference *137*) and was concluded to be of no safety concern at estimated dietary exposures.
Epoxides				
2144	Methyl β-phenylglycidate	85	10–12% ethyl β-phenylglycidate	Ethyl β-phenylglycidate (No. 1576) was evaluated by the Committee at the sixty-fifth meeting (Annex 1, reference *178*) and was concluded to be of no safety concern at estimated dietary exposures.
2148	2,3-Epoxyheptanal	94	2–3% *trans*-2-heptenal	*trans*-2-Heptenal (No. 1360) was evaluated by the Committee at the sixty-third meeting (Annex 1, reference *173*) and was concluded to be of no safety concern at estimated dietary exposures.
2149	2,3-Epoxydecanal	94	2–3% *trans*-2-decenal	*trans*-2-Decenal (No. 1349) was evaluated by the Committee at the sixty-third meeting (Annex 1, reference *173*) and was concluded to be of no safety concern at estimated dietary exposures.

Evaluation of certain food additives

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, including flavouring agents, with a view to concluding as to safety concerns and to preparing specifications for identity and purity.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of and assessment of dietary exposure to food additives, including flavouring agents. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for five food additives (magnesium dihydrogen diphosphate; mineral oil (medium and low viscosity) classes II and III; 3-phytase from *Aspergillus niger* expressed in *Aspergillus niger*; serine protease (chymotrypsin) from *Nocardiopsis prasina* expressed in *Bacillus licheniformis*; and serine protease (trypsin) from *Fusarium oxysporum* expressed in *Fusarium venenatum*) and 16 groups of flavouring agents (aliphatic and aromatic amines and amides; aliphatic and aromatic ethers; aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers containing furan substitution; aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters; amino acids and related substances; epoxides; furfuryl alcohol and related substances; linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters; miscellaneous nitrogen-containing substances; phenol and phenol derivatives; pyrazine derivatives; pyridine, pyrrole and quinoline derivatives; saturated aliphatic acyclic branched-chain primary alcohols, aldehydes and acids; simple aliphatic and aromatic sulfides and thiols; sulfur-containing heterocyclic compounds; and sulfur-substituted furan derivatives).

Specifications for the following food additives were revised: ethyl cellulose, mineral oil (medium viscosity), modified starches and titanium dioxide.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures to and toxicological evaluations of the food additives and flavouring agents considered.

ISBN 978-92-4-120974-8